25th Symposium on Biotechnology for Fuels and Chemicals

Hosted by the National Renewable Energy Laboratory

BIOTECHNOLOGY

Program & Abstracts

Beaver Run Resort
Breckenridge, Colorado
MAY 4-7, 2003

Organizing Committee Members:

Mark Finkelstein, Conference Chairman
National Renewable Energy Laboratory
Golden, Colorado

Brian Davison, Conference Co-chairman
Oak Ridge, National Laboratory
Oak Ridge, Tennessee

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Dartmouth College
Hanover, New Hampshire

Guido Zacchi
Lund University
Lund, Sweden

Gisella M. Zanin
State University of Maringa
Maringa, Parana, Brazil

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 25th 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 25th Symposium, we continue the tradition of providing an informal, congenial atmosphere that our participants find conducive to technically discussing program topics. This year technical topics include:

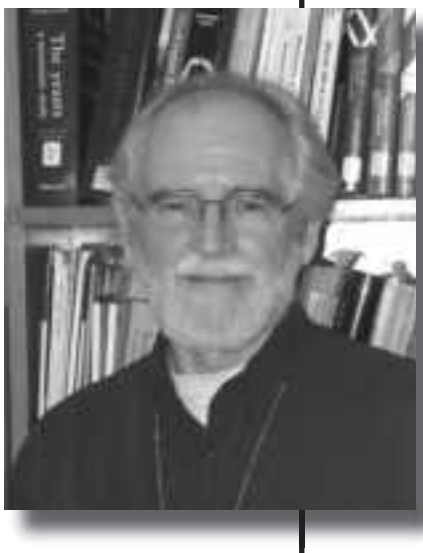
Session 1A.	Feedstock Supply, Logistics, Processing, and Composition
Session 1B.	Enzyme Catalysis and Engineering
Session 2.	Microbial Catalysis and Engineering
Session 3.	Bioprocessing including Separations
Session 4.	Biotechnology for Fuels and Chemicals – Past, Present, and Future
Session 5.	Biobased Industrial Chemicals
Session 6A.	Biomass Preteatment and Hydrolysis
Session 6B.	Plant Biotechnology and Feedstock Genomics
Special Topics A.	Microbial Pentose Metabolism
Special Topics B.	International Bioenergy Agency Bioethanol Meeting

This year we will augment our technical program with an exciting presentation by Dr. J. Craig Venter, Genome Sequencer, Entrepreneur, and Chief Executive Officer. The title of his presentation is "*Genomic Approaches to the Environment*."

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 Biotechnology Symposium 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 BSFC to recognized persons who have distinguished themselves in the area of biotechnology to produce fuels and chemicals. See page 'i' for information on this years winner.

As always, we have included events to provide you with opportunities to socialize with your colleagues. You will also have opportunities to explore Colorado's Rocky Mountains. Ask at the registration desk for more information.

Charles D. Scott Award



Through the course of his professional career, Tom Jeffries has worked on many aspects of bioenergy and biomass conversion. Tom's fundamental interest in primary productivity is reflected in his MS work with photosynthetic bacteria from 1969-1971 and in his work at on the biophotolytic production of hydrogen by blue-green algae. During doctoral studies on yeast cell wall lysis at Rutgers University from 1972-1975, Tom had the good fortune of working with James D. Macmillan, Elwin Reese, Mary Mandels and Doug Eveleigh, all of whom spurred a life-long interest in enzymatic hydrolysis of cellulose, hemicellulose and complex polysaccharides. While at the Lawrence Livermore National Laboratory from 1976-1977, Tom worked with a highly interdisciplinary team to assess the magnitude of the U.S. biomass resource. In post-doctoral studies with Harry Gregor in the Department of Chemical Engineering at Columbia University from 1978-1979, Tom developed membrane-coupled anaerobic fermentations of cellulose for the production of methane and short chain organic acids. In 1979, Tom joined Kent Kirk at the Forest Products Laboratory (FPL) where he worked for several years on the regulation of lignin biodegradation. In the early 1980's the FPL ethanol program under the guidance of Jerry Saeman enabled Tom to return to studies on yeast physiology and regulation. His research into the oxygen requirements for yeast xylose fermentations has continued to this day. This has led to increased knowledge of xylose metabolism and improved recombinant yeasts, especially *Pichia stipitis*. Tom has also mentored many students both at FPL and the University of Wisconsin. In this Symposium, Tom has served in many roles since the earliest meetings. Tom's life has been blessed by the support and encouragement of his wife, Giovanna Miceli-Jeffries and by the love of their three daughters, Angelica, Carla and Francesca.

25th Symposium on Biotechnology for Fuels and Chemicals
Hosted by the National Renewable Energy Laboratory

Conference Program

Sunday, May 4, 2003

10:00 am - 5:00 pm	Registration/Editor's Desk Open	Foyer, 3rd Floor
10:00 am - 8:00 pm	Poster Session/Exhibits Set-up	Breckenridge Ballroom
1:00 am - 1:10 pm	Opening Remarks	Peaks 1 - 5, 3rd Floor
1:10 pm - 5:00 pm	**Session 1A and Session 1B (Concurrent Sessions)**	**Peaks 1 - 5, 3rd Floor**
5:30 pm - 8:30 pm	Welcoming Reception	Lower Pool Area

Monday, May 5, 2003

7:30 am - 5:00 pm	Registration/Editor's Desk Open	Foyer, 3rd Floor
7:15 am - 8:00 am	Continental Breakfast	South Foyer, 3rd Floor
7:15 am - 8:00 am	Speakers' Breakfast	Peak 6 - 7, 2nd Floor
8:00 am - 12:00 pm	**Session 2**	**Peaks 1 - 5, 3rd Floor**
12:00 pm - 1:00 pm	Lunch On Your Own	
2:00 pm - 4:00 pm	**Special Topics**	**Peaks 1 - 5, 3rd Floor**
	Session A and Session B (Concurrent Sessions)	
5:30 pm - 10:00 pm	Poster Reception	Breckenridge Ballroom

Tuesday, May 6, 2003

7:30 am - 1:00 pm	Registration/Editor's Desk Open	Foyer, 3rd Floor
7:15 am - 8:00 am	Continental Breakfast	South Foyer, 3rd Floor
7:15 am - 8:00 am	Speakers' Breakfast	Peak 6 - 7, 2nd Floor
8:00 am - 12:00 pm	**Session 3**	**Peaks 1 - 5, 3rd Floor**
12:00 pm - 1:30 pm	Organizing Committee Luncheon and Meeting	Peak 6 - 7, 2nd Floor
	Free Afternoon	
3:00 pm - 10:00 PM	Poster Exhibit Removal	Breckenridge Ballroom
7:00 pm - 9:30 pm	**Session 4**	**Peaks 1 - 5, 3rd Floor**

Wednesday, May 7, 2003

7:30 am - 3:00 pm	Registration/Editor's Desk Open	Foyer, 3rd Floor
7:15 am - 8:00 am	Continental Breakfast	South Foyer, 3rd Floor
7:15 am - 8:00 am	Speakers' Breakfast	Peak 6 - 7, 2nd Floor
8:00 am - 12:00 pm	**Session 5**	**Peaks 1 - 5, 3rd Floor**
8:00 am - 11:00 am	Final Poster Exhibit Removal	Breckenridge Ballroom
12:00 pm - 1:00 pm	Lunch On Your Own	
1:00 pm - 5:00 pm	**Session 6A and Session 6B (Concurrent Sessions)**	**Peaks 1 - 5, 3rd Floor**
5:30 pm - 6:30 pm	Pre-Banquet: Cocktails in the Foyer	Foyer, 3rd Floor
6:30 pm - 10:00 pm	Banquet Speaker/Awards/Entertainment	Peaks 1 - 5, 3rd Floor

Sunday, May 4, 2003

Session 1A – Feedstock Supply, Logistics, Processing, and Composition

Chair: Jim Hettenhaus, C.E.A. Inc.
Co-Chair: David Morris, Institute for Local Self-Reliance

25 minute talks with 5 minutes for questions

1:00 p.m. Opening remarks
Symposium Chair, Session Chair/Co-Chair

1:15 p.m. Oral Presentation 1A-01. **What Have We learned from Federal R&D Programs in Biomass?** *David Morris*, Institute for Local Self-Reliance, Minneapolis, MN

1:45 p.m. Oral Presentation 1A-02. **Rainfall and Wind Erosion-Based Sustainable Residue Removal Analysis and Resource Assessment for Corn Stover and Wheat Straw for Eight Selected Cropping Rotations in the United States,** *Richard G. Nelson*, Enersol Resources & Kansas State University, Manhattan, KS

2:15 p.m. Oral Presentation 1A-03. **Single Pass Whole-plant Corn Harvesting for Biomass: Comparison of Single and Versus Multiple Harvest Streams,** Kevin J. Shinners, Biological Systems Engineering Dept, University of Wisconsin, Madison, WI and *Philippe Savoie*, Agriculture and Agri-Food Canada, Sainte-Foy, Quebec, Canada

2:45 p.m. Break

3:15 p.m. Oral Presentation 1A-04. **Pipeline Transport of Biomass,** *Amit Kumar*, Jay B. Cameron, P. C. Flynn, Department of Mechanical Engineering, University of Alberta, Edmonton, Alberta, Canada

3:45 p.m. Oral Presentation 1A-05. **The Effect of Corn Stover Composition on Ethanol Process Economics,** *Mark F. Ruth*, Steven R. Thomas, NREL, Golden, CO

4:15 p.m. Oral Presentation 1A-06. **Corn Stover Feedstock Learnings: Kearney Area Ag Producers Alliance Removal Project,** John Love, Kearney, NE

4:45 p.m. Closing remarks – Chair/Co-Chair

Session 1B – Enzyme Catalysis and Engineering

Chair: Liisa Viikari, VTT
Co-Chair: Michael Himmel, NREL

25 minute talks with 5 minutes for questions

1:00 p.m. Opening Remarks
25th Symposium Chair, Session Chair/Co-Chair

1:15 p.m. Oral Presentation 1B-01. **Strain and Process Improvement of Whole Cellulase by *Trichoderma reesei,*** Elizabeth Bodie, *Tim Dodge*, Genencor International, Palo Alto, CA

1:45 p.m. Oral Presentation 1B-02. **Thermostability, Substrate Specificity and Hydrolysis of Cellulose by Endoglucanases from Families 5, 7, and 45 of Glycoside Hydrolases,** *Elena Vlasenko*, Feng Xu, Joel Cherry, Novozymes Biotech, Inc., Davis, CA

2:15 p.m. Oral Presentation 1B-03. **An Accelerated Evolutionary Route to Enzyme Fitness,** Nisha Palackal, Walt Callen, Gerhard Frey, Shaun Healey, Young Kang, Keith Kretz, Edward Lee, Eric Mathur, Dan Robertson, Jay Short, Xuqiu Tan, Geoff Tomlinson, John Verruto, Ya-Li Yang, *Brian Steer*, Diversa Corporation, San Diego, CA

2:45 p.m. Break

3:15 p.m. Oral Presentation 1B-04. **Impact of Binding Domains in Cellulase Activity Assays and in Hydrolysis of Crystalline Cellulose,** *Liisa Viikari*, Matti Siika-aho, Maija Tenkanen, VTT Biotechnology, Finland

3:45 p.m. Oral Presentation 1B-05. **Molecular Modeling of the *T. reesei* CBH I Linker,** *Tauna Rignall* and Clare McCabe, Dept of Chemical Engineering, Colorado School of Mines, Golden, CO, Dick Woods and John Brady, Department of Food Science, Cornell University, Ithaca, NY, and Michael Himmel, National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO

4:15 p.m. Oral Presentation 1B-06. **Selection of Endoxylanases for Depolymerization of Glucuronoxylan from Hemicellulose,** *James F. Preston*, Franz J. St. John, John D. Rice, University of Florida, Gainesville, FL

4:45 p.m. Closing Remarks – Chair/Co-Chair

Monday, May 5, 2003

Session 2 – Microbial Catalysis and Engineering

Chair: Tom Jeffries, University of Wisconsin
Co-Chair: Lee Lynd, Dartmouth College

25 minute talks with 5 minutes for questions

8:00 a.m. Opening Remarks – Session Chair/Co-Chair

8:15 a.m. Oral Presentation 2-01. **Genomics for Industrial Yeast Physiology and Vice Versa,** *Antonius J.A. van Maris,* Jan-Maarten A. Geertman, Viktor Boer, Johannes P. van Dijken, Jack T. Pront, Kluyverlaboratory of Biotechnology, Delft University of Technology, The Netherlands

8:45 a.m. Oral Presentation 2-02. **Evaluation of Recombinant Microorganism Ethanol Fermentation of Corn Fiber Hydrolysate,** *Eric Dennison,* Charles Abbas, Archer Daniels Midland Research Department Decatur, IL

9:15 a.m. Oral Presentation 2-03. **Conversion of Non-native Glucans by Strains of Saccharomyces cerevisiae Expressing Heterologous Enzymes,** *Willem H. Van Zyl,* Daniel C. La Grange, Johanna J. Zietsman, Dept of Microbiology, Sharath Gundllapalli, Ricardo Cordero Otero, Isak S. Pretorius, Institute for Wine Biotechnology, University of Stellenbosch, South Africa, and John McBride, Lee R. Lynd, Thayer School of Engineering, Dartmouth College, Hanover, NH

9:45 a.m. Break

10:15 a.m. Oral Presentation 2-04. **Bioelectrosynthesis of Chemicals and Fuels Using Microbes,** *J. Greg Zeikus*, D. H. Park, Michigan State University, East Lansing, MI

10:45 a.m. Oral Presentation 2-05. **Second Generation Biocatalysts for Production of Fuels and Chemicals from Biomass,** Milind Patel, Mark Ou, L.O. Ingram, *K.T. Shanmugam*, University of Florida, Dept of Microbiology and Cell Science, Gainesville, FL

11:15 a.m. Oral Presentation 2-06. **Manipulating *Saccharomyces cerevisiae* Redox Metabolism for Improved Xylose Consumption,** *Lisbeth Olsson*, Christophe Roca, Christina Lauritzen, Jens Nielsen, Center for Process Biotechnology, BioCentrum-DTU, Lyngby, Denmark

11:45 a.m. Closing Remarks – Session Chair/Co-chair

Special Topics Discission Group

2:00 p.m. - 4:00 p.m. (Concurrent Sessions)

Session Special Topics A: Microbial Pentose Metabolism

Chair: Bärbel Hahn-Hägerdal, Lund University, Sweden
Co-Chair: Neville Pamment, University of Melbourne, Austria

Invited Speakers:

- Lonnie Ingram, University of Florida
- Bruce Dien, USDA
- Min Zhang, NREL
- Tom Jeffries, University of Wisconsin
- Bob Benson, Tembec
- Jessica Becker, Institute for Microbiology, Frankfurt
- Annelie Nilsson, Lund University
- Lee Lynd, Dartmouth College

Session Special Topics B: International Bioenergy Agency Bioethanol Meeting-Current State of Fuel Ethanol Commercialization

Chair: Jack Saddler, University of British Columbia, Vancouver, BC

Invited Speakers:

- Gary Punter, British Sugar
- Greg Luli, BC International
- David Glassner, Cargill Dow
- Kendall Pye, Lignol
- Quang Nguyen, Abengoa Bioenergy
- Guido Zacchi, Swedish Bioethanol
- Bob Benson, Tembec
- Shiro Saka, Kyoto University
- Warren Mabee, UBC Process Development Unit

Tuesday, May 6, 2003

Session 3 – Bioprocessing, Including Separations

Chair: Dale Monceaux, Katzen International, Inc.
Co-Chair: David R. Short, DuPont

20 minute talks with 5 minutes for questions

8:00 a.m. Opening Remarks – Session Chair/Co-Chair

8:10 a.m. Oral Presentation 3-01. **Development and Application of Computational Fluid Dynamics Models for Scale-up of Biocommodity Processes,** *Xiongjun Shao,* Colin Hebert, Zhiliang Fan, Charles E. Wyman, Lee Lynd, Thayer School of Engineering, Dartmouth College, Hanover, NH, and Andre Bakker, Fluent Corporation, Lebanon, NH

8:35 a.m. Oral Presentation 3-02. **Identification of Microbial Inhibitory Functional Groups in Corn Stover Hydrolysate by 13C NMR Spectroscopy**, *F. A. Agblevor*, J. Fu, B. Hames, J.D. McMillan, Dept of Biological Systems Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA, and NREL, Golden, CO

9:00 a.m. Oral Presentation 3-03. **Nisin and Lactic Acid Simultaneous Production from Cheese Industry Byproducts: Optimization of Fermentation Conditions Through Statistically Based Experimental Designs,** *Chuanbin Liu*, Yan Liu, Wei Liao, Zhiyou Wen, Dongmei Wang, Shulin Chen, Dept of Biological Systems Engineering, Washington State University, Pullman, WA

9:25 a.m. Oral Presentation 3-04. **High-Rate Thermophilic Methane Fermentation on Short-Chain Fatty Acids,** Masahiro Tatara, *Akira Yamazawa,* Yoshiyuki Ueno, Hisatomo Fukui, Masafumi Goto, Dept of Environmental Engineering, Kajima Technical Research Institute, Tokyo, Japan

9:50 a.m. Break

10:15 a.m. Oral Presentation 3-05. **A Separative Bioreactor: Direct Product Capture and pH Control,** Yupo J. Lin, Michelle B. Arora, Jamie A. Hestekin, Edward J. St. Martin, Cynthia S. Millard, Mark Donnelly, *Seth W. Snyder,* Chemical and Biological Technology, Argonne National Laboratory, IL

10:40 a.m. Oral Presentation 3-06. **Optimization of Xylose Fermentation in Spent Sulfite Liquor by Saccharomyces cerevisiae 259ST,** *Steven S. Helle*, Sheldon J. B. Duff, Dept of Chemical & Biological Engineering, UBC Vancouver, BC and David R. Cameron, Robert Benson, Tembec Chemical Products Group, Temiscaming, Quebec, Canada

11:05 a.m. Oral Presentation 3-07. **Development of a Fermentation-Based Process for 1,3-Propanediol: Highlights of a Successful Path from Corn to Textile Fiber,** *Tyler T. Ames*, DuPont Central Research & Development, Wilmington, DE, and Catherine H. Babowicz, DuPont Bio-Based Materials Business Unit, Wilmington, DE

11:30 a.m. Closing Remarks – Session Chair/Co-Chair

Session 4 -Biotechnology for Fuels and Chemicals – Past, Present, and Future

Chair: Charles D. Scott, ORNL Retired
Co-Chair: Charles E. Wyman, Dartmouth College

7:00 p.m. Oral Presentation 4-01. **Origins and Changes in Annual Symposium on Biotechnology for Fuels and Chemicals,** *Charles D. Scott,* Retired Director, Bioprocessing Research and Development Center, ORNL, Oak Ridge, TN

7:20 p.m. Oral Presentation 4-02. **Improvements in Applied Microbiology and Biochemistry for Bioprocessing,** *Tom Jeffries*, USDA, University of Wisconsin, Madison, WI

7:35 p.m. Oral Presentation 4-03. **Evolution of Industrial Bioprocessing,** Don Johnson, Retired Consultant, Hertford, NC

7:50 p.m. Oral Presentation 4-04. **Startup of a New Business in Processing of Biomass to Chemicals,** *David Glassner,* Cargill Dow LLC, Minnetonka, MN

8:05 p.m. Oral Presentation 4-05. **Synergies, and Challenges for Biological Processing of Cellulosic Biomass to Fuels and Chemicals,** *Charles Wyman,* Dartmouth College, Hanover, NH

8:20 p.m. Oral Presentation 4-06. **Government Priorities and Support: Past, Present, and Future,** *Richard Moorer,* U.S. Department of Energy, Washington, DC

8:35 p.m. Oral Presentation 4-07. **Advanced Technology Scenarios for Production of Fuels and Chemicals,** *Lee Lynd*, Dartmouth College, Hanover, NH

8:50 p.m. Oral Presentation 4-08. **A Future for the Carbohydrate Economy?** *David Morris,* Institute for Local Self Reliance

9:05 p.m. Closing Remarks, Session Chair/Co-Chair

Wednesday, May 7, 2003

Session 5 - Biobased Industrial Chemicals

Chair: Doug Carmeron, Cargill, Inc.
Co-Chair: Marion Bradford, Tate & Lyle, Retired
25 minute talks with 5 minutes for questions

8:00 a.m. Opening Remarks – Session Chair/Co-Chair

8:15 a.m. Oral Presentation 5-01. **Opportunities in the Biobased Products Industry,** *Tracy M. Carole*, Joan Pellegrino, Energetics, Inc. Columbia, MD and Mark Paster, DOE, Washington DC

8:45 a.m. Oral Presentation 5-02. **DuPont Sorona[R] 3GT – First of a Family from Bio-Based Materials,** *Ray W. Miller*, E. I DuPont de Nemours and Company, Inc., Wilmington, DE

9:15 a.m. Oral Presentation 5-03. **3-hydroxypropionic Acid-A New Intermediate Platform,** *Rich Zvosec*, Cargill Inc., Wayzata, MN

9:45 a.m. Break

10:15 a.m. Oral Presentation 5-04. **Biobased Succinic Acid as a Building Block for Fuels and Chemicals,** Ulrika Rova, *Kris A. Berglund*, Lulea University of Technology, Sweden

10:45 a.m. Oral Presentation 5-05. **PHAs–A Versatile Family of Biobased Performance Polymers**, *Oliver Peoples*, *James Barber*, Metabolix Inc., Cambridge, MA

11:15 a.m. Oral Presentation 5-06. **Novel Thermochemical Pathways for Converting Glutamic Acid to Value Added Products,** *Johnathan E. Holladay*, Todd A. Werpy, Pacific Northwest National Laboratory, Richland, WA

Session 6A: Biomass Pretreatment and Hydrolysis

Chair: Y.Y. Lee, Auburn University
Co-Chair: Bruce Dale, Michigan State University
25 minute talks with 5 minutes for questions

1:00 p.m. Opening Remarks – Chair/Co-Chair

1:15 p.m. Oral Pre sentation 6A-01. **Understanding Factors that Limit Enzymatic Hydrolysis of Biomass: Characterization of Pretreated Corn Stover,** *Lizbeth Laureano-Perez,* Farzaneh Teymouri, Hasan Alizadeh, Bruce E. Dale, Michigan State University, East Lansing, MI

1:45 p.m. Oral Presentation 6A-02. **Comparison of SHF and SSF of Two-step Steam Pretreated Softwood for Ethanol Production,** *Joanna Soderstrom*, Mats Galbe, Guido Zacchi, Lund University, Sweden

2:15 p.m. Oral Presentation 6A-03. **Can We Produce an "Ideal" Substrate from Softwood for Enzymatic Hydrolysis?** *Xiao Zhang*, Zhizhuang Xiao, David Gregg, John Saddler, Forest Products Biotechnology, University of British Columbia, Vancouver, BC, Canada

2:45 p.m. Break

3:15 p.m. Oral Presentation 6A-04. **Corn Fiber Pretreatment Scale-up and Evaluation in an Industrial Corn to Ethanol Facility,** Nathan S. Mosier, Richard Hendrickson, Gary Welch, Richard Dreschel, Bruce Dien, *Michael Ladisch,* Purdue University, West Lafayette, IN; Williams Bioenergy, Pekin, IL; and USDA NCAUR Laboratories, Peoria, IL

3:45 p.m. Oral Presentation 6A-05. **Potential to Improve Dry Mill Economics by Increasing Ethanol Yield from Corn Fiber Residue,** *Nick Nagle*, Melvin P. Tucker, Bruce Dien, Kevin Hicks, Quang Nguyen, NREL, Golden CO; USDA-NCAUR, Peoria, IL; USDA-ERRC, Wyndmoor, PA; High Plains Corporation, St. Louis, MO

4:15 p.m. Oral Presentation 6A-06. **Comparative Data from Application of Leading Pretreatment Technologies to Corn Stover,** Bruce E. Dale, Richard T. Elander, Mark Holtzapple, Michael R. Ladisch, Y.Y. Lee, Tim Eggeman, *Charles E. Wyman*, Michigan State University, Lansing, MI; NREL, Golden, CO; Texas A&M University, College Station, TX; Purdue University, West Lafayette, IN; Auburn College, Auburn, AL; Neoterics International, Lakewood, CO; Dartmouth College, Hanover, NH

4:45 p.m. Closing Remarks – Chair/Co-Chair

Wednesday, May 7, 2003 *(continued)*

Session 6B – Plant Biotechnology and Feedstock Genomics

Chair: Jim McLaren, Inverizon International
Co-Chair: Steve Thomas, NREL

25 minute talks with 5 minutes for questions

1:00 p.m. Opening Remarks – Session Chair, Co-Chair

1:15 p.m. Oral Presentation 6B-01. **Improving Feedstocks for Energy Production Through the Acquisition of Complete Crop Gene Sequence by Genethresher™ Methylation Filtering Technology,** *Nathan Lakey,* Muhammad A. Budiman, Andrew N. Nunberg, Robert W. Citek, Dan Robbins, and Joseph A. Bedell, Orion Genomics, St. Louis, MO

1:45 p.m. Oral Presentation 6B-02. **Molecular Evolution as a Tool to Manipulate Plant Metabolism for Biomass Conversion,** *Michael Lassner,* Verdia Inc., Redwood City, CA

2:15 p.m. Oral Presentation 6B-03. **Discovery and Evolution of Enzymes for Modification of Oil Composition in Plants,** *Justin T. Stege*, Leslie Hickle, Timothy Hitchman, David P. Weiner, Jay M. Short, Diversa Corporation, San Diego, CA

2:45 p.m. Break

3:15 p.m. Oral Presentation 6B-04. **Transfer of Microbial Polyhydroxybutyrate (PHB) and Cellulase Genes to Maize for Production of Biodegradable Plastic, Fermentable Sugar, and Other Chemicals,** *Mariam Sticklen,* Heng Zhong, Shahina Maqbool, Farzaneh Teymouri, Bruce Dale, Robab Sabzikar, Hesham Oraby, Michigan State University, East Lansing, MI

3:45 p.m. Oral Presentation 6B-05. **Near-Infrared Spectroscopy as a Genetic Screening Tool for Corn Stover Cell Wall Chemistry,** *Steven R. Thomas,* Tammy K. Hayward, Amie D. Sluiter, David W. Templeton, Kent W. Evans, Bonnie R. Hames, NREL, Golden, CO

4:15 p.m. Oral Presentation 6B-06. **Expression of UDP-glucose Dehydrogenase Reduces Cell-wall Polysaccharide Concentration and Increases Xylose Content in Alfalfa Stems,** *Deborah A. Samac*, Hans-Joachim G. Jung, USDA-ARS Plant Science Research Unit, St. Paul, MN and Lynn Litterer, David A. Somers, University of Minnesota, St. Paul, MN and Glena G. Temple, Viterbo College, LaCrosse, WI

4:45 p.m. Closing Remarks – Session Chair/Co-Chair

List of Poster Presentations

Monday Evening, May 6, 2003 5:30 - 10:00 pm

Chair: Mildred Newman
National Renewable Energy Laboratory

All posters should be up by Monday afternoon, May 5, 2003.
Presenters should be near their respective posters as below:

5:30 pm - 7:30 pm	Sessions 3, 4, 6A, 6B
8:00 pm - 10:00 pm	Sessions 1A, 1B, 2, 5
5:30 pm - 10:00 pm	Social gathering with buffet

This evening's program will showcase the full range of topics for the areas of the formal sessions. Presenters are encouraged to set up their posters by 1:00 pm on Monday, May 5. The posters format is most appropriate for presentations that benefit from detail descriptions and direct interaction between author and other participants.

Feedstock Supply, Logistics, Processing, and Composition

Poster 1A-07. **Perspectives for Bioethanol Production in the Netherlands: Feedstock Selection and Pretreatment Options** *Ed de Jong*, Robert R.Bakker, Wolter Elbersen, Ruud A. Weusthuis, and Ronald H.W. Maas. Institute for Agrotechnological Research (ATO), Wageningen University and Research Centre the Netherlands; J.H. Reith, H. den Uil, and H. van Veen. Energy Research Centre of the Netherlands(ECN) Unit Biomass, Petten, the Netherlands; W.T.A.M. de Laat, J.J. Niessen. Royal Nedalco B.V., Bergen op Zoom, the Netherlands.

Poster 1A-08. **Hydrodynamic Separation of Grain and Stover Components in Corn Silage.** Philippe Savoie. Agriculture and Agri-Food Canada, Sainte-Foy, Quebec, Canada; Kevin J. Shinners. Biological Systems Engineering Department, University of Wisconsin, Madison, WI.

Poster 1A-09. **Harvest and Storage of Wet and Dry Corn Stover as a Biomass Feedstock.** Kevin J. Shinners and Ben N. Binversie. Biological Systems Engineering Department University of Wisconsin, Madison, WI; Philippe Savoie. Agriculture and Agri-Food Canada, Quebec, Canada.

Poster 1A-10. **Storage and Characterization of Cotton Gin Waste for Ethanol Production.** F.A. Agblevor, C. Mingle, W. Li and J.S. Cundiff. Biological Systems Engineering Department, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Poster 1A-14. **Biomechanics of Straw and Corn Stover Stem Separation.** Eric D. Steffler, *J. Richard Hess,* Peter Pryfogle, Thomas H. Ulrich, and Jeff Lacey. Idaho National Engineering and Environmental Laboratory, Idaho Falls, ID.

Poster 1A-15. **Image-Based Flow Characterization and Measurement for Biomass Separation Technologies.** *J. Richard Hess*, Kevin L. Kenney, and Christopher T. Wright. Idaho National Engineering and Environmental Laboratory, Idaho Falls, ID.

Poster 1A-16. **Understanding the Role of Lignin Synthesis on the Harvestibility of Higher Value Cereal Crop Residues.** Jeffrey A. Lacey, Thomas H. Ulrich, J. Richard Hess, and David N. Thompson. Idaho National Engineering and Environmental Laboratory, Idaho Fall, ID; Robert S. Zemetra, Philip H. Berger, and Marc S. Cortese. University of Idaho, Moscow, ID.

Poster 1A-17. **A New Class of Plants for a Biofuel Feedstock Energy Crop.** James Kamm. University of Toledo, Scott Park Campus, Toledo, OH.

Poster 1A-18. **Modeling Soil Carbon and Nitrogen Dynamics Under Various Cropping Systems: Can Corn Residue Removal be Sustainable?** *Bruce E. Dale* and Seungdo Kim. Dept of Chemical Engineering and Material Science, Michigan State University, East Lansing, MI.

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Poster 1A-21. **Wet Storage & Transport—The Past is Prologue** Joseph E. Atchison. Atchison Consultants, Inc., Sarasota, FL; James Hettenhaus. c.e.a. Inc., Charlotte, NC.

Poster 1A-22. **Modeling of Biomass Supply Logistics.** *Shahab Sokhansanj*. Oak Ridge National Laboratory, Environmental Sciences Division, Oak Ridge, TN.

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Poster 1A-25. **Fungal Upgrading of Wheat Straw for Straw-Thermoplastics Production.** *Tracy P. Houghton*, David N. Thompson, J. Richard Hess, and Jeffrey A. Lacey. Idaho National Engineering and Environmental Laboratory, Idaho Falls, ID; Michael P. Wolcot, Anke Schirp, Karl Englund, David Dostal, and Frank Loge. Washington State University, Pullman, WA.

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Poster 1B-09.**Cellulase Production in *Trichoderma reesei* using Sophorose.** *George England* and Aaron Kelley. Genencor International, Inc., Palo Alto, CA.

Poster 1B-10. **Cellulase Production by Glucose Grown Cultures of *Trichoderma reesei* RUT C-30 as a Response to Cellulose Feed.** *Nóra Szijártó*, Zsolt Szengyel, and Kati Réczey. Department of Agricultural Chemical Technology, Budapest University of Technology and Economics, Budapest, Hungary; Gunnar Lidén. Department of Chemical Engineering II, Lund University, Sweden.

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Poster 1B-13. **Properties of a Recombinant -Glucosidase from the Polycentric Anaerobic Fungus *Orpinomyces* PC-2 and Its Application for Cellulose Hydrolysis**. *Xin-Liang Li.* Fermentation Biotechnology Research, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL; Lars G. Ljungdahl. Department of Biochemistry & Molecular Biology and Center for Biological Resource Recovery, The University of Georgia, Athens, GA.

Poster 1B-14. **Development and Application of an Integrated System for Ethanol Monitoring.** E.M. Alhadeff, A.M. Salgado, N. Pereira Jr., B. Valdman. Dep. Engenharia Química, Escola de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Poster 1B-15. **Model Based Soft-Sensor for On-Line Determination of Substrate.** Salgado, A.M.; Valdman, B. Dep. Engenharia Química, and Escola de Química. Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil; Valero, F. Dep. D´Enginyeria Química ETSE, Universitat Autonoma de Barcelona, Bellaterra, Spain.

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Poster 1B-17. **4-Chlorophenol Degradation by Chloroperoxidase: Isolation, Purification and Identification of Oxidized Products** Adriana S. De Oliveira, Camilo E. La Rotta H., and Elba P.S. Bon. Laboratório de Tecnologia Enzimática, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.

Poster 1B-18. **Characterization and Performance of Immobilized Amylase and Cellulase.** B.A. Saville, M. Khavkine, G. Seetharam, B. Marandi, and Y-L. Zuo. Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada.

Poster 1B-19. **Oligosacharides Production by Free and Immobilized Enzyme in Organic Media.** Fernanda V. Alves, Maria I. Rodrigues, and *Francisco Maugeri*. Food Engineering Department, UNICAMP, SP, Brazil.

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Poster 1B-21. **Effect of *Trichoderma* Endoglucanases on Secondary Fiber Properties**. *Dóra Dienes*, Anita Egyházi, and Kati Réczey. Department of Agricultural Chemical Technology, Budapest University of Technology and Economics, Budapest, Hungary.

Poster 1B-22. **Overexpression of Xylanase B of A. Niger in *Pichia pastoris*.** Ling-xiang Zhu, Ai-jun Mao, Wei-feng Liu, and Zhi-yang Dong. Institute of Microbiology, Chinese Academy of Sciencand es, Beijing. PR China.

Poster 1B-23. **Evaluation of Lipase Production by Solid State Fermentation by *P. simplicissimum* using Soy Cake** Fernando Capra, Najara P. Ribeiro, Débora de Oliveira, and Marco Di Luccio. Food Engineering Department, RS, Brazil; *Denise M. G. Freire*. Biochemistry Department – IQ/UFRJ, Centro de Tecnologia, Rio de Janeiro, Brazil.

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Poster 1B-28. **Asparaginase II Fermentation Kinetics of *Saccharomyces cerevisiae ure2dal80* Mutant: Effect of Nitrogen Source and pH.** Maria Antonieta Ferrara and Josiane M. V. Mattoso. Far-Manguinhos – Fiocruz, Av. Sizenando Nabuco; *Elba P. S. Bon.* Instituto de Química – Universidade Federal do Rio de Janeiro; Nei Pereira, Jr. Escola de Química – Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brzsil.

Poster 1B-29. **The Effect of Temperature, Pressure and Depressurization Rates on Lipase Activity in $SCCO_2$.** *Débora de Oliveira*, Marcelo Lanza, Wagner Luiz Príamo, Cláudio Dariva, and José Vladimir de Oliveira. Department of Food Engineering, URI-Campus de Erechim, RS, Brazil.

Poster 1B-30. **Immobilized Enzyme Studies in a Micro-scale Bioreactor.** Zonghuan Lu and *Bill B. Elmore*. Department of Chemical Engineering, Louisiana Tech University, Ruston, LA; Francis Jones. Chemical and Environmental Engineering, The University of Tennessee at Chattanooga, Chattanooga, TN.

Poster 1B-31. **Studies on Lipase Immobilization in Hydrophobic Sol-Gel Matrix**. Cleide Mara Faria Soares, Flávio Faria de Moraes, and *Gisella Maria Zanin.* Chemical Engineering Department, State University of Maringá,Maringa (PR), Brazil; Heizir Ferreira de Castro Faculdade de Engenharia Química de Lorena, Lorena-SP, Brazil.

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Poster 1B-33. **CMCase and Xylanase Production by *Thermoascus aurantiacus* in Solid State Fermentation in Different Residues.** *Roberto da Silva*; Ellen S. Lago, Carolina W. Merheb, Mariana M. Macchione, Eleni Gomes, and Yong K Park. Laboratório de Bioquímica e Microbiologia Aplicada – IBILCE – UNESP, Sao Jose do Rio Preto-SP, Brazil.

Poster 1B-34. **The Effect of pH on the Cellulase Production of *Trichoderma reesei* RUT C30**. Tamás Juhász, *Zsolt Szengyel*, and Kati Réczey. Department of Agricultural Chemical Technology, Budapest University of Technology and Economics, Budapest, Hungary.

Poster 1B-35. **Production of Galactooligossaccharides by β-galactosidase from *Scopulariopsis sp.*** *Gláucia M. Pastore*. Food Biochemistry Laboratory, Faculty of Food Engineering. Mareci Mendes de Almeida. Zootechny and Food Technology Department, Ponta Grossa State University, Brazil, Laboratório de Bioquímica de Alimentos – DCA/FEA/UNICAMP, São Paulo, Brazil.

Poster 1B-36. **Characterization and Purification of Cyclodextrin Glycosyltransferase (CGTase) Produced by Thermophilic Strain H69-3.** Heloiza F. Alves-Prado and Roberto Da Silva. State University of São Paulo - UNESP, Chemistry and Geociencies Department, São José do Rio Preto, SP; Eleni Gomes. State University of São Paulo - UNESP, Biology Department, São José do Rio Preto, SP, Brazil.

Poster 1B-37. **Enhanced Production of CGTase by Optimization of Culture Medium Using Response Surface Methodology.** *Heloiza F. Alves-Prado*, Daniela A. Bocchini, and Roberto Da Silva. State University of São Paulo - UNESP, Chemistry and Geociencies Department, São José do Rio Preto, SP; Eleni Gomes. State University of São Paulo - UNESP, Biology Department, São José do Rio Preto, SP; Luis C. Baida. State University of São Paulo - UNESP, Computation Sciences and Statistic Department, São José do Rio Preto, SP.

Poster 1B-38. **Heterologous Expression, Purification, and Characterization of a Cellobiohydrolase from *Penicillium funiculosum***.*Yat-Chen Chou*, William S. Adney, Stephen R. Decker, John O. Baker, and Michael E. Himmel. Biotechnology for Fuels and Chemicals Division, National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO.

Poster 1B-39. **Towards the Discovery of New Enzymes Involved in Biomass Degradation: Combination of SSH and Microarray Technologies to Identify *Trichoderma reesei* Biomass-induced Genes.** *Elena V. Bashkirova* and Randy M. Berka. Novozymes Biotech, Inc., Davis, CA.

Poster 1B-40. **The Effect of Lignin Modifying Enzymes on the Molecular Weight Distribution of Kraft Lignin.** Stephen R. Decker, Michael E. Himmel, and Todd B. Vinzant. National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO. *Aarti Gidh.* Department of Chemical Engineering, University of Mississippi, University, MS.

Poster 1B-41. **Secretion Signal Swapping for Improved β-glucosidase Expression in *Trichoderma reesei* and *Saccharomyces cerevisiae*.** *Suchindra Maiyuran*, Ana Fidantsef, Elena Vlasenko, Feng Xu, and Howard Brody. Novozymes Biotech Inc., Davis, CA.

Poster 1B-42. **Insights from Quantitative Modeling into the Mode of Action of Fungal Cellulases.** Yiheng Zhang and Lee Lynd. Thayer School of Engineering, Dartmouth College, Hanover, NH; Biological Sciences, Dartmouth College, Hanover, NH.

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Poster 1B-45. **Ester Synthesis Catalyzed by *Mucor miehei* Lipase Immobilized onto Polysiloxane-Polyvinyl Alcohol Magnetic Particles**. Laura M. Bruno. Embrapa Agroindústria Tropical, Rua Dra. Sara Mesquita, Brazil; José L. de Lima Filho and Eduardo H. de M. Melo. Biochemistry Department, LIKA, Federal University of Pernambuco, RECIFE-PE, Brazil; Heizir F. de Castro. Department of Chemical Engineering, Faculty of Chemical Engineering of Lorena, Lorena-SP, Brazil.

Poster 1B-47. **Chloroperoxidase Stabilization by Covalent Binding in Mesoporous Sol-gel Glass.** *Brian H. Davison*, Sheng Dai, and Abhijeet Borole. Oak Ridge National Laboratory, Oak Ridge, TN; Ping Wang. Department of Chemical Engineering, The University of Akron, Akron, OH; Catherine L. Cheng. Eli Lilly and Company, Indianapolis, IN.

Poster 1B-49. **Discovery of Novel Alkaline and Thermophilic Cellulases and Their Industrial Applications**. Janne Kerovuo, Flash Bartnek, Jack Clark, Katie Kline, Rod Fielding, Debra Mertes, Kevin Gray, Dan Robertson, Mark Burk, Geoff Hazlewood, Eric Mathur, Martin Keller, Karsten Zengler, Steven Wells, and Jay Short. Diversa Corporation, San Diego, CA.

Poster 1B-50. **Coriolopsis SP Ligninases Production and Characterization in Different Substrates.** C.C. Carvalho, S. Xavier-Santos, R.Da Silva, E. Gomes. Biochemistry and Applied Microbiology Lab., IBILCE-UNESP – S. J. Rio Preto, SP.

Poster 1B-51. **Cloning, Expression and Characterization of a Novel Family 74 Xyloglucanase from *Trichoderma reesei.*** *Elizabeth J. Golightly*, Jeffery A. Haas; Randy M. Berka, Elena Bashkirova, and Michael W. Rey. Novozymes Biotech, Inc., Davis, CA.

Poster 1B-52. **Enzymatic Activities of *Ceriporiopsis subvermispora* Acting on Sugarcane Bagasse.** Adilson R. Gonçalves and Sirlene M. Costa. Depto. Biotecnologia-FAENQUIL, SP-Brazil; Elisa Esposito. Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes, Brazil.

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Poster 1B-54. **Increased Thermal Tolerance of *T. fusca* β-Glucosidase *via* Directed Evolution.** *Eric E. Jarvis*, William S. Adney, Stephen R. Decker, John O. Baker, and Michael E. Himmel. National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO.

Poster 1B-55. **Using HPLC/ELSD Methods to Quantify Oligosaccharides and Determined Cellulose Molecular Weight Distribution During Enzymatic Hydrolysis of Cellulose.** *Xiao Zhang*, Dan Xie and John Saddler. Forest Products Biotechnology, University of British Columbia, Vancouver BC, Canada.

Poster 1B-56. **Immobilization of β-glucosidase on Eupergit C for Cellulose Hydrolysis.** Maobing Tu, *Xiao Zhang* and John N. Saddler. Forest Products Biotechnology, University of British Columbia, Vancouver BC, Canada.

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Poster 1B-58. **Review of Enhanced Production of *R*-Phenylacetylcarbinol (*R*-PAC) Through Enzymatic Biotransformation.** Peter Rogers and Bettina Rosche. University of New South Wales, School of Biotechnology and Biomolecular Sciences, Sydney, Australia.

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Poster 2-08. **Directed Evolution of a Thermostable *A. oryzae* β-Glucosidase Utilizing a *Saccharomyces cerevisiae* Expression System and Robotic Screening.** *A.L. Fidantsef*, B. Gorre-Clancy, J.H. Yi, S.A. Teter, A.M. Lamsa, M.H. Lamsa, J. R. Cherry. Novozymes Biotech, Inc., Davis CA.

Poster 2-09. **Isolation and Performance Optimization of Cultures Capable of Converting Syngas to Ethanol.** *Mark E. Zappi*, W. Todd French, Christine E. Morrison, Katherine Taconi, and Emily R. Easterling. Dave C. Swalm School of Chemical Engineering, Mississippi State University, MS; Lewis R. Brown, Magan E. Green. Department of Biological Sciences, Mississippi State University, MS.

Poster 2-10. **Microarray-Analysis of Xylose-Growing Recombinant *Saccharomyces cerevisiae* Strains.** *Marie Jeppsson,* Christer Larsson, Bärbel Hahn-Hägerdal, and Marie F. Gorwa-Grauslund. Applied Microbiology, Lund University, Lund, Sweden; Fredrik Wahlbom. Novozymes A/S, Bagsværd, Denmark.

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Poster 2-13. **Effect of Organic Acids and Aldehydes on the Growth and Fermentation of *K. marxianus***. J.M. Oliva, I. Ballesteros, M.J. Negro, P. Manzanares, A.Cabañas, and *M. Ballesteros*. CIEMAT, Madrid, Spain.

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Poster 2-18. **Effect of Carbon: Nitrogen Ratio and Substrate Source on Glucose-6-Phosphate Dehydrogenase Production by *Saccharomyces cerevisiae* W 303 181**. *Eliane Dalva Godoy Danesi*, Doriela Herek Ferreira, João Carlos Monteiro de Carvalho, and Adalberto Pessoa, Jr. Biochemical and Pharmaceutical Technology Department, FCF/USP, São Paulo-SP, Brazil.

Poster 2-19. **The Effect of Viscosity Change on the Rate and Extent of *Zymomonas mobilis* Cellulose Fermentation.** *Natalia V. Pimenova*, Byung-Hwan Um, and Thomas R. Hanley. Department of Chemical Engineering, University of Louisville, Louisville, KY.

Poster 2-20. **Construction of Recombinant *Escherichia coli* Strains for Poly-(3-hydroxybutyrate-co-3-Hydroxyvalerate) Production.** Kin-Ho Law, Pui-Ling Chan, Yun-Chung Leunh, Hong Chua, Thomas Wai-Hung Lo, and *Peter Hoifu Yu*. Open Laboratory of Chirotechnology of the Institute of Molecular Technology for Drug Discovery and Synthesis and Applied Biology and Chemical Technology, Hong Kong Polytechnic University, Hong Kong, China.

Poster 2-21. **Conversion of Corn Fibrous Material into Ethanol** *Bruce S. Dien*, Nancy N. Nichols, Xin-Liang Li, and Michael A. Cotta. National Center for Agricultural Utilization Research, Peoria, IL; Rodney J. Bothast. National Corn to Ethanol Research Pilot Plant, Edwardsville, IL.

Poster 2-23. **Xylitol Production by Flocculating Yeast, *Candida* sp. HY200.** *Heui-Yun Kang* and Yeon-Woo Ryu. Department of Molecular Science and Technology, Ajou University, Suwon, Korea; Jin-Ho Seo. Department of Food Science and Technology, Seoul National Univerity, Suwon, Korea.

Poster 2-24. **Biosynthesis of (*R*)-(-)-3-Hydroxybutyric Acid by Metabolically Engineered *Escherichia coli***. *Sang Yup Lee.* Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Republic of Korea; Young Lee. ChioBio Inc., Rublic of Korea.

Poster 2-25. **Synthesis of Polyhydroxyalkanoate (PHA) by Microorganisms from Activated Sludge.** W.S. Tong, T.C. Ma, Hong Chua, Thomas Wai-Hung Lo, Ken Man Cheung, and *Peter Hoifu Yu.* Open Laboratory of Chirotechnology of the Institute of Molecular Technology for Drug Discovery and Synthesis, Applied Biology and Chemical Technology, Hong Kong Polytechnic University, Hong Kong, China.

Poster 2-26. **Molecular Weight Distribution Study of Microbial-produced Polyhydroxyalkanoates by Improved Viscosity Method.** Phoeby Ai-Ling Wong, Hong Chua, Thomas Wai-Hung Lo, Ken Man Cheung, and *Peter Hoifu Yu*. Open Laboratory of Chirotechnology of the Institute of Molecular Technology for Drug Discovery and Synthesis Applied Biology and Chemical Technology, Hong Kong Polytechnic University, Hong Kong, China.

Poster 2-27 Student. **Real Time Estimation and Neuro-Fuzzy Control of Fed-Batch Baker's Yeast Cultivation.** *Thiago de Castilho M. Campos*, Roberto de Campos Giordano, and Antonio J. G. Cruz. Chemical Engineering Department, São Carlos Federal University, São Carlos-SP, Brazil.

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Poster 2-30. **Gata Factors Regulation of *Saccharomyces cerevisiae* Invertase: Role of Ure2P and the Gln3P and Nil1P Transcriptional Factors.** Edna M.M. Oliveira, José João Mansure, and *Elba PS Bon*. Chemistry Institute - FedPPeral University of Rio de Janeiro, Rio de Janeiro, Brazil.

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Poster 2-32. **Proteomic Analysis of Physiological Properties Between *Candida magnoliae* Wild and its Mutant Strain by two Dimensional Electrophoresis and Microsequencing**. Lee, Do-Yup, *Hyo-Jin Kim*, Won-Gi Min, and Jin-Ho Seot. Department of Food Science and Technology, Research Center for New BioMaterials in Agriculture, Seoul National University, Suwon, Korea; Yeon-Woo Ryut. Department of Molecular Science and Technology, Ajou University, Suwon, South Korea.

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Poster 2-35. **Production of Biosurfactant by *Bacillus subtilis* Using a Cassava-Processing Effluent.** *Glaucia M. Pastore*, and Marcia Nitschke. Food Biochemistry Laboratory, Faculty of Food Engineering, UNICAMP, Laboratório de Bioquímica de Alimentos – DCA/FEA/UNICAMP, Campinas-SP, Brasil.

Poster 2-36. **Comparative Statistical Metabolic Control Analysis of Ethanol Production in *E. coli* and *S. cerevisiae*.** Inanc Birol, Liqing Wang, and *Vassily Hatzimanikatis.* Department of Chemical Engineering, Northwestern University, Evanston, IL.

Poster 2-37. **Development and Application of Genetic Systems for Anaerobic, Thermophilic, Ethanol-Producing Bacteria.** Michael V. Tyurin, Sunil G. Desai, and Lee R. Lynd. Thayer School of Engineering, Dartmouth College, Hanover, NH.

Poster 2-38. **Cell immobilization on the Production and Utilization of Lactic Acid Probiotic Bacteria in the Dairy Industry.** *Enrique Durán-Páramo*, Marcos Morales-Contreras, Marco A. Brito Arias, and Fabián Robles-Martínez. Departamento de Bioprocesos, UPIBI, Instituto Politécnico Nacional, México.

Poster 2-39. **Cell Immobilization Application on the Maintenance of Viability of Lactic Bacteria.** *Enrique Durán-Páramo*, Marcos Morales-Contreras, Marco A. Brito-Arias, and Fabián Robles-Martínez. Departamento de Bioprocesos, UPIBI, Instituto Politécnico Nacional, Ticomán, México.

Poster 2-40. **Cell Immobilization on the Gibberellic Acid Production.** *Enrique Durán-Páramo*, Fabián Robles-Martínez, Marcos Morales-Contreras, and Marco A. Brito-Arias. Departamento de Bioprocesos, UPIBI, Ticomán, México.

Poster 2-41 Student. **Cellulase and Hemicellulase Production in *Penicillium brasilianum* When Changing the Substrate Composition.** *Kristian Krogh* and Lisbeth Olsson. Center for Process Biotechnology, BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark.

Poster 2-42. **A Miracle "Chimeric" Gene Enabling the *Saccharomyces* Yeast to Convert Cellulosic Biomass to Ethanol, Lactic Acid, and ….** Nancy W. Y. Ho. Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, IN.

Poster 2-43. **Kinetic Modeling to Investigate the Interactions of the Engineered Pentose Pathway with the Glycolytic (ED) Pathway in *Zymomonas mobilis*.** *Mete Altintas* and Dhinakar S. Kompala. Department of Chemical Engineering, University of Colorado, Boulder CO; Chris Eddy, Min Zhang, and Jim McMillan.National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO.

Poster 2-44. **The Production of Ethanol from Cellulosic Biomass Hydrolysates Using Genetically Engineered Saccharomyces Yeast Capable of Co-Fermenting Glucose and Xylose.** *Miroslav Sedlak* and Nancy W. Y. Ho. Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, IN.

Poster 2-45. **^{31}P Nuclear Magnetic Resonance Studies of Sugar Metabolismin *Zymomonas mobilis*.** *M. Mete Altintas* and Dhinakar S. Kompala. Department of Chemical Engineering, University of Colorado, Boulder, CO; Mark Davis, Christina Eddy, Min Zhang, and James D. McMillan. National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO.

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Poster 2-47. **Measurement of Xylose Transport in Xylose-fermenting *Zymomonas mobilis*.** *Christina Eddy,* James D. McMillan, and Min Zhang. Biotechnology Division for Fuels and Chemicals, National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO; Mete Altintas and Dhinakar S. Kompala. Department of Chemical Engineering, University of Colorado, Boulder, CO.

Poster 2-48. **Bridging Between Global Gene Expression and Metabolic Phenotype of Recombinant *Saccharomyces cerevisiae* During Xylose Fermentation.** *Yong-Su Jin* and Yi Mutt. Department of Food Science. University of Wisconsin, Madison, WI; Jose M. Laplazat and Thomas W. Jeffriest. Institute for Microbial and Biochemical Technology, USDA, Forest Service, Forest Products Laboratory, Madison, WI.

Poster 2-49. **Cellulase Production on Pure Cellulose Using *Trichoderma reesei* Strains L27, RL-P37, Rut C-30, and MTC-a-13.** *Daniel J. Schell*, Jenny Hamilton, Millie Newman, Nancy Dowe, and James D. McMillan. National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO; Juan Carlos Sáez. University of Puerto Rico, Mayagüez Campus, Mayagüez, Puerto Rico; Arun Tholudur. Covance Biotechnology Services, Inc., Cary, NC.

Poster 2-50. **Utilization of Dynamic Secondary Membranes for Flux Optimization in Membrane Microfiltration.** *Parag R. Nemade* and Robert H. Davis. Department of Chemical Engineering, University of Colorado, Boulder, CO.

Poster 2-51. **Xylanase Production and Oxygen Limited Xylose Utilization of *Neurospora crassa*.** Xiao Zhang, Dongqing Zhu, Jianqiang Lin, and *Yinbo Qu*. State Key Laboratory of Microbial Technology, School of Life Science, Shandong University, Jinan, China; Dan Wang. School of Chemical Engineering, Nanjing Forestry University, Nanjing, China; College of Science, Shandong Agricultural University, Taian, China.

Poster 2-52. **Micro Pilot Anaerobic Reactor with Axial Flow** M. Morales Contreras, J. Gonzáles Martínez, E. Duran Páramo, J. Aranda Barradas, and F. Robles Martínez. Departamento de Bioprocesos, UPIBI, Instituto Politécnico Nacional, Ticomán, México.

Poster 2-53**. Bioenergetics of Microbial Cellulose Utilization of *Clostridium thermocellum*.** Yiheng Zhang and Lee Lynd. Thayer School of Engineering, Dartmouth College, Hanover, NH; Biological Sciences, Dartmouth College, Hanover, NH.

Poster 2-54. **Optimization from the Inside Out: Multivariant Metabolic Engineering for Biocatalyst Development.** *Thomas W. Jeffries* and Jose Laplaza. Institute for Microbial and Biochemical Technology, USDA, Forest Service, Forest Products Laboratory, Madison, WI; Yong-Su Jin. Department of Food Science; Haiying Ni. Department of Bacteriology, University of Wisconsin, Madison, WI.

Poster 2-55. **Enzymatic Synthesis of Monolaurin.** C.C.B. Pereira, M.A.P. Silva. Escola de Química, Universidade Federal do Rio de Janeiro, Centro de Tecnologia, Rio de Janeiro, Brazil; Langone, M.A.P., Lisboa, J.S. Instituto de Química, Universidade do Estado do Rio de Janeiro, Brazil.

Poster 2-56. **Strain Development for the Complete Utilization of Mixed Carbohydrates in Lignocellulosic Biomass.** *Jae-Han Kim*. Department of Food Science and Technology; David A. Mills, David E. Block. Department of Viticulture and Enology; Sharon P. Shoemaker. California Institute of Food and Agricultural Research, University of California, Davis, CA.

Poster 2-57. **Evaluation of Newly Developed Integrants of *rZymomonas* mobilis for Ethanol Production Process with Corn Stover Hydrolysate.** *Ali Mohagheghi,* Nancy Dowe, Dan Schell, Yat-Chen Chou, Christina Eddy, and Min Zhang. Biotechnology Division for Fuels and Chemicals, National BioEnergy Center, National Renewable Energy Laboratory. Golden, CO.

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Poster 2-58 Student. **Kinetic Studies of a Metabolically Engineered *Zymomonas mobilis* Fermenting Glucose and Xylose Mixtures.** *Juan Carlos Sáez* and Lorenzo Saliceti Piazza. Department of Chemical Engineering, University of Puerto Rico, Mayagüez Campus, Mayagüez, Puerto Rico; James D. McMillan. Biotechnology for Fuels and Chemicals Division, National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO.

Poster 2-59. **A Novel Ethanologenic Yeast for the Fermentation of Lignocellulosic Hexose Sugars.** *J.D. Keating*, J. Robinson, J.N. Saddler, and S.D. Mansfield. Forest Products Biotechnology, Department of Wood Science, University of British Columbia, Vancouver, BC, Canada; R.J. Bothast. National Corn-to-Ethanol Research Pilot Plant, Southern Illinois University Edwardsville, Edwardsville, IL.

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Poster 3-08. **The Effect of Process Parameters in the Production of a Biopolymer by R*hizobium* sp.** Flávia Pereira Duta, Francisca Pessôa de França, and Eliana Flávia Camporese Servulo. Universidade Federal do Rio de Janeiro, Centro de Tecnologia, Departamento de Engenharia Bioquímica, Rio de Janeiro, Brazil; Léa Maria de Almeida Lopes. Universidade Federal do Rio de Janeiro, Instituto de Macromoléculas. Prof. Eloisa Mano; Antonio Carlos Augusto da Costa. Universidade Estadual do Rio de Janeiro, Brazil.

Poster 3-09. **Design of Nanostructured Catalysts for the Conversion of Biorenewable Feedstocks.** *Brent H. Shanks* and Isa Mbaraka. Chemical Engineering Department, Iowa State University, Ames, IA.

Poster 3-10. **Production of Butanol from Concentrated Lactose/ Whey Permeate Using *Clostridium acetobutylicum* and Removal by Perstraction.** N. Qureshi. University of Illinois, Biotechnology & Bioengineering Group, Department of Food Science, Urbana, IL; I.S. Maddox. Institute of Technology and Engineering, Massey University, Palmerston, New Zealand.

Poster 3-11. **Effect of Pretreatment on the Extraction of Silymarins from Milk Thistle.** Senthil Subramaniam, Danielle Julie Carrier, and *Edgar C. Clausen*. University of Arkansas, Department of Chemical Engineering, Fayetteville, AR.

Poster 3-12. **Extraction of High Value Co-products from Energy Crops.** *Ching-Shaun Lau*, Danielle Julie Carrier, Luke R. Howard, and Edgar C. Clausen. University of Arkansas, Department of Chemical Engineering, Fayetteville, AR.

Poster 3-13. **Silymarin Extraction from Milk Thistle Using Hot/Liquid Water.** Lijun Duan, Danielle Julie Carrier, and *Edgar C. Clausen.* University of Arkansas, Department of Chemical Engineering, Fayetteville, AR.

Poster 3-14. **High Productivity Continuous Biofilm Reactor for Butanol Production: Effect of Acetic and Butyric Acids and CSL on Bioreactor Performance.** *P. Karcher*, N. Qureshi, and H.P. Blaschek. University of Illinois, Biotechnology & Bioenginering Group, Department Food Science & Human Nutrition, Urbana, IL.

Poster 3-15. **Optimization of an Integrated Process for the Production of Inulinase by *Kluyveromyces marxianus.*** Helen Treichel, Yemiko Makino, Juliana Macedo, Maria I. Rodrigues, and*Francisco Maugeri*. Food Engineering Department, UNICAMP, Campinas-SP, Brazil.

Poster 3-16**. Influence of Culture Conditions on the Expression of Green Fluorescent Protein (*GFPuv*) by *Escherichia coli.*** Thereza Christina Vessoni Penna, *Marina Ishii*, Luciana Cambricoli de Souza, and Adalberto Pessoa, Jr. Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Science, University of São Paulo, Brazil; Olivia Cholewa. Molecular Probes, Inc., Eugene, OR**.**

Poster 3-17. **Thermal Stability of Recombinant Green Fluorescent Protein *(GFPuv)* at Various pH Conditions.** *Thereza Christina Vessoni Penna*, Marina Ishii, Luciana Cambricoli de Souza, and Adalberto Pessoa, Jr. Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Science, University of São Paulo, Brazil; Olivia Cholewa. Molecular Probes, Inc., Eugene, OR.

Poster 3-18. **Recovery of Acetic Acid from Byproduct Streams** Maohong Fan, Yonghui Shi and Robert C Brown. Center for Sustainable Environmental Technologies, Iowa State University, Ames, IA.

Poster 3-19. **Two-stage Membrane Bioreactor System to Ethanol Production from Lignocellulosic Biomass.** *Jun Seok Kim*. Department of Chemical Engineering, Kyonggi University, Suwon, Korea; Hyunjoon Kim, and Suk-In Hong. Department of Chemical and Biological Engineering, Korea University, Seoul, Korea.

Poster 3-20. **Characterization of Xylose Reductase Extracted by CTAB-reversed Micelles from *Candida guilliermondii* Homogenate.** *Michele Vitolo*, Adalberto Pessoa-Jr., and Ely Vieira Cortez. University of São Paulo, School of Pharmaceutical Sciences, Department of Biochemical and Pharmaceutical Technology, São Paulo, Brazil; Maria das Graças de Almeida Felipe, Inês Conceição Roberto. Department of Biotechnology, Lorena, Brazil.

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Poster 3-21 Student. **Determination of the Rheological Properties of Distillers' Grains Slurries Using a Helical Impeller Viscometer** Tiffany L. Houchin and Thomas R. Hanley. Department of Chemical Engineering, Speed Scientific School, University of Louisville, Louisville, KY.

Poster 3-22 Student. **The Effect of Toxic Products on Ethanol Production from Concentrated Corn Stover Hydrolysates in a Three-Liter Bench Scale Bioreactor.** *Byung-Hwan Um* and Thomas R. Hanley. Department of Chemical Engineering, Speed Scientific School, University of Louisville, Louisville, KY.

Poster 3-23. **Separation of Xylose Reductase and Xylitol Dehydrogenase from *Candida guilliermondii* Homogenate by BDBAC Reversed Micelles.** Michele Vitolo, Adalberto Pessoa-Jr., and Ely Vieira Cortez. University of São Paulo, School of Pharmaceutical Sciences, Department of Biochemical and Pharmaceutical Technology, São Paulo, SP, Brazil; Maria das Graças de Almeida Felipe and Inês Conceição Roberto. Department of Biotechnology, Lorena, Brazil.

Poster 3-25 Student. **Techno-Economic Evaluation of Ethanol from Softwood Potential of Energy Savings in an SSF Process.** *Anders Wingren*, Mats Galbe, and Guido Zacchi. Department of Chemical Engineering 1, Lund University, Lund, Sweden.

Poster 3-26. **CFD Simulation and Redesign of a Vertical Screw Conveyor Reactor.** *Yinkun Wan* and Thomas R. Hanley. Department of Chemical Engineering, Speed Scientific School, University of Louisville, Louisville, KY.

Poster 3-27. **Production of Biodiesel Fuel by Transesterification of Rapeseed Oil**. Gwi-Taek Jeong, Ki-Young Byun, Choon-Hyoung Kang, and Woo-Tai Lee. Faculty of Chemical Engineering; *Don-Hee Park*. Institute of Bioindustrial Technology; Chung-han Yoon. Department of Mineral and Energy Resources Engineering; Byung-Chul Choi. School of Automotive Engineering, Chonnam National University, Kwangju, Korea; Hae-Sung Kim. Department of Chemical Engineering, Myongji University, Korea; Un-Taek Lee. Onbio Corporation, Pucheon-Si, Kyunggi-Do, Korea.

Poster 3-28. **Recovery of Tocopherols Through Molecular Distillation Process.** *Moraes, E. B.*, Batistella, C.B. and Wolf Maciel, M. R. Separation Process Development Laboratory, Faculty of Chemical Engineering. State University of Campinas, UNICAMP, Campinas-SP, Brazil.

Poster 3-29**. Optimization of an Extractive Alcoholic Fermentation Process.** *Aline C. da Costa* and Rubens Maciel Filho. DPQ/FEQ/UNICAMP, Campinas-SP, Brasil.

Poster 3-30. **Controlled Fed-batch Fermentations on Dilute Acid Hydrolysate in PDU-scale.** *Andreas Rudolf*, Mats Galbe, and Gunnar Lidén. Chemical Engineering, Lund University, Lund, Sweden.

Poster 3-31. **The Modeling of Alcoholic Fermentation at High Glucose Concentrations.** Amauri A. Ferreira, Valéria V. Murata, and *Eloízio J. Ribeiro.* School of Chemical Engineering, Federal University of Uberlândia, MG. Brazil.

Poster 3-32. **Production of Lactic Acid in a pH-Controlled Separative Bioreactor.** *Edward J. St.Martin,* Yupo J. Lin, Jamie A. Hestekin, Michelle B. Arora, Cynthia Y. Sanville-Millard, Seth W. Snyder, and Mark Donnelly. Argonne National Laboratory, Argonne, IL.

Poster 3-33. **Use of Deterministic Model and Artificial Neural Networks in the Fermentation Process for Ethanol Production.** Vera Lúcia Reis de Gouvêia. School of Chemical Engineering, State University of Campinas – UNICAMP, Laboratory of Optimization, Design and Advanced Process Control (LOPCA), Campinas-SP, Brazil; Rubens Maciel Filho. School of Chemical Engineering, State University of Campinas – UNICAMP, Laboratory of Optimization, Design and Advanced Process Control (LOPCA), Campinas-SP, Brazil.

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Poster 3-35. β**-Galactosidase Production Using *Kluyveromyces marxianus* and Enhanced Cheese Whey by Fermentation.** Adilson J. de Assis, Patrícia A. Santiago, Vicelma L. Cardoso and *Eloízio J. Ribeiro*. School of Chemical Engineering, Federal University of Uberlândia, Uberlandia-MG, Brazil.

Poster 3-36. **Starch Hydrolysis with Thermopressurized Aqueous Phosphoric Acid # and Free Maltosugars Bioconversion to Oxygenated Carotenoids.** *José D. Fontana* and Mauricio Passos. Biomass/Biotechnology Laboratory, Department of Pharmacy UFPR - Federal University of Paraná, Curitiba-PR, Brazil.

Poster 3-37. **Development of a Methodology for the Determination of Viable Cells in a Three Phase Fluidized Bed Reactor.** Thaís Letícia Bioni, Ígor Tadeu Lazzarotto Bresolin, Rosane Rosa de Souza, and *Marcelino Luiz Gimenes*. Maringá State University, Chemical Engineering Department (UEM-DEQ), Maringá – PR, Brazil; Benedito Prado Dias Filho. Maringá State University – Clinic Analysis Department (UEM-DAC).

Poster 3-38 Student. **Scale-up of Anaerobic Processes Involving Soluble Substrates: The Use of CFD Analysis for Experimental Design.** *Colin Hebert*, Charles E. Wyman, and Lee Lynd. Thayer School of Engineering, Dartmouth College, Hanover, NH; Andre Bakker. Fluent Corporation, Lebanon, NH.

Poster 3-39. **Biological Hydrogen Production from Synthesis Gas: Preliminary Techno-Economics & Reactor Design Issues.** *Edward J. Wolfrum*, Andrew S. Watt, and Wade Amos. National Bioenergy Center, National Renewable Energy Laboratory, Golden CO.

Poster 3-40. **A Green Process to Obtain Acethaldehyde: Oxidation of Ethyl Alcohol.** Eduardo Coselli Vasco de Toledo, and Rubens Maciel Filho. Faculty of Chemical Engineering, State University of Campinas – UNICAMP, Campinas, SP, Brazil.

Poster 3-41. **Evaluation of the Agitation Effective Power in Aerated and Non-Aerated Systems for Yeast and Filamentous Fungi Suspensions.** Luiz Claudio S. Carlos, Maria Antonieta P. Gimenes, Eliana M. Alhadeff, *Nei Pereira, Jr.* Departamento de Engenharia Bioquímica, Universidade Federal Rio de Janeiro, Centro de Tecnologia Escola de Química, Rio de Janeiro, Brazil.

Poster 3-42. **Comparison of Strain Performance in Fed-batch Fermentation of Hydrolyzates**. *Anneli Nilsson* and Gunnar Lidén. Department of Chemical Engineering II, Lund Institute of Technology, Lund, Sweden; Marie-Francoise Gorwa-Grauslund, and Bärbel Hahn-Hägerdal. Department of Applied Microbiology, Lund Institute of Technology, Lund, Sweden.

Poster 3-43. **The Effect of Temperature on the Anaerobic Methane Fermentation Process for the Digestion of Organic Wastes.** Jung Kon Kim, Joon Hwuy Kim, Geon Hyung Cho, Young Nam Chun, and *Si Wouk Kim*. Research Center for Proteineous Materials and Department of Environmental Engineering, Chosun University, Gwangju, Korea; Jung Heon Lee. Department of Chemical Engineering, Chosun University, Gwangju, Korea; Don-Hee Park. Department of Chemical Engineering, Chonnam National University, Gwangju, Korea.

Poster 3-44. **Biorefinery Optimization Tools – Development & Validation.** *John J. Marano*. University of Pittsburgh, Pittsburgh, PA; John L. Jechura. National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO.

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Poster 3-46. **Biobased Products via Syngas Fermentation** *Theodore J. Heindel,* Alan A. DiSpirito, Robert C. Brown, and Basil J. Nikolau. Department of Mechanical Engineering, Center for Sustainable Environmental Technologies, Iowa State University, Ames, IA.

Poster 3-47. **Biodegradation Studies on Chemical Laboratory Effluent.** Cláudia T. Benatti, *Célia R.G. Tavares*, and Mateus P. Gaspar. Departamento de Engenharia Química, Universidade Estadual de Maringá, PR, Brazil; Benedito P. Dias Filho. Departamento de Análises Clínicas, Universidade Estadual de Maringá, PR, Brazil.

Poster 3-48. **Use of Anion Exchange Resin to Recover Succinic Acid from a Continuous Fermentation.** *Robert Hanchar*, Elankovan Ponnampalam, and Deborah Burgdorf. MBI International, Lansing, MI.

Poster 3-49. **Ethanol Production from Hydrolyzates of Steam Exploded Biomass by Immobilized-cell Bioreactors.** I. De Bari, D. Cuna, F. Nanna, and G. Braccio. ENEA, Biomass Laboratories, Policoro (MT), Italy.

Poster 3-50 Student. **Optimisation of Steam Pre-Treatment of H_2SO_4-Impregnated Corn Stover for Enhance Enzymatic Digestibility.** *Enik Varga*, and Kati Réczey. Budapest University of Technology and Economics, Department of Agricultural Chemical Technology, Hungary; Guido Zacchi. Lunds University, Department of Chemical Engineering 1, Lund, Sweden.

Poster 3-51. **Controlled Immobilization of Biocatalytic Enzymes in Separative Bioreactors.** *Michelle B. Arora*, Jamie A. Hestekin, Yupo J. Lin, Hend Samaha, Jonathon Davila, William H. Eschenfeldt, Mark Donnelly, Edward J. St.Martin, and Seth W. Snyder. Argonne National Laboratory (ANL), Argonne, IL.

Poster 3-52. **Potential of Ethanol Production by Immobilized Thermophilic Bacteria *hermoanaerobacter* HY10 at Extreme Loading Rates.** Mads Torry-Smith, Ioannis V. Skiadas, and *Birgitte K. Ahring*. BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark.

Poster 3-53. **Modeling of Biomass Conversion to Mixed Alcohol Fuels (MixAlco Process) Using ASPEN-Plus.** Kemantha Jayawardhana and *G. Peter van Walsum*. Department of Environmental Studies, Baylor University, Waco, TX.

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Poster 3-56. **Xylitol Production by Immobilized Yeasts from Sugarcane Bagasse Using a Fluidized Bed Reactor:Influence of Aeration and Mass of Carrier.** Júlio C. Santos, Walter de Carvalho, Solange I. Mussatto, *Silvio S. da Silva*. Departamento de Biotecnologia - Faculdade de Engenharia Química de Lorena - Rodovia Itajubá-Lorena, Lorena-SP, Brazil.

Poster 3-57. **A Hollow-Fiber Membrane Extraction Process for Recovery and Purification of Lactic Acid from Fermentation Broth.** *Hanjing Huang.* Environmental Energy Inc., Blacklick, OH; and Shang-Tian Yang. Department of Chemical Engineering, The Ohio State University, Columbus, OH.

Poster 3-58. **Immobilization of Whole Yeast Cells on Alternative Matrices for the Production of Sugar Inverted Syrup.** Heizir F. de Castro and Daniele Urioste. Department of Chemical Engineering; Manuela L. Martines, *M. Bernadete de Medeiros.* Department of Biotechnology, Faculty of Chemical Engineering of Lorena, Lorena-SP, Brazil.

Poster 3-59. **Organosolv-Based Biorefining – Process Considerations and Product Profiles.** *Claudio Arato*, and Kendall Pye. Lignol Innovations Corp, Vancouver, BC, Canada.

Poster 3-60. **The Effect of β-Glucosidase on the Foamability of Cellulase.** William D. Lambert, Vorakan Burapatana, Aleš Prokop, and *Robert D. Tanner*. Chemical Engineering Department, Vanderbilt University, Nashville, TN.

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Poster 3-62. **Succinic Acid Adsorption from Fermentation Broth and Regeneration.** *Brian H. Davison*, Gerald L. Richardson. Oak Ridge National Laboratory, Oak Ridge, TN; Nhuan P. Nghiem. Michigan Biotechnology Insititute, East Lansing, MI.

Poster 3-63. **A Technique to Quantify the Population of Viable Cells in a Three Phase Fluidized Bed Reactor.** Thaís Letícia Bioni, Ígor Tadeu Lazzarotto Bresolin, Rosane Rosa de Souza, and *Marcelino Luiz Gimenes*. Maringá State University, Chemical Engineering Department (UEM-DEQ), Maringá – PR, Brazil; Benedito Prado Dias Filho. Maringá State University, Clinical Anayalsis Department, Maringá – PR, Brazil.

Poster 3-64. **Ethanol/Water Pulping of Sugarcane Bagasse: Kinetic Studies and Enzymatic Bleachability of the Pulps Obtained.** Regina Y. Moriya, Lísia A. Cintra, Adilson R. Gonçalves. Departamento de Biotecnologia - FAENQUIL, Lorena-SP, Brazil.

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Poster 3-67. **Comparison of Different Methods Used for Detoxification of Rice Straw Hydrolysate and Their Influence on Xylitol Production.** *Solange I. Mussatto*, Júlio C. Santos, and Inês C. Roberto. Department of Biotechnology, Faculty of Chemical Engineering of Lorena, Lorena–SP, Brazil.

Poster 3-68. **Increase of Tannase Production in Solid State Fermentation by Aspergillus Niger 3T5B8.** *Gustavo A.S. Pinto*, and Laura M. Bruno. Embrapa Tropical Agroindustry, Fortaleza/CE, Brazil; Mariana S. Hamacher, Selma C. Terzi, and Sonia Couri. Embrapa Food Agroindustry, Rio de Janeiro, Brazil; Selma G.F. Leite. Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Poster 3-69. **Cellulase Retention and Sugar Removal by Membrane Ultrafiltration During Lignocellulosic Biomass Hydrolysis**. *Jeffrey S. Knutsen*, Robert H. Davis. University of Colorado, Department of Chemical Engineering, Boulder, CO.

Poster 3-70. **Selection of Anion Exchangers for Detoxification of Dilute Acid Hydrolysates.** *Nils-Olof Nilvebrant* and Anders Sjöde. STFI AB, Swedish Pulp and Paper Research Institute, Stockholm, Sweden; Ilona Sárvári Horváth. Department of Chemical Reaction Engineering, Chalmers University of Technology, Gothenburg, Sweden; Andrei Zagorodny. Depart-ment of Material Science and Engineering, Royal Institute of Technology, Stockholm, Sweden; Leif J. Jönsson. Biochemistry, Division for Chemistry, Karlstad University, Karlstad, Sweden.

Poster 3-71. **Engineered Fluid Transporting Fractals for Improving Bioprocessing Efficiencies.** *Mike Kearney*. Amalgamated Research Inc., 2531 Orchard Drive East, Twin Falls, ID.

Poster 3-72. **Removal of VOCs Using Biotricking Filters.** Jin-Myeong Cha and Young-Seon Jang. B&E TECH Co. Ltd, Kwangju Universty, Kwangju, Korea; In-Wha Lee. Department of Environmental Engineering, Chosun University, Kwangju, Korea; Gwang-Weon Lee. Dong-A College, Chonnam, Korea; Min-Ha Oh and Don-Hee Park. Faculty of Applied Chemical Engineering, Chonnam National University, Kwangju, Korea.

Poster 4-15. **Bioethanol Co-location with a Coal-Fired Power Plant.** *Bob Wallace*. National Renewable Energy Laboratory, National BioEnergy Center, Golden, CO; Mark Yancey. BBI International, Evergreen, CO; James Easterly. Easterly Consulting, Fairfax, VA.

Poster 4-16. **Two-Step Preparation for A Catalyst-Free Biodiesel Production; Hydrolysis and Methyl Esterification**. *Dadan Kusdiana* and Shiro Saka. Graduate School of Energy Science, Yoshida Honmachi Sakyo-ku, Kyoto University, Japan.

Poster 4-17. **Biodiesel Fuel from Vegetable Oil by Various Supercritical Alcohols**. Youichiro Warabi, Dadan Kusdiana and *Shiro Saka*. Graduate School of Energy Science, Yoshida Honmachi Sakyo-ku, Kyoto University, Kyoto, Japan.

Poster 4-18. **Stabilization of Photochemical Energy Conversion Potentiality of Cyanobacterial Thylakoids by Osmolytes and Immobilization. S**. *N. Tripathi* and Kusum Lata Dwivedi. Department of Botany, Banaras Hindu University, Varanasi 221 005, India.

Poster 4-19. **Conversion of Municipal Solid Waste to Carboxylic Acids Using A Mixed Culture of Mesophilic Microorganisms.** *Cateryna Aiello-Mazzarri*. Universidad del Zulia. Facultad de Ingeniería. Departamento de Química, Maracaibo, Venezuela; Mark T. Holtzapple. Texas A&M University, Department of Chemical Engineering, College Station, TX.

Poster 4-20. **Complete Recycling of Effluent from the Bottom of Ethanol Distillation Column in Ethanol Continuous Fermentation Using Self-Flocculating Yeast**. *Chuanbin Liu*. Department of Biological Systems Engineering, Washington State University, Pullman, WA; Fengwu Bai, Dongxia Li, and Jian Xie. Institute of Biochemical Engineering, Dalian University, Dalian, China.

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Poster 4-10. **Optimization of Biodiesel Enzymatic Production from Castor Oil in Organic Solvent Medium.** *Débora de Oliveira*, Marco Di Luccio, Carina Faccio, Clarissa Dalla Rosa, João Paulo Bender, Nádia Lipke, Silvana Menoncin, Cristiana Amroginski, and José Vladimir de Oliveira. Department of Food Engineering, URI-Campus de Erechim, Erechim, RS, Brazil.

Poster 4-11. **Exhaust Emissions and Performance of Diesel Engine Operating on Biomass-based Oil Blends and their Water-fuel Emulsions**. Takaaki Morimune, Takayuki Nakata, and *Takayuki Morino*. Department of Mechanical Engineering, Shonan Institute of Technology, Kanagawa, Japan.

Poster 4-12. **Pre-Esterification of Free Fatty Acids by Solid Acid Catalysts.** S. H. Kim, *J. S. Lee,* D. K. Kim. KIER, Taejeon, Korea; H. J. Kim, and K. Y. Lee. Korea University, Seoul, Korea; H. S. Kim. Myung-Ji University, Yongin, Korea.

Poster 4-13. **Efficient Ethanol Production by a Novel Bioprocess** *Hideo Kawaguchi*, Kaori Nakata, Masayuki Inui, and Hideaki Yukawa. Research Institute of Innovative Technology for the Earth (RITE), Kyoto, Japan.

Poster 4-14. **Production of Lactic Acid by Novel Bioprocess Using Coryneform Bacteria.** *Shohei Okino*, Kaori Nakata, Naoko Okai, Masayuki Inui, Crispinus A. Omumasaba, and Hideaki Yukawa. Research Institute of Innovative Technology for the Earth (RITE), Kyoto, Japan.

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Poster 5-07**. Using Landfill Gas Energy as a Source of Renewable Power, Localized Biodiesel Production and Hydrogen Vehicle Refueling Stations.** *C. John Villella*. DTE Biomass Energy, Inc., Cassadaga, FL; Russel Teall. Biodiesel Industries, Inc., Santa Barbara, CA.

Poster 5-08. **Conversion of Rice Straw for Production of Bioethanol and Other Valuable Products using The Danish Bioethanol Concept.** *Frank Haagensen* and Birgitte K. Ahring. Environmental Microbiology & Biotechnology Research Group, BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark; Anne B. Thomsen. Plant Biology & Biogeochemistry, RISØ National Laboratories, Roskilde, Denmark.

Poster 5-09. **Application of the Danish Bioethanol Concept to Different Types of Biomass Waste.** *Birgitte K. Ahring*. BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark; Mads Torry-Smith, and Frank Haagensen. BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark; Anne Belinda Thomsen. Plant Biology and Biogeochemistry, Risoe National Laboratory, Roskilde, Denmark.

Poster 5-10. **Extraction of Low Grade Energy in the Form of Hot Water at 65 to 70 Degrees Celsius from Compostable Materials Such as Municipal Solid Waste (MSW) pre Human Consumption Plant and Vegetable Waste and Animal Manures.** *Joseph Ouellette.* Agrilab Technologies Inc., Windsor, Ontario, Canada.

Poster 5-11. **Utilization of Lignin Biomass Component in Composites with Polyolefins**. *Bozena Kosikova*, Adriana Gregorova. Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia; Pavol Alexy. Department of Plastics and Rubber, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia; Maria Mikulasova. Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia.

Poster 5-12. **Vegetable Oil as Substitute for Mineral Oils** *Yves Bertrand*, and Lê Chiên Hoang. EDF R&D, France.

Poster 5-13. **Production of Succinic Acid by *Anaerobiospirillum Succiniciproducens* from Wood Hydrolysate.** *Sang Yup Lee*, Soon Ho Hong, Pyung Cheon Lee, and Ho Nam Chang. Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Republic of Korea.

Poster 5-14. **Batch and Continuous Cultures of *Mannheimia succiniciproducens* MBEL55E for the Production of Succinic Acid from Whey and Corn Steep Liquor**. *Sang Yup Lee*, Soon Ho Hong, Pyung Cheon Lee, and Ho Nam Chang. Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Republic of Korea.

Poster 5-15. **High Level Production of Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by Fed-batch Culture of Recombinant *Escherichia coli* in a Pilot Scale Fermentor.** *Sang Yup Lee*, Si Jae Park, and Jong-il Choi. Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Republic of Korea.

Poster 5-16. **High Level Production of_-polyglutamic Acid by Fed-batch Culture of *Bacillus licheniformis*.** *Sang Yup Lee*, Nagendra Takur, and Ho Nam Chang. Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Republic of Korea.

Poster 5-17. **Characterization of Surfactin from *Bacillus subtilis* for Application as an Agent for Enhanced Oil Recovery.** *Kastli D. Schaller*, Greg A. Bala. Idaho National Engineering and Environmental Laboratory, Idaho Falls, ID.

Poster 5-18. **The Manufacture Of Synthesis Gas From Biomass And Production Of Alcohols And Electric Power Using The Pearson Thermo-Chemical Steam Reforming And Catalytic Conversion Processes.** Stanley R. Pearson. Pearson Technologies, Inc., Aberdeen, MS.

Poster 5-19. **The Effect of Germ and Fiber Removal on the Production of Ethanol from Corn.** *Elankovan Ponnampalam*, D. Bernie Steele, Deborah Burgdorf, and Darold McCalla. MBI International, Lansing, MI.

Poster 5-20. **Kinetics of *Monascus ruber* Secondary Metabolites Production on Rice, Under Variable Initial Cell Concentration.** Gisele Y. Miyashira, Rogerio Rodrigues, and Beatriz V. Kilikian. Department of Chemical Engineering – EPUSP, São Paulo, Brazil.

Poster 5-21. **Relationship Between Secondary Metabolites Production and Batch Growth Kinetics of *Monascus purpureus* sp.** *Daniela Gerevini Pereira*, Sandra Bilbao Orozco, and Beatriz V. Kilikian. Department of Chemical Engineering – EPUSP, São Paulo, Brazil.

Poster 5-22. **Optimizing the Three Precursor Concentration for Aureomicin Biosynthesis Through Solid Fermentation.** *Marcela Segura-Granados*, Samuel Dorantes-Alvarez, Ignacio Robledo-Bautista, and Oscar García-Kirchner. Departamento de Bioprocesos, Ticomán. México.

Poster 5-23 Student. **Effects of Xylose Reductase Sources on Xylitol Production in Recombinant *Saccharomyces cerevisiae*.** Myoung-Dong Kim, Young-Sok Jeun, *Sung-Gun Kim*, Tae-Hee Lee, and Jin-Ho Seo. Department of Food Science and Technology, Research Center for New Bio-Materials in Agriculture, Seoul National University, Suwon, South Korea; Yeon-Woo Ryu. Department of Molecular Science and Technology, Ajou University, Suwon, South Korea.

Poster 5-24. **Development of Itaconic Acid Fermentation by *Aspergillus terreus*.** Bálint Kupcsulik, Szabolcs Halmos, *Zsolt Szengyel*, and Béla Sevella. Department of Agricultural Chemical Technology, Budapest University of Technology and Economics, Budapest, Hungary.

Poster 5-25. **Development of a Brewery's Spent Grain Dilute-Acid Hydrolyzate Media for Polyols Production by *Debaryomyces hansenii* CCMI 941.** *Florbela Carvalheiro*, Luis C. Duarte, Raquel Medeiros, and Francisco M. Gírio. INETI, Departmento de Biotecnologia, Lisboa, Portugal.

Poster 5-26. **Genetic Engineering of Glucose/Xylose Co-fermenting *Saccharomyces* Yeast for Co-production of Ethanol and Various Industrial Enzymes.** *Miroslav Sedlak*, Zhengdao Chen, and Nancy Y.W. Ho. Laboratory of Renewable Resources Engineering (LORRE), Purdue University, West Lafayette, IN; Yanfang Pang and Todd Applegate. Department of Animal Sciences, Purdue University.

Poster 5-27. **Extraction of Oryzanols and Corn Oil from Distillers Dried Grains and Solubles.** Robert Hanchar, Elankovan Ponnampalam, Rebecca Powell, and D. Bernie Steele. MBI International, Lansing MI.

Poster 5-28. **Applications of a Specialty Polymer Derived from a Biobased Monomer.** William McDonald, *Robert Hanchar*, Sunil Dourado, Joel Dulebohn, and Amy Koren. MBI International, Lansing, MI.

Poster 5-29 Student. **Expression of Yeast Alcohol Acetyltransferase Genes in *Escherichia coli* and *Clostridium acetobutylicum* for the Production of CoA Esters.** *Catherine Emily Horton*, Frederick B. Rudolph, and George N. Bennett. Rice University, Houston, TX.

Poster 5-30. **Continuous Production of Butanol by *Clostridium acetobutylicum* Using a Fibrous Bed Bioreactor.** *Wei-Cho Huang*, and Shang-Tian Yang. Department of Chemical Engineering, Ohio State University, Columbus, OH; David E. Ramey. Environmental Energy Inc., Blacklick, OH.

Poster 5-31. **Peroxidase as the Biocatalyst Agent in the Biotransformation of Isosafrole into Piperonal Effected by *Paecilomyces variotii*.** A.S. Santos. Departamento de Engenharia Química,Universidade Federal do Pará; O.A.C. Antunes. Instituto de Química, Universidade do Brasil; N. *Pereira Jr.* Departamento de Engenharia Bioquímica, Universidade do Brasil; M.I. Sarquis. Departamento de Micologia, Laboratório de Coleção de Cultura de Fungos, IOC.

Poster 5-32. **Peptidelipid Surfactant Production By *Bacillus subtilis* Grown On Low Cost Raw Materials**. Fabíula Andréa Sena Leal Reis, Maria Aparecida Nóbrega de Almeida, Eliana Flavia Camporese Sérvulo. Escola de Química, Universidade Federal do Rio de Janeiro, Centro de Tecnologia, Rio de Janeiro, Brazil.

Poster 5-33. **A Computational Framework for the Discovery of Novel Biobased Industrial Chemicals.** Chunhui Li, Justin A. Ionita, Linda J. Broadbelt, *Vassily Hatzimanikatis*. Department of Chemical Engineering, Northwestern University, Evanston, IL.

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Poster 5-37. **Succinic Acid Production from Glucose by Two-Step Bioconversion Process**. Se-Kwon Moon, Young-Jung Wee, Jong-Sun Yun, and *Hwa-Won Ryu*. Faculty of Applied Chemical Engineering, Institute of Bioindustrial Technology, Chonnam National University, Gwangju, South Korea.

Poster 5-38. **The Effects of Trace Contaminants on Catalytic Processing of Biomass-Derived Feedstocks**. *Douglas C. Elliott*, Keith L. Peterson, Eric V. Alderson, Todd R. Hart, and Gary G. Neuenschwander. Pacific Northwest National Laboratory, Richland, WA.

Poster 5-39. **Anaerobic Fermentation of Biomass Generated Producer Gas to Ethanol and Other Useful Products.** *Rohit P. Datar*, Rustin M. Shenkman, and Randy S. Lewis. School of Chemical Engineering, Oklahoma State University, Stillwater, OK; Ralph S. Tanner and Jack Liou. Department of Botany and Microbiology, University of Oklahoma, Norman, OK; B. G. Cateni, D. D Bellmer, T. J. Bowser, and R. L. Huhnke. Biosystems and Agricultural Engineering, Oklahoma State University, Stillwater, OK.

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Poster 5-41. **The Higher Alcohols Biorefinery: Improvement of the Catalyst for Ethanol Conversion.** *Edwin S. Olson*, Ramesh K. Sharma, and Ted R. Aulich. Energy & Environmental Research Center, University of North Dakota, Grand Forks, ND.

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Poster 6A-08**. Ammonia Fiber Explosion (AFEX) for Pretreatment of Corn Stover: Recent Research Results.** *Bruce E. Dale*, Lizbeth Laureano-Perez, Farzaneh Teymouri, and Hasan Alizadeh. Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI.

Poster 6A-09. **The Development of a Pilot-Scale Hydrogen Energy System From Livestock Wastes and Raw Garbage.** *Pomin Li*. Doctoral Program of Agricultural Science, University of Tsukuba, Japan; Zhenya Zhang, Norio Sugiura. Institute of Agricultural and Forest Engineering University of Tsukuba, Japan; Takeo Inoue.Bioelex Corporation, Japan; Takaaki Maekawa. Institute of Agricultural and Forest Engineering University of Tsukuba, Japan.

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Poster 6A-11. **Predicting Performance of Batch, Flowthrough, and Mixed Batch Hemicellulose Hydrolysis by Coupled Mass Transfer and Reaction Models.** *Michael A. Brennan* and Charles E. Wyman. Thayer School of Engineering, Dartmouth College, Hanover, NH.

Poster 6A-12. **Fed-batch of SO_2-Impregnated and Steam Pretreated Spruce in Simultaneous Saccharification and Fermentation for Production of Ethanol.** Mats Galbe, Sara Månsson and Christian Roslander. Department of Chemical Engineering, Lund University, Lund, Sweden.

Poster 6A-13. **Hydrothermal Pretreatment for Barley Straw Conversion to Ethanol.** M.J. Negro, P. Manzanares, I. Ballesteros, J.M. Oliva, F.Sáez and *M. Ballesteros*. CIEMAT, Madrid, Spain.

Poster 6A-14. **Kinetics of Xylooligosaccharides Hydrolysis** Xia Li, Alvin O. Converse and Charles E. Wyman. Thayer School of Engineering, Dartmouth College, Hanover, NH.

Poster 6A-15. **Impact of Fluid Velocity and Contact Time on Corn Stover Pretreatment in a Flowthrough Reactor.** *Chaogang Liu* and Charles E. Wyman. Dartmouth College, Hanover, NH.

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Poster 6A-17. **Dilute-Acid Hydrolysis of Hemicellulose from Agricultural Renewable Biomass.** Dae-Il Oh, Sung-Kap Hong, Gi-Sub Choi, and Yeon-Woo Ryu. Department of Molecular Science and Technology, Ajou University San 5 Woncheon-dong Paldal-gu, Suwon, Korea; Jin-Ho Seo. Department of Food Science and Technology and Research Center for New Bio-Materials in Agriculture, Seoul National University, Suwon, Korea.

Poster 6A-18 Student. **Extraction of Sugar from the Fiber Fraction in Wheat Grain to Enhance Fuel Ethanol Production.** *Marie Linde,* Mats Galbe and Guido Zacchi. Department of Chemical Engineering 1, Lund University, Lund, Sweden.

Poster 6A-19. **Ammonia Fiber Explosion Process (AFEX): A Rapid and Flexible Laboratory Scale Unit.** *Hasan Alizadeh*, Farzaneh Teymouri, Lizbeth Laureano-Perez, and Bruce E. Dale. Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI.

Poster 6A-20. **Production of Succinic Acid from Ammonia Fiber Explosion (AFEX) Pretreated Biomass Hydrolysates.** Nhuan P. Nghiem, Elankovan Ponnampalam, David Slomczynski, Tonya Tiedje, and Rebecca Powell. MBI International, Lansing, MI.

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Poster 6A-22. **Dilute Acid Hydrolysis of SEDAP Treated Oak Wood** Sang Woo Baek, Myoung Hoon Park, and *Kyeong Keun Oh*. Department of Industrial Chemistry, Dankook University, Cheonan, Korea; Jin-Suk Lee and Soon-Chul Park. Korea Institute of Energy Research, Taejeon, Korea.

Poster 6A-23. **Softwood Hydrolysis in a Shrinking Bed Flow-through Reactor**. *Pär O. Pettersson*. Mid Sweden University, Örnsköldsvik, Sweden; Robert W. Torget and Edward W. Jennings. National Renewable Energy Laboratory, Golden, CO; Robert Eklund. Umeå University, Umeå, Sweden.

Poster 6A-24 Student. **Optimisation of Pretreatment of SO_2 Impregnated Corn Stover for Ethanol Production.** *Karin Öhgren*, Mats Galbe and Guido Zacchi. Department of Chemical Engineering 1, Lund University, Lund, Sweden.

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Poster 6A-26. **Pretreatment of Barley Husks for Ethanol Production.** Beatriz Palmarola-Adrados, Laura Diego-Meliveo, María Learra-Martínez, Mats Galbe, and Guido Zacchi. Department of Chemical Engineering 1, Lund University, Lund, Sweden.

Poster 6A-27. **Application of Xylanase from *Thermomyces lanuginosus* OC-4145 in Enzymatic Hydrolysis of Corncob and Sugar Cane Bagasse.** Monica Caramez Triches Damaso, Aline Machado de Castro, Raquel Machado Castro, and *Nei Pereira, Jr.* Escola de Química da Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; Carolina Maria M. C. Andrade. White Martins Gases Industriais LTDA, Rio de Janeiro, Brazil.

Poster 6A-28. **A Modified Polymer Depolymerization Kinetics Model to Describe the Thermochemical Hydrolysis of Hemicellulose.** *Todd Lloyd* and Charles E. Wyman. Thayer School of Engineering, Dartmouth College, Hanover, NH.

Poster 6A-29. **Enhancement of Enzymatic Digestibility of Used Newspaper by Surfactant Addition in Ammonia-Hydrogen Peroxide Pretreatment.** Jin Won Chun and *Sung Bae Kim*. Division of Applied Chemical Engineering and ERI, Gyeongsang National University, Jinju, Korea.

Poster 6A-31. **Study on Methane Fermentation and Production of Vitamin B12 from Alcohol Waste Fluid.** *Zhenya Zhang*. Institute of Agricultural and Forest Engineering University of Tsukuba, Japan; Taisheng Quan. Master Program of Biosystem Studies, University of Tsukuba, Japan; Yansheng Zhang. Water Conservancy and Civil Engineering College, China Agricultural University, Peking, China; Pomin Li. Doctoral Program of Agricultural Science, University of Tsukuba, Japan; Norio Sugiura and Takaaki Maekawa. Institute of Agricultural and Forest Engineering University of Tsukuba, Japan.

Poster6A-32. **Recirculation of Condensate Streams in Fuel Ethanol Production from Softwood Based on SSF.** Malek Alkasrawi, Mats Galbe, and Guido Zacchi. Department of Chemical Engineering 1, Lund University, Lund, Sweden.

Poster 6A-33. **Enzyme Activity in Fed Batch Simultaneous Saccharification and Fermentation.** D. Barisano, G. Braccio, M. Cardinale, E. Viola, F. Zimbardi. ENEA, Biomass Laboratory, Policoro, Italy.

Poster 6A-34. **Evaluation of Post-Hydrolysis Processes of Brewery's Spent Grain Autohydrolysis Liquor to Produce a Pentose-Containing Culture Media.** Luís C. Duarte, *Florbela Carvalheiro*, Sónia Lopes, Susana Marques, and Francisco M. Gírio. INETI, Departamento de Biotecnologia, Lisboa, Portugal.

Poster 6A-35. **Optimization of Brewery's Spent Grain Dilute-Acid Hydrolysis for the Production of Pentose-Rich Culture Media.** *Florbela Carvalheiro*, Luis C. Duarte, Raquel Medeiros, and Francisco M. Gírio. INETI, Departmento de Biotecnologia, Lisboa, Portugal.

Poster 6A-36. **Comparison of the Microbial Inhibition and Enzymatic Hydrolysis Rates of Liquid and Solid Hydrolysates Produced from Pretreatment of Biomass with Carbonic Acid and Liquid Hot Water.** *Damon Yourchisin* and G. Peter van Walsum. Department of Environmental Studies, Baylor University, Waco, TX.

Poster 6A-37. **Modeling of Carbonic Acid Pretreatment Process Using ASPEN-Plus.** Kemantha Jayawardhana, and *G. Peter van Walsum*. Department of Environmental Studies, Baylor University, Waco, TX.

Poster 6A-38. **Generation of Coproducts Derived from a Modified Hot Water Pretreatment of Corn Stover.** *Richard Hendrickson*, Nathan S. Mosier, and Michael R. Ladisch. Purdue University, West Lafayette IN.

Poster 6A-39. **Enhanced Enzymatic Hydrolysis of Steam-Exploded Douglas Fir by Alkaline Oxygen Post-treatment.** *Xuejun Pan*, Xiao Zhang, David J. Gregg and John N. Saddler. University of British Columbia, Vancouver, BC, Canada.

Poster 6A-40. **The Effect of Modified Pretreatment and Delignification Parameters on the Bioconversion Process.** I. Culllis, John N. Saddler, and S.D. Mansfield. University of British Columbia, Vancouver, BC, Canada.

Poster 6A-41. **SO_2-catalysed Steam Explosion of Corn Fibre for Ethanol Production.** *R. Bura*, S.D. Mansfield, and John N. Saddler. Forest Products Biotechnology, Department of Wood Science, University of British Columbia, Vancouver, BC, Canada; R.J. Bothast. National Corn-to-Ethanol Research Pilot Plant, Edwardsville, Il.

Poster 6A-42 Student. **Enzymatic Hydrolysis of Cellulose to Improve Pre-hydrolysate Sugar Concentration.** *J. Robinson,* J.D. Keating, S.D. Mansfield and John N. Saddler. The University Of British Columbia, Vancouver, BC, Canada.

Poster 6A-43. **A Quantitative approach to Studying the Effects of Sugar Inhibition on Cellulase and β-glucosidase During Enzymatic Hydrolysis of Softwood Substrates.** *Zhizhuang Xiao*, Xiao Zhang, David Gregg and John N. Saddler. University of British Columbia, Vancouver BC, Canada.

Poster 6A-44. **High Consistency Hydrolysis of Softwood Substrates.** *Zhizhuang Xiao*, Xiao Zhang, David Gregg, and John N. Saddler University of British Columbia, Vancouver, BC, Canada.

Poster 6A-45. **Saccharification of Sugar Cane Bagasse Pith by a Heterogenerous Cellulase.** *Oscar García-Kirchner*. Departamento de Bioprocesos UPIBI-IPN, Ticomán, D.F. México; Carlos Huitrón-Vargas and Rosalba Pérez-Villalva. Departamento de Biotecnologia, Instituto de Investigaciones Biomédicas, D.F. México.

Poster 6A-46. **Evaluation of the Fermentation Conditions for Beta-glucosidase Production with *A. niger* Using Different Lignocellulosic Materials.** *Oscar García-Kirchner*, Marcela Segura-Granados, Samuel Suazo-Abarca, and Ignacio Robledo-Bautista. Departamento de Bioprocesos UPIBI-IPN, Ticomán, D.F. México.

Poster 6A-47. **Rapid Biomass Analysis: New Analytical Methods Supporting Biomass Pretreatment Research.** *Amie D. Sluiter*, Raymond O. Ruiz, and Bonnie R. Hames. National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO.

Poster 6A-48. **Methods for Quantitative Analysis of Uronic Acids in Biomass.** *David K. Johnson*. National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO.

Poster 6A-49. **Effects of Dilute Acid Hydrolyzate Components on Glucose Degradation.** *David K. Johnson*. National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO.

Poster 6A-50. **Hot-Washing of Pretreated Corn Stover Using Integrated Sunds Horizontal Screw and Jaygo Pretreatment Reactors with Pneumapress Automatic Pressure Filter.** *Melvin P. Tucker*, Nicholas J. Nagle, Edward Jennings, Robert Lyons, and Richard Elander. Biotechnology Division for Fuels and Chemicals, National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO.

Poster 6A-51. **Production and Hydrolysis of Cellulose from the PureVision Biomass Fractionation Process.** Richard C. Wingerson, and Ed Lehrburger. PureVision Technology, Inc., Fort Lupton, CO; *Frank D. Guffey.* Western Research Institute, Laramie, WY.

Poster 6A-52. **Computer Simulations of Water Structuring Adjacent to Microcrystalline Cellulose Iβ Surfaces**. Cathy Skopec and *John Brady*. Department of Food Science, Cornell University, Ithaca, NY; Tauna Rignall and Clare McCabe. Department of Chemical Engineering, Colorado School of Mines, Golden CO; Michael Himmel. National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO.

Poster 6A-53. **Pretreatment of Corn Stover by Low-Liquid Ammonia Percolation Process.** *Tae Hyun Kim* and Y. Y. Lee. Department of Chemical Engineering, Auburn University, Auburn, AL.

Poster 6A-54**. Effects of Residual and Soluble Lignin on Enzymatic Hydrolysis of Cellulose .***Tae Hyun Kim* and Y. Y. Lee. Department of Chemical Engineering, Auburn University, Auburn, AL.

Poster 6A-55. **Kinetics of Glucose Decomposition Under Extremely Low Acid and High Temperature Conditions** Qian Xiang and *Y. Y. Lee*. Department of Chemical Engineering, Auburn University, Auburn, AL; Robert W. Torget. National BioEnergy Center, National Renewable Energy Laboratory**,** Golden, CO.

Poster 6A-56. **Acid Hydrolysis of Corn Stover Hemicellulose by Low-Liquid Percolation Process.** *Yongming Zhu* and Y. Y. Lee. Department of Chemical Engineering, Auburn University, AL; Richard Elander. National BioEnergy Center, National Renewable Energy Laboratory, Golden CO.

Poster 6A-57. **Hydrolyzed Distiller's Grain Production, Fermentation and Animal Feeding Trials.** Melvin P. Tucker, Nicholos J. Nagle, Edward Jennings, Quang A. Nguyen, Kyoung H. Kim, Kelly Ibsen, and Sally Noll. Biotechnology Division for Fuels and Chemicals, National BioEnergy Center, National Renewable Energy Laboratory, Golden, CO.

Poster 6A-58 Student. **Measuring and Modeling Oligomer Solubilities in Hemicellulose Hydrolysis**. *Matthew C. Gray*, Alvin O. Converse, and Charlie E. Wyman. Thayer School of Engineering, Dartmouth College, Hanover, NH.

Poster 6A-60. **The Role of Solids Concentration and Acetylation in Uncatalyzed Batch Hydrolysis of Corn Stover Hemicellulose.** *Suzanne Stuhler* and Charles E. Wyman. Thayer School of Engineering, Dartmouth College, Hanover, NH.

Poster 6A-61. **Softwood to Ethanol Process Design and Optimization.** *Olga Mirochnik* and Sheldon J. B. Duff. Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, British Columbia, Canada; Xiao Zhang, David J. Gregg, John N. Saddler. Department of Wood Science, University of British Columbia, Vancouver, British Columbia, Canada; Claudio Arato. Lignol Innovations Corp., Vancouver, British Columbia, Canada.

Plant Biotechnology and Feedstock

Poster 6B-07**. Effects of Pretreatment on the Activity of Plant-Produced Cellulase and Xylanase Enzymes.** *Farzaneh Teymouri*, Lizbeth Laureano-Perez, Hasan Alizadeh, Mariam B. Sticklen, and Bruce E. Dale. Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI.

Poster 6B-08. **Effective Digestion of Untreated Corn Stover by Microbial Enzymes.** *Brian Vande Berg*, Duncan Taylor, Jill Burdette, Toby Osofsky, Sharon Mason, Brian Carr, Nick Duck, and Mike Koziel. Athenix Corp., Durham, NC.

Poster 6B-09. **Biorefining Reproductive Organs of Plant Crops.** *George H. Robertson*, Dominic Wong, Charles Lee, and William J. Orts. Western Regional Research Center, USDA-ARS, Albany, CA.

Poster Presentation 6B-10. **Transgenic Maize-Produced Cellulases for Biomass Conversion.** Elizabeth E. Hood. Plant Biotechnologist, Texas A&M University, College Station, TX.

Poster Presentation 6B-11. **Plant Genomics at the Service of Energy Crops.** Neal Gutterson and Pierre Broun. Mendel Biotechnology, Hayward CA.

Poster 6B-12. **Modifying Lignin Composition to Enhance Ethanol Production from Maize Stover.** Wilfred Vermerris. Department of Agronomy and Department of Agricultural & Biological Engineering; Siobhán Bout. Department of Agronomy; Michael Ladisch. Department of Agronomy and Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, IN.

Poster 6B-13. **The Effect of Inoculum Conditions on the Growth of Hairy Root of *Panax ginseng* C.A. Meyer.** Gwi-Taek Jeong (Institute of Bioindustrial Technology), *Don-Hee Park*, and Hwa-Won Ryu. Faculty of Chemical Engineering; Baik Hwang. Department. of Biological Sciences, Chonnam National University, Kwangju, Korea; Je-Chang Woo. Department of Biology, Mokpo National University, Chonnam, Korea.

Session Special Topics A:

Microbial Pentose Metabolism

Session Special Topics B:

International Bioenergy Agency

Bioethanol Meeting-Current State of Fuel Ethanol Commercialization

Abstracts for Oral Presentations

Oral Presentation 1A-01

What Have We Learned from Federal R&D Programs in Biomass?

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The federal biomass program is over 25 years old. Total expenditures for R&D are over $1 billion. Tax expenditures are over $8 billion. What have we learned from its successes and failures? What public policies and expenditures are necessary to make the transition to an economy where plants are a significant source of fuels and industrial products and where the cultivation of those plants benefits the cultivators and rural communities here and abroad?

Oral Presentation 1A-02

Rainfall and Wind Erosion-Based Sustainable Residue Removal Analysis and Resource Assessment for Corn Stover and Wheat Straw for Eight Selected Cropping Rotations in the United States

Richard G. Nelson

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A methodology was developed and utilized to assess sustainable removal of corn stover and small-grain (wheat, barley, oat, and rye) residues/straws from agricultural cropland, subject to rainfall and wind erosion constraints. Specifically, predictions of average annual removable residue quantities (tons of residue at harvest per year) from continuous corn, wheat, and oats, and corn-soybean, wheat-soybean, corn-wheat, corn-oats, and wheat-oats rotations were made for all land capability class I-VIII cropland soils in each county of 40 states, subject to the constraint of not exceeding the NRCS-mandated tolerable soil loss limit. Field management practices of conventional tillage, conservation/mulch tillage, and no-till were applied to each rotation.

County-level removal residue quantities were tabulated from removable residue amounts on each LCC soil type for all rotations and each field management practice. Results associated with employing this soil erosion-based removal residue methodology indicate significant quantities of corn stover and small-grain residues exist and can be removed for alternative purposes.

Oral Presentation 1A-03

Single Pass Whole-Plant Corn Harvesting for Biomass: Comparison of Single and Versus Multiple Harvest Streams

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Corn grain has been a major feedstock to produce a variety of bioproducts such as ethanol, starch, corn syrup, fructose, corn oil, gluten and several specialty chemicals. More and more interest has been expressed in using the rest of the corn plant, known as stover, to produce other bioproducts. The amount of dry matter (DM) provided by stover, which includes the stalk, cob, leaf and husk, can be almost as high as the DM provided by grain per unit area. Recent data have shown that the stover to grain DM ratio varied from 1.10 to 0.78 during the potential harvest period between 122 and 181 days after planting (Aug. 27 to Oct. 25). The paper compares the five following harvest systems: (1) harvesting grain with a combined harvester and immediately chopping or baling wet stover in two sequential streams (grain and wet stover); (2) harvesting grain with a combined harvester and baling dry stover after a field wilting period in two sequential streams (grain and dry stover); (3) harvesting grain and chopping stover at the same time with an innovative harvester that produces two simultaneous but separate streams (grain and wet stover); (4) snapping the corn and separately creating three streams simultaneously (grain, cob and the rest of the wet stover); and (5) harvesting and chopping the whole crop in a single stream (chopped grain and stover stored together as silage). Each system will be described in terms of cost of harvest, storage, handling and separation of components prior to processing.

Oral Presentation 1A-04

Pipeline Transport of Biomass

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This paper presents a detailed cost analysis of pipeline transport of biomass. Transportation cost is one of the main components in the biomass power cost. A previous study by the authors estimated that for power generated at optimum size from truck delivered whole forest biomass (whole tree in the form of chips), forest residues (chipped limbs and tops left on the roadside after logging operations) and agricultural residues (straw and chaff) in western Canada, the transportation cost is US$10 per dry tonne, $38 per dry tonne, and $20 per dry tonne respectively. This makes up 14%, 38% and 25% of the biomass power cost respectively. It is highest for forest residues because of the large transportation distance of 330 kms at the optimum size (137 MW). Truck transportation would also lead to road congestion (for example a 450 MW biomass power plant would require 10-15 trucks per hour). Fossil fuel power plants in western Canada do not rely on highway truck delivery.

Pipeline economics are first evaluated by a study of the transport of biomass for a long distance from a remote harvest location, using water or oil as the carrying liquid. Forest and/or agricultural biomass is transported from an inlet point at the harvest site to the biomass power plant, where it would be piled and drained before utilization as fuel. This study evaluates the feasibility of a pipeline system for transporting biomass, a capital and operating cost estimate for the pipeline transport of biomass, the cost of biomass transportation for pipeline compared to truck transport and the cost of power from biomass using pipeline delivery of biomass. This study is then extended to a biomass power plant located in the center of its biomass collection area and supported by pipelines arrayed like spokes.

The issues related to carrying medium are also be evaluated. In the case of oil as a carrying medium, drained oil is filtered and recycled by a return pipeline. In the case of water as a carrying medium, water would either be discharged as surface runoff or recycled by a return pipeline.

Oral Presentation 1A-05

The Effect of Corn Stover Composition on Ethanol Process Economics

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During the last fifteen years, the National Renewable Energy Laboatory (NREL) has modeled biomass-to-ethanol processes and estimated capital and operating economics for the processes. Modeling and economic estimates are based on parameters that are typically entered as single values; however, feedstock composition and other parameters vary considerably and would be better modeled as probability density functions. In a Monte Carlo analysis, random values (fitting the appropriate probability density functions) are assigned for multiple parameters, the simulation is run, and the results are recorded. The iterative process of value assignment, calculation, and recording is used to develop resulting distributions that are reported in histograms and cumulative probability curves.

In 2002, NREL published an updated design report for the co-current dilute acid prehydrolysis and enzymatic hydrolysis process with a single corn stover composition. Additional simulations have been run using the compositions of 600 unique corn stover samples to show the effect of those compositions on ethanol yield, total project investment, and minimum ethanol selling price. The results indicate that feedstock composition can modify the minimum ethanol selling price by $0.20/gal. Also, a Monte Carlo analysis was run using probability density functions for the following: feedstock composition, pretreatment, enzymatic saccharification, and fermentation yields to show the effect of multiple variables on the process.

Oral Presentation 1A-06

Corn Stover Feedstock Learnings: Kearney Area Ag Producers Alliance Removal Project

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A large scale corn stover collection project is described based on the 1996 experiences of the Kearney Area Ag Producers Alliance (KAAPA). Over 4,000 mostly irrigated high yielding acres of cornstalks were baled in a 3-month period as a demonstration for a proposed corn stover to paper pulp project. A large quantity of these bales was stored for 18 months.

This project was a demonstration to generate equity investment, alleviate both producer and processor concerns, generate realistic cost estimates and advance the collection, storage and transport learning curve. The dirt removal strategies and improved baling processes developed were superior to prior collection methods.

Strategies for various types of cropping practices were developed as well to provide ground cover, prevent soil erosion and deal with no till where the irrigation ridges had been destroyed by the baling process.

Precautions needed to insure that collected stover bales remained in good condition at the end of the storage period were determined, along with the weather risks and the critical parts of the process that need to be fine tuned to make sure it is reliable, efficient, sustainable, functional and economic.

Oral Presentation 1B-01

Strain and Process Improvement for the Production of Whole Cellulase by *Trichoderma reesei*

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Genencor International was awarded a subcontract from the National Renewable Energy Laboratory for cellulase cost reduction for biomass conversion to fermentable sugars. The goal of this three-year program was to reduce the cost of cellulase by ten-fold. Reaching this aggressive target requires improvements in both the production, and in the specific performance, of the cellulase mixture. This presentation will focus on efforts to improve the *Trichoderma reesei* host strain and the production process for the manufacture of whole cellulase. Efforts included 1) various mutagenesis and selection/screening methods for improved cellulase producing strains, 2) replacement of lactose with lower cost carbon sources for cellulase production, 3) fermentation process improvements and 4) reducing the cost of post-fermentation processes. Results of these efforts and the overall effect on cellulase cost reduction will be discussed.

Oral Presentation 1B-02

Thermostability, Substrate Specificity and Hydrolysis of Cellulose by Endoglucanases from Families 5, 7, and 45 of Glycoside Hydrolases

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Hydrolysis of the cellulose component of lignocellulose requires the synergistic action of a multi-enzyme complex, which generally include endoglucanase, exoglucanase (cellobiohydrolase), and cellobiase (Himmel et al., 1997). Of these enzymes, the cellobiohydrolases appears to be the key class of enzyme for degradation of crystalline cellulose (Schülein, 1997). However, addition of even small quantities of endoglucanases usually results in drastic increases in saccharification yields due to synergistic interaction between endoglucanases and cellobiohydrolases (Thomas et al., 1995).

Several purified endoglucanases from glycoside hydrolase families 5, 7, and 45 were evaluated in order to identify enzymes with greater thermostability and better hydrolysis of insoluble cellulose as compared to endoglucanase Cel7B (EGI) from filamentous fungus *Trichoderma reesei*.

Thermostability of endoglucanases was determined by measuring residual activity towards carboxymethylcellulose (CMC) after 3-h incubation at 40°C-80°C. Specific activities of endoglucanases on various substrates, including CMC, p-nitrophenyl-β-D-cellobioside (PNPC), p-nitrophenyl-β-D-lactoside (PNPL), and phosphoric-acid-swollen cellulose (PASC) were measured. Important correlations between the sequence-based family number and thermostability/substrate specificity of the enzymes were observed.

Temperature activity profiles were obtained by measuring specific activity of endoglucanases on PASC at 40°C-70°C. Temperature dependence of the hydrolysis yield was determined by measuring the degree of PASC conversion to reducing sugar (RS) over extended reaction times at 40°C-70°C. Several endoglucanases with high specific activity on PASC and high thermostability were identified.

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Oral Presentation 1B-03

An Accelerated Evolutionary Route to Enzyme Fitness

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Directed evolution technologies were used to fine-tune a selected phenotype of an enzyme, without significantly changing other biochemical properties. The enzymatic breakdown of hemicellulose would generate fermentable pentose sugars and aid in the pretreatment of biomass for the separation of the cellulosic and hemicellulosic fractions. Thermal tolerant xylanases would have utility in the hydrolysis of arabinoxylan, the major component of the hemicellulose fraction of biomass. This presentation will describe the tandem use of Gene Site Saturation Mutagenesis™ (GSSM) and GeneReassembly™ technologies to dramatically improve the thermal properties of a family 11 xylanase. The power of combining GSSM and GeneReassembly to obtain enzyme variants with the highest degree of fitness will be shown. To our knowledge, the shift in the melting temperature transition midpoint (T_m) of this xylanase is the largest shift yet reported for directed evolution of an enzyme.

Oral Presentation 1B-04

Impact of Binding Domains in Cellulase Activity Assays and in Hydrolysis of Crystalline Cellulose

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The complete hydrolysis of cellulose is a complex process, which requires the simultaneous synergistic action of several different enzymes. The main cellulolytic enzyme produced by *T. reesei* is CBH I (Cel7a), which generally accounts for about half of the total protein produced. The amount of CBH II (Cel6a) is about 20%. The two main endoglucanases, EG I (Cel7b) and EG II (Cel5a), both represent about 10% of the produced protein. All these four major enzymes have a modular structure consisting of a separate catalytic domain (core protein) and a cellulose-binding domain (CBD) separated by a linked peptide. The presence of CBDs is especially important to the action of CBHs on crystalline cellulose, whereas the hydrolysis of soluble cellulose derivatives or cello-oligosaccharides is not drastically affected by the presence or absence of CBDs.

The determination of cellulase activities is also a complicated task, as the degradation of water insoluble cellulose is not linear with time and different enzyme dosages. Therefore, several artificial substrates are normally used. Soluble cellulose derivatives, such as carboxymethyl cellulose (CMC) and hydroxyethyl cellulose (HEC), are routinely used to measure endoglucanase activity. These substrates are rather specific to EGs, as CBHs are not generally able to degrade substituted celluloses. The activity of CBHs is often measured by small fluorogenic substrates, such as methylumbelliferyl cellobioside (MUC).

In this work, the role of CBDs on different activities, especially on the FPU activity, in cellulase mixtures was evaluated. The core proteins of the four major cellulases of *T. reesei* were produced and their effect on the cellulase activities by different assays were determined. The performance of these enzymes (individual core or intact enzymes in designed cellulase mixtures) in the hydrolysis of microcrystalline cellulose was also evaluated.

Oral Presentation 1B-05

Molecular Modeling of the *T. reesei* CBH I Linker

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Cellobiohydralase I (CBH I), from *Trichoderma reesei,* is a thermostable cellulase enzyme that hydrolyzes cellulose in a processive manner, liberating cellobiose residues. CBH I is a multi-domain enzyme consisting of a catalytic domain containing an active site tunnel and a small cellulose binding domain joined to one another by a 27 amino acid residue linker. Although the spatial conformation adopted by the linker peptide is yet to be determined, it is thought to play an important role in the enzymatic hydrolysis of cellulose. Since the protein linker 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 preliminary computer simulations of the glycosylated linker peptide in an aqueous environment in order to gain insight into the role of the linker in cellulose hydrolysis.

Oral Presentation 1B-06

Selection of Endoxylanases for Depolymerization of Glucuronoxylan from Hemicellulose

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The hemicellulose fraction represents as much as 25% of the lignocellulosic biomass of hardwoods and crop residues, and thus represents a significant and underutilized resource for conversion to alternative fuels and biobased products. The predominant polymer in these hemicellulose fractions is 4-O-methylglucuronoxylan (MeGAXn), a linear beta-1,4-D-xylan with regular substitutions by alpha-1,2-linked 4-O-methyl-D-glucuronic acid (MeGA) residues, with a ratio of MeGA to xylose of 1:5 to 1:15, depending on the source. The enzymatic digestion of MeGAXn to fermentable sugars requires the action of endoxylanases that release MeGA-substituted oligsaccharides that can be taken up by bacteria and further metabolized. To select the most effective endoxylanases for digestion of MeGAXn, endoxylanases representing the different glycohydrolase families, GH5, GH10, and GH11, have been compared with respect to generation of products that serve as substrate for further metabolism. All of the endoxylanases appear to initiate a cleavage of the xylan backbone that is directed by the substitution of a MeGA residue. Only members of the GH10 enzymes have been shown to generate aldotetrauronic acid, MeGAX3, that can be taken up and further metabolized. The gene encoding an endoxylanase derived from an aggressive xylanolytic *Paenibacillus sp.* has been cloned and sequenced, revealing a GH10 catalytic module, three cellulose binding modules, and three surface layer homology modules. In this respect, it is similar in structure to GH10 endoxylanases produced by species of *Clostridium* and *Thermoanaerobacterium*. As multi-domain enzymes with the ability bind to cellulose and receptors on the surface of the bacterial cell, the GH10 endoxylanases offer the potential to generate products for vectoral transport and fermentation.

Oral Presentation 2-01

Genomics for Industrial Yeast Physiology and Vice Versa

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The use of various 'omics' for fundamental and applied research on industrial organisms is now well established. Whereas at the basis of metabolic engineering for fuels and chemicals is a rational genetic modification of the microorganism, further optimization of its industrial performance requires fine tuning of the metabolic network to suit the production process via 'omics'. These processes are characterized by hostile environments (high product concentrations, extreme pH values, presence of inhibitory substances) that result in low growth rates. Therefore, in studies on the optimization of the microbial production of antibiotics, organic acids (poly)alcohols, heterologous proteins etc. it is important to apply cultivation techniques that mimic the industrial environment.

In our studies on the above applications, chemostat cultivation is used as a tool for genomics studies aimed at industrial application of microbial physiology because:

1. Different physiological conditions can be studied at the same growth rate
2. Growth rate can be manipulated and kept constant
3. Nutrient-limited growth can be studied with respect to all the medium constituents
4. Also other environmental parameters such as osmotic pressure, pH, temperature, dissolved oxygen concentration, effects of inhibitors can be studied at the same growth rate.
5. Mutants can be selected, under appropriate selective pressure, that show improved performance. This can be used to apply reverse metabolic engineering: via 'omics' the changes in the microorganism may be traced and further, directed genetic engineering may be applied.

In our contribution we will elaborate in the use of chemostat cultivation and DNA arrays for the production of organic acids with engineered *Saccharomyces cerevisiae*. As will be shown, these compounds can be produced in large amounts by the yeast, provided that 'traditional' metabolic engineering is applied in combination with natural selection of mutants.

Oral Presentation 2-02

Evaluation of Recombinant Microorganism Ethanol Fermentation of Corn Fiber Hydrolysate

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A recombinant microorganism, capable of cofermentation of glucose and xylose to ethanol was tested in untreated corn fiber hydrolysate (CFH) by batch, fed-batch and continuous fermentation. The CFH was prepared from corn hulls obtained from the Archer Daniels Midland Company (ADM) and was concentrated to 30-35% solids prior to fermentation. Fermentation conditions were pH, 4.5; temperature, 31° C; 150 rpm agitation with a single Rushton type impeller and no aeration. Cultures were grown in batch phase in media consisting of 10-25% (v/v) corn steep liquid and 20% (v/v) CFH, followed by controlled feeding of undilited CFH concentrate for fed-batch and continuous harvest fermentation at dilution rate 0.005 to 0.03. Batch fermentation consisted of 50% (v/v) CFH and 50% (v/v) ADM corn plant blender mix used for fuel ethanol fermentation. Fermentation broth was treated with amylase to convert any residual complex carbohydrate to fermentable monomer subunits. Ethanol concentration ranged from 35-75 g/L, with yield from available carbohydrate 25-44% and 25-75% available xylose utilized.

Oral Presentation 2-03

Conversion of Non-native Glucans by Strains of *Saccharomyces cerevisiae* Expressing Heterologous Enzymes

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The yeast Saccharomyces cerevisiae has been domesticated for millennia by man for the conversion of fermentable hexoses to ethanol, and rightly so in light of its high ethanol productivity and robustness in industrial fermentation processes. 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 using engineered strains of *S. cerevisiae*.

The enzymatic hydrolysis of starch and cellulose requires the simultaneous production of several hydrolytic enzymes, collectively called amylases and cellulases. Expression and co-expression of genes encoding amylases and cellulases will be reported, along with evaluation of recombinant strains expressing different combinations of these genes in batch fermentations on non-native glucan substrates. Results to be presented include the expression of a chimeric gene encoding the efficient secretion signal of the β-xylanase II of *Trichoderma reesei* fused to the β-glucosidase of *Saccharomycopsis fibuliger* from a multicopy plasmid in the laboratory *S. cerevisiae* strain Y294. This recombinant strain grew equally well on 5 g/L cellobiose or 5 g/L glucose (growth rate of 0.15 h-1 in defined medium). Furthermore, expression of the cellobiohydrolase gene cbh1-4 of *Phanerochaete chrysosporium* from a multicopy plasmid in an autoselective recombinant *S. cerevisiae* strain Y294 yielded about 200 U/L activity on bacterial microcrystalline cellulose as substrate. Both these milestones represent incremental achievements towards the engineering of *S. cerevisiae* for efficient cellulose degradation. Results for amylase expression in *S. cerevisiae* will also be presented, and strategies and prospects for future work will be discussed.

Oral Presentation 2-04

Bioelectrosynthesis of Chemicals and Fuels Using Microbes

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Our lab has recently developed novel bioelectrochemical reactors and electrodes containing different microbial electron mediators. Neutral red, a chemical mimic of menaquinone, and manganese were shown to reduce or oxidize pyridine nucleotides (e.g. NAD/H) and to enable electricity to enter or leave diverse microbes in these bioreactor systems.

Results presented will include demonstration of electrical enhancement of bioconversion-fermentations for: glucose plus CO_2 into succinate by *A. succinogenes*, cellulose into ethanol by *C. thermocellum*; CO_2 into CH_4 by methanogenic granules, and tetralone into tetralol by yeast. In addition, the production of electricity by microbial fuel cells degrading organic wastes was demonstrated using pure or mixed cultures of diverse microbes. The results show that microbes can produce or consume electricity and function as devices that can be electrically manipulated to produce diverse chemicals and fuels.

Oral Presentation 2-05

Second Generation Biocatalysts for Production of Fuels and Chemicals from Biomass

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Simultaneous saccharification and fermentation (SSF) of biomass-derived sugars is a cost-effective process for generating fuel ethanol as well as commodity chemicals. However, the optimal conditions for growth of and fermentation by biocatalysts, such as the ethanologenic *Escherichia coli* KO11, yeast as well as *Lactobacillus*, significantly differ from that of the fungal cellulase needed for depolymerization of cellulose to glucose. The objective of this study is to isolate new biocatalysts whose growth and fermentation conditions match those of the fungal cellulases (50EC and pH 5.0) to reduce the cost of SSF. Towards this goal we have isolated close to 400 bacterial isolates capable of growing at 50EC (and higher) and at pH 5.0 (and lower). All these isolates utilized glucose and xylose, the two major sugars present in biomass and produced L-(+) lactate as the main fermentation product with close to 99% optical purity. Based on a number of physiological characteristics, four isolates were selected for detailed study (isolates 17C5, 36D1, P4-74B and P4-102B). The conversion efficiency of sugars to lactate exceeded 85% and with xylose small amounts of acetate and ethanol were also detected in the broths. Isolates 17C5 and 36D1 also fermented over-limed hemicellulose hydrolysate with a conversion efficiency of close to 90%. In batch fermentations, isolate P4-102B produced about 42 g/L of lactate. Based on 16S rRNA sequence, all the tested isolates belong to a new group of *Bacillus* and the closest relative is *B. coagulans.* The utility of these unique second generation biocatalysts in SSF will be presented and discussed.

Oral Presentation 2-06

Manipulating *Saccharomyces cerevisiae* Redox Metabolism for Improved Xylose Consumption

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In order to ensure an efficient conversion of lignocellulosic hydrolysates into ethanol, it is necessary to improve xylose fermentation. By introduction of the genes encoding xylose reductase and xylitol dehydrogenase from *Pichia stipitis* and over-expression of the endogenous gene for xylulokinase, *Sacchromyces cerevisiae* has been shown to be able to convert xylose to ethanol. However, resulting xylose consumption rates and ethanol yields are sub-optimal. Amongst others, the limitation in efficient xylose conversion has been attributed to limited transport capacity of xylose as well as redox problems in the cell. The two first steps during xylose metabolism utilize NAD(P)H and NAD^+, respectively, and an redox imbalance is therefore created.

Redox equivalents in the form of NADH and NADPH participate in many cellular reactions and utilization and production of these co-factors needs to be balanced in all part of the cell. By introduction of xylose metabolism, two fundamental problems are imposed on the cellular metabolism: firstly a large carbon flux has to be processed via the pentose phosphate pathway, which normally carries a low flux in yeast, and secondly, an imbalance in the formation of the redox factors NADPH and NADH is created. In order to ensure a high flux of xylose towards ethanol it is important to ensure that the cell can balance the net formation of these co-factors, and it is therefore necessary to engineer parts of the metabolism that carry high fluxes. In our work we have mapped the key redox fluxes in the cell and used these to evaluate different strategies to manipulate the redox metabolism in *Saccharomyces cerevisiae* in order to improve the xylose metabolism, and in the presentation results of this work will be discussed.

Oral Presentation 3-01

Development and Application of Computational Fluid Dynamics Models for Scale-Up of Biocommodity Processes

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Large-scale conversion of cellulosic biomass to fuels and chemicals is likely to employ fermentors of 1 million gallons and more. Issues arising in the course of scale-up from smaller laboratory and pilot-scale systems have not been addressed in a systematic fashion, and are an impediment to commercialization of first-of-a-kind technology.

In this presentation, we report the development of a new scale-up tool based on computational fluid dynamics (CFD) using the FLUENT platform. Modification of the Simultaneous Saccharification and Fermentation (SSF) model of South et al. (1995) will be described with the objectives of: a) accommodating intermittent as well as fully continuous feeding, and b) eliminating iterative loops that increase computational time and are thus difficult to incorporate into a CFD context. Results will be presented for the modified kinetics model in combination with a large-scale CFD-based reactor model, and the impact of various factors and operational variables examined. Experimental data for SSF of pretreated mixed hardwood and paper sludge will be presented and considered in the context of the combined kinetics and CFD models.

Oral Presentation 3-02

Identification of Microbial Inhibitory Functional Groups in Corn Stover Hydrolysate by ^{13}C NMR Spectroscopy

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Dilute acid biomass hydrolysates contain biomass degradation products that inhibit cell growth and product formation. Overliming with calcium hydroxide has been shown to be one of the most effective methods for detoxifying the dilute acid hydrolysate for ethanol production. However, the mechanism of overliming is not well understood. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy was used to elucidate the functional groups involved in the overliming reaction. The ^{13}C NMR spectra showed that the overliming process removed aliphatic and aromatic acids, and other aromatic and aliphatic compounds. Most aliphatic acids were completely removed but trace amounts of the aromatic carboxylic acids were still present in the spectrum. The overliming caused about 3 ppm shift in the acetic acid signals in both the aliphatic and carbonyl regions. This is the first time that ^{13}C NMR has been used to elucidate the overliming reaction.

Oral Presentation 3-03

Nisin and Lactic Acid Simultaneous Production from Cheese Industry Byproducts: Optimization of Fermentation Conditions Through Statistically Based Experimental Designs

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Whey and whey permeate, by-products of the cheese industry, retain many nutrients of milk including lactose, soluble proteins, lipids, and mineral salts. The large quantity of nutrients are not fully utilized due to the seemingly inadequate concentration of nutrients and low value of whey products. A biorefinery process which utilizes these cheese industry by-products as substrates to simultaneously produce nisin (a natural food preservative) and lactic acid (raw material for biopolymer production) has been developed. This presentation will include the study of the fermentation process and will emphasize the optimization of fermentation conditions through statistically-based experimental designs.

A 12-run Plackett-Burman design was used to screen the important factors for nisin and lactic acid production by *Lactococcus lactis* subsp. *Lactis* (ATCC 11454) in whey-based mediums. Supplement of nutrients including yeast extract, peptone, $MgSO_4$, and KH_2PO_4, were found to be the important factors affecting nisin and lactic acid formation. In the following step, a 30-run central-composite design was applied to optimize those factors. Second order polynomial models were developed to quantify the relationship between nisin and lactic acid production and the variables. The optimal values of these variables were also determined. Finally, a verification experiment was performed to confirm the optimal values that were predicted by the models. The models fit well with the experiment results.

Oral Presentation 3-04

High-Rate Thermophilic Methane Fermentation on Short-Chain Fatty Acids

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For the purpose of enhancing microbial recovery of energy resources from organic wastes, an R&D work on a novel two-stage anaerobic digestion system has been initiated; the first stage is for liquefaction of solid matters and hydrogen production, and the second stage for high-rate methane production from dissolved organic compounds.

In order to maximize the efficiency of the second stage, experiments on thermophilic methane production in a continuous flow packed-bed reactor were conducted. Defined growth media containing acetic acid and butyric acid as the major carbon sources at a fixed molar ratio were supplied to the reactor at the organic loading rates ranging from 0.2 to 10.0 g-CODcr/L-reactor/day, and the hydraulic retention times (HRTs) from 20 days to 6 hours.

As the results, a stable methane production was observed even at relatively short HRTs of less than 6 hours while maintaining the total CODcr removal ratio of 85% or greater, and almost complete utilization of the short-chain fatty acids.

The project was conducted as a High Efficiency Bioenergy Conversion Project supported by the NEDO (New Energy and Industrial Technology Development Organization of Japan) grant. General outline and future prospect of the project will be also introduced in the presentation.

Oral Presentation 3-05

A Separative Bioreactor: Direct Product Capture and pH Control

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Production of fuels and chemicals from renewable feedstocks are typically based on fermentation or enzymatic processes. Product separations in these biological processes often offer significantly more complexity than traditional chemical separations due to dilute substrate and product streams, relatively little change in molecular structure during conversion, and pH, solvent, and temperature limitations. In nature, however, cells perform these separations extremely well by using an electrochemical pump to export metabolic products (e.g., organic acids) as they are generated. Starting from this concept of cellular metabolic control, we designed a Separative Bioreactor to capture biocatalytically-derived organic acids. The Separative Bioreactor is based on electrodeionization (EDI) – electrochemically-driven transport across ion exchange membranes. As an extension to electrodialysis, EDI utilizes ion exchange resins in the feed channel between the ion exchange membranes to facilitate ion capture and electrical conduction. In the Separative Bioreactor, we molded these ion exchange resins with specific biocapture resins into a porous wafer. The EDI Separative Bioreactor performs extremely efficient electrochemical separations capable of removing dilute organic acids (ppb range) as they are produced. Electrochemical protonation and transport generates the organic acid, not its salt, in the product stream, and controls or maintains the pH of the reaction compartment near the optimal range without buffering or neutralization. In addition, the reactions are conducted by feeding only sugar without addition of buffers, salts, or other nutrients. We have demonstrated the feasibility of the Separative Bioreactor with both enzymatic systems (specifically engineered to coordinate to the biocapture resins) and microbial systems at reasonably high productivities. The biocapture resin technology enables *in situ* removal of degraded enzyme an

Oral Presentation 3-06

Optimization of Xylose Fermentation in Spent Sulfite Liquor by *Saccharomyces cerevisiae* 259ST

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Spent sulfite pulping liquor (SSL) is a high-organic content byproduct of acid bisulfite pulp manufacture which is fermented to make industrial ethanol. Tembec Inc., Temiscaming Quebec, produces 14 million litres per year of industrial alcohol by fermenting hexose sugars in SSL. Challenges associated with fermenting SSL are similar to those for acid hydrolysates of other lignocellulosic substrates, such as forestry and agricultural residues, and include elevated osmotic strength, low sugar content (3-4%), inhibitors, and a large proportion of pentose sugars (up to 60% in hardwood SSL). To date only robust industrial strains of Saccharomyces cerevisiae have been applied successfully for SSL fermentation. These strains are unable to ferment the pentose fraction of the substrate.

In this work xylose fermentation in hardwood and softwood SSL by a pentose fermenting recombinant yeast strain (*S. cerevisiae* 259ST) was optimized using shake flask experiments. The effect of a number of process variables (including pH, temperature, aeration rate, osmotic strength, yeast concentration, nutrient amendment, sugar concentration, and SSL pretreatment) were examined by partial factorial design experiments. Xylose was fermented in undiluted SSL (22 wt% solids) if the initial pH was 6 or greater, otherwise dilution of the SSL and/or nutrient amendment was required. Large initial viable yeast concentrations (10 g/L) gave the best results. Under optimum conditions, up to 80% of the xylose was converted to ethanol.

Oral Presentation 3-07

Development of a Fermentation-Based Process for 1,3-Propanediol: Highlights of a Successful Path from Corn to Textile Fiber

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DuPont and its collaboration partner Genencor have undertaken an extensive effort over the past seven years to develop a biocatalyst which would enable a fermentation-based process for 1,3-propanediol (PDO). PDO is a key ingredient in Sorona™ DuPont's newest advanced polymer platform. In addition to benefits of softness, stretch recovery, vibrant color, and stain resistance, textiles made with Sorona™ produced from fermentation-based PDO will have the dimension of a naturally sourced material.

Since 2000, DuPont and Tate & Lyle have been jointly developing the commercial manufacturing process for fermentation-based PDO. The partnership meshes DuPont's expertise in biocatalyst engineering and polymer processes with Tate & Lyle's expertise in commercial fermentation and carbohydrate processing. While the collaboration team is geographically diverse, a dedicated pilot-plant facility at Tate & Lyle's Decatur, Illinois site has served the project focal point.

Reflecting on accomplishments in process development and piloting, this presentation will provide an overview of the fermentation-based PDO program and highlight key success factors. Focus will be on technical and organizational issues of importance for development of routes to industrial polymers and fibers via fermentation of renewable carbohydrate feedstocks.

Oral Presentation 4-01

Origins and Changes in the Symposium Series on Biotechnology for Fuels and Chemicals

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More than 25 years ago, a group of research engineers and applied scientists with interest in energy applications got together to plan for a symposium series. It was decided for the first symposium to specifically target energy production and conservation. The Department of Energy (DOE) indicated an interest in supporting such a symposium series and the Bioprocessing Research Group in the Oak Ridge National Laboratory (ORNL) agreed to help organize the effort. An appropriate location was desired that would allow 150 to 300 participants to have uninterrupted and intense interactions on a scientific, technical, and social basis.

The first meeting entitled "Biotechnology in Energy Production and Conservation" was held in 1978 at Gatlinburg, TN. This same location and title was used for the first four annual meetings; however, it became obvious that the areas of fuels and chemicals were of most interest. Therefore, the fifth and succeeding symposia were entitled "Biotechnology for Fuels and Chemicals."

There was wide acceptance of these meetings with participants from the government, academia, and the commercial sector. Over 25 different nations were represented. Sponsorship and organization expanded to include several companies and other DOE laboratories. At that point it was decided to share the hosting duties between ORNL and the Solar Energy Research Institute, with the annual meetings alternating between the mountain areas of Tennessee and Colorado.

Oral Presentation 5-01

Opportunities in the Biobased Products Industry

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Approximately 200 billion pounds of organic chemicals, lubricants, and greases are produced annually in the U.S. About 12 billion pounds are currently produced using domestic biomass as the feedstock. The rest is produced from petroleum. The development of new bioproducts has the potential to displace our use of petroleum resources, reduce our dependence on imported oil, and improve energy security. Research is ongoing to develop new products derived from lignocellulosic material, oils/lipids, and protein. Advances in biotechnology are leading to new and improved fermentation organisms as well as the ability to modify renewable feedstocks for higher contents of desired components or the addition of metabolic pathways to plants to produce desired compounds in their tissue.

Lowered production costs of biobased chemicals such as lactic acid and succinic acid are further enabling their use as chemical intermediates and creating opportunities for the displacement of petrochemicals. A more recent development, 3-hydroxypropionic acid, also has the potential to address high volume chemical markets. In oilseeds, researchers are pursuing fatty acid compositions that are more favorable to applications such as lubricants and polymers. The development of plants as factories is being led by polyhydroxyalkanoates.

There is substantial opportunity for growth in the biobased industry. In an effort to help illuminate the bright future for biobased products, this report reviews some of the emerging and potential biobased products. Estimated market penetration of the products by 2020 is included.

Oral Presentation 5-02

DuPont Sorona® 3GT – First of a Family from Bio-Based Materials

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Since the 1940's and the discovery of macromolecules, DuPont scientists have been aware of the unique properties of a family of polyesters based on the 3-carbon glycol (1,3 propanediol). The most common of these polymers, the analog of PET known as "3GT", has almost 3 times the stretch-recovery of nylon, has softer aesthetics, and is dyeable without chemical carrier at the boil. However, only recently were commercial sources of the glycol available. This talk will highlight the history of this new family of polymers and how they can be used in a wide variety of applications. Most significantly, the talk will explain how the glycol will be made using a "miracle of science" from DuPont: a bio-based process that uses a recombinant microorganism and renewable feedstocks rather than a traditional petrochemical process.

Oral Presentation 5-03

3-hydroxypropionic Acid–A New Intermediate Platform

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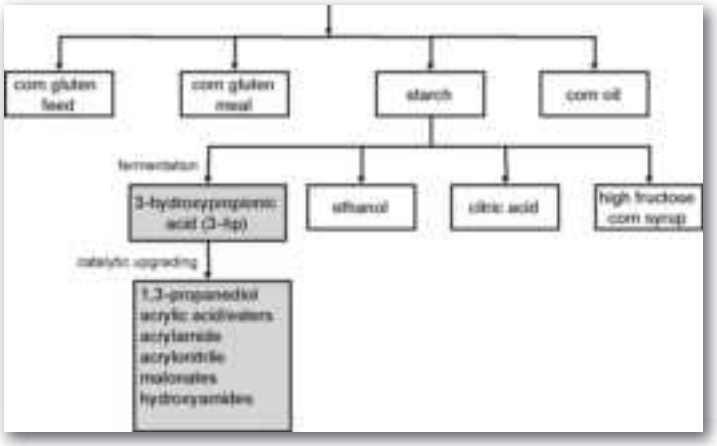
To match the scale, flexibility and efficiency of the petrochemical industry, it will be necessary for the biorefining industry to develop a set of versatile chemical building blocks, or platform intermediates, from which a wide range of products can be derived. Such platform intermediates can be competitively produced by fermentation; the two most well know examples being lactic (2-hydroxypropionic) acid and succinic (butanedioic) acid. Cargill is developing a third important building block, 3-hydroxypropionic acid (3-hp), a platform intermediate that can be produced at a theoretical yield of 100% from glucose. By integrating fermentation with chemical processing, this novel intermediate can then be cost-effectively used to make other commercially valuable chemicals such as 1,3-propanediol, acrylic acid, malonic acid, and acrylamide.



Recently, Cargill has developed the scientific foundation for the microbial conversion of glucose to 3-hp. The key genes have been cloned and sequenced and several microbial strains have been constructed and shown to produce 3-hp. The basic conversions of 3-hp to industrial chemicals have also been demonstrated. The scope of this presentation is to introduce the technical and commercial opportunities present in this development program.

Oral Presentation 5-04

Biobased Succinic Acid as a Building Block for Fuels and Chemicals

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The progress of succinic acid development as a building block for fuels and chemicals will be described. The development of the fermentation technology by the USDOE will reviewed as it relates to current activities for commercialization. A critical element of succinic acid's entry into the market is the expanded potential for new products. Succinic acid's use in solvents, deicers, and water treatment chemicals hold the promise for a wide range of applications that can lead to process economy. Data on the performance of succinic acid and derivatives in these new applications will be presented.

Oral Presentation 5-05

PHAs–A Versatile Family of Biobased Performance Polymers

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Metabolix has applied cutting edge bioengineering to develop economic methods for sustainably producing a wide range of biologically derived materials, polyhydroxyalkanoates (PHAs). The company's near term focus is on commercializing these products based on fermentation using plant-derived sugars and oils with longer-term research directed at direct production in crop plants. Metabolix's patented developments forge a significant new link between large-scale, low-cost, sustainable agricultural production and the existing polymer processing and chemical industries.

PHA biopolyesters range in properties from strong, moldable thermoplastics to highly elastic materials to soft, tacky compositions. They can be made as resins or as aqueous dispersions with excellent film-forming characteristics. PHAs are biodegradable, and in some cases extend the performance envelopes achievable with conventional plastics. PHAs offer a sustainable and environmentally friendly alternative to petroleum-derived synthetic materials.

Oral Presentation 5-06

Novel Thermochemical Pathways for Converting Glutamic Acid to Value Added Products

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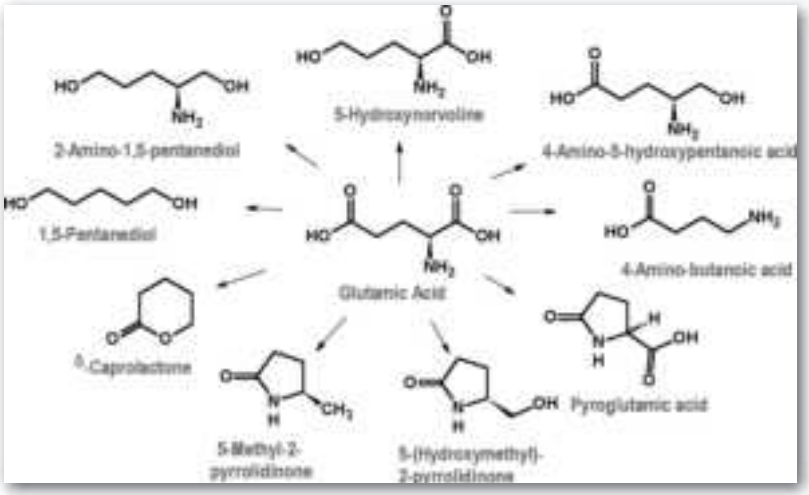
Technology to convert biomass to chemical building blocks provides an opportunity to displace fossil fuels and increase the economic viability of bio-refineries. Coupling fermentation capability with aqueous phase catalysis provides novel routes to monomers and chemicals, including those not accessible from petrochemical routes. In this presentation we will discuss novel pathways to C-4 and C-5 compounds that utilize aqueous phase catalysis of glutamic acid, a fermentation product of glucose.

Glutamic acid provides a platform to numerous compounds through thermal chemical approaches including, hydrogentation, cyclyization, decarboxylation and deamination (see Figure 1). Hydrogenation of amino acids also provides access into chiral compounds with high enantio-purity.



This presentation will detail thermochemical processes that we have developed leading to valuable chemical intermediates from glutamic acid, including compounds not shown in Figure 1. In addition, ^{13}C NMR and MALDI Mass spec data will be provided to give mechanistic picture of the reactions. The results show that hydrogenation of glutamic acid has unique characteristics from other amino acids and that paradigms in the literature do not hold up for this reaction.

Oral Presentation 6A-01

Understanding Factors that Limit Enzymatic Hydrolysis of Biomass: Characterization of Pretreated Corn Stover

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Pretreatment of lignocellulosic biomass is necessary to obtain high sugar yields by enzyme catalysis. However, the fundamental characteristics of biomass that limit its enzymatic conversion are not clearly understood. A better fundamental understanding of these factors would help improve pretreatment/hydrolysis systems. Toward this end of improved fundamental understanding, leading biomass pretreatment techniques are being studied in an integrated multi-university research project funded by the U. S. Department of Agriculture's Initiative for Future Agriculture and Food Systems (IFAFS). As part of this joint research effort, Michigan State University is using spectroscopy and other methods to characterize corn stover pretreated by a variety of pretreatment approaches including aqueous ammonia recycle percolation (ARP), uncatalyzed hydrolysis, dilute acid hydrolysis, controlled pH, lime and ammonia fiber explosion (AFEX).

Spectroscopic characterization of both untreated and treated material is being performed in order to determine changes in the biomass and the effects of pretreatment on crystallinity, lignin content, selected chemical bonds and depolymerization of hemicellulose and lignin. The methods used are X-Ray diffraction for determination of cellulose crystallinity (CrI); diffusive reflectance infrared (DRIFT) for changes in C-C and C-O bonds; and fluorescence to determine lignin content. Raman spectroscopy is also being evaluated to determine cellulose crystallinity. Changes in spectral characteristics and crystallinity are statistically correlated with enzymatic hydrolysis results to identify and better understand the fundamental features of biomass that govern its enzymatic conversion to monomeric sugars.

Oral Presentation 6A-02

Comparison of SHF and SSF of Two-step Steam Pretreated Softwood for Ethanol Production

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In two previous studies two-step steam pretreatment of SO_2 and H_2SO_4 impregnated softwood was investigated and the optimal conditions selected. In the present study the yield of sugar and ethanol was determined in a process development unit, where the pretreatment was performed in a 10-L reactor and the simultaneous saccharification and fermentation (SSF) and enzymatic hydrolysis were performed in 30-L reactors. The effect of the larger scale was investigated.

Two pretreatment combinations were studied. In one study the first pretreatment step was performed at 180ºC for 10 min with 0.5% H_2SO_4 and the second step at 210ºC for 2 min with 1% H_2SO_4. In a second study, SO_2 was used for impregnation and the first step was performed at 190ºC for 2 min followed by a second step at 210ºC and 5 min. The concentration was 3% of SO_2 in both steps.

Enzymatic hydrolysis and SSF of the whole slurry after the second pretreatment step were performed to determine the yield of sugars and ethanol. The liquid after the first pretreatment step was also fermented. The overall yield for the four process configurations was evaluated to determine the best process alternative. The results from this study will be presented.

Oral Presentation 6A-3

Can We Produce an "Ideal" Substrate from Softwood for Enzymatic Hydrolysis?

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Lignocellosic substrates derived from softwoods are known to be much more recalcitrant to enzyme hydrolysis than those obtained from agricultural residues and hardwoods. Part of the reason for this recalcitrance is the high lignin content and high condensed lignin ratio found in pretreated softwoods. Most of the pretreatment methods currently employed are not suitable for use with softwood substrates. Our research has focused on developing effective pretreatment methods to fractionate softwood materials in order to enhance their digestibility to enzyme hydrolysis. Several pretreatment methods, including steam explosion and organosolv cooking, will be discussed in this paper, for their potential to break down softwood substrates and to recover cellulosic materials. The characteristics of different substrates at fiber/fibril, microfibril and molecular levels were assessed. The efficiencies of enzyme hydrolysis on these substrates were also determined. The correlation between the physio-chemical properties of different substrates and their hydrolysability were investigated. The cellulosic matrix produced from organosolv pretreatment has shown a great susceptibility to hydrolysis by cellulase enzymes. The properties of this type of substrate were elucidated. A strategy to produce an "ideal" substrate from softwood will be described.

Oral Presentation 6A-04

Corn Fiber Pretreatment Scale-up and Evaluation in an Industrial Corn to Ethanol Facility

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The design and scale-up of an effective pretreatment process has been tested in a commercial fuel ethanol plant using commercial scale equipment. This pretreatment process requires multiple steps and unit operations. First the substrate feed enters a mixing tank where the solids (corn fiber at 60% moisture) and liquid (stillage) are combined. The resulting slurry is heated through a combination of stem injection and heat exchange. The heat exchanger transfers heat from the fiber/stover stream leaving the pretreatment reactor to the fiber/stover entering the pretreatment reactor, and the second injection served to attain the final operating temperature 160°C. The hot fiber/stover stream entered a plug-flow reactor (snake coil) and was held for less than a half hour. This was sufficient to dissolve about 60% of the dry weight of the corn fiber. The resulting oligosaccharides gave both hexoses and pentoses upon enzyme hydrolysis. Subsequent fermentation trials showed that the hydrolysate was fermentable without any obvious inhibition. This paper reports physical and compositional changes of the fiber as a consequence of the pretreatment, as well as material and energy balances for the process. A model that relates pretreatment conditions to changes in the fiber structure, composition, and hydrolysis upon subsequent addition of enzyme is described. A first estimate of the economics for the process will also be discussed.

Oral Presentation 6A-05

Potential to Improve Dry Mill Economics by Increasing Ethanol Yield from Corn Fiber Residue

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Annual production of fuel ethanol is expected to reach five billion gallons by the year 2012. Currently two methods are used to produce fuel ethanol – wet milling or dry milling of corn. The dry milling process is becoming more common due to the lower capital costs required to build and operate these plants. One pathway to increasing efficiency and yield for the dry mill process is to remove the non-fermentable portion of the corn prior to fermentation, thereby increasing the fermentation capacity. The "Quick Germ-Quick Fiber Process" developed at the Univ. of Illinois separates the corn germ and fiber prior to fermentation by soaking the kernels in hot water followed by germ and fiber milling. The residual corn fiber can be a potential source of co-products or further hydrolyzed to solubilizing carbohydrates for ethanol fermentation.

This paper details the pretreatment and bioconversion of Quick Fiber to ethanol. Quick Fiber was pretreated using a batch Zipperclave reactor in a series of experiments to evaluate the effect of acid concentration, temperature and time on sugar release. The pretreated material was then evaluated for the bioconversion potential using yeast under SSF conditions. Yields of ethanol reached 86% of theoretical for the C6 sugars, in 72 hours. Co-products evaluation from the pretreated material focused on corn oil as the primary product.

Results indicate that Quick Fiber can be pretreated under modest severity and achieve high yields of ethanol based on the release of hydrolyzed sugars. These promising results need to be evaluated further for their effect on process economics for ethanol production.

Oral Presentation 6A-06

Comparative Data from Application of Leading Pretreatment Technologies to Corn Stover

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Biological processing of cellulosic biomass to fuels and chemicals would open up major new agricultural markets and provide powerful societal benefits, but pretreatment operations essential to high yields have a major impact on costs. Leading pretreatment options of ammonia explosion, aqueous ammonia recycle, controlled pH, dilute acid, flowthrough, lime, and autocatalyzed hydrolysis are being applied to a common source of corn stover to facilitate technology selection. Material balances will be summarized based on compositions of the fluid and solid streams from each pretreatment when characterized by shared analytical methods that participants monitor to insure consistency. In addition, comparative data will be presented on the digestibility of cellulose in the pretreated solids using a controlled source of cellulase, and some initial information will be reported on the fermentability of the liquid fractions. Preliminary estimates of the cost of making a common product, ethanol, based on a single process model will then be summarized. Some important performance and cost similarities and differences observed at this stage in the project will be presented, and sensitivity analyses will identify possible opportunities to improve each system. In addition, promising directions for developing advanced pretreatments will be postulated based on the insight gained from these results.

Oral Presentation 6B-01

Improving Feedstocks for Energy Production Through the Acquisition of Complete Crop Gene Sequence by Genethresher™ Methylation Filtering Technology

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Crops, which have undergone thousands of years of domestication, have been improved by breeders to be ideal sources of food for humans and domestic animals. Presently, these same crops are now being utilized for the production of bio-fuels and a range of bio-based products. Breeding efforts have not yet targeted traits that enhance yield in these new bio-energy applications. Genomics technology now promises to rapidly develop and bring to market new "energy traits" in major crops like corn and sorghum, improving feed stocks and greatly reducing the cost of bio-energy production. To maximize the tools of genomics, possessing a complete list of the genes within a genome of interest is essential. This is quite difficult in crop genomes as they are large and complex. For example the corn genome is roughly the size of the human genome while the wheat genome is five times larger, and these large genome sizes place the cost of complete genome sequencing out of reach.

Orion Genomics™ has developed a proprietary technique that rapidly reduces the time and cost of discovering all of the genes in a plant genome of interest. The technique takes advantage of a chemical difference between repetitive junk DNA, which is methylated and comprises ~90% of the crop genome, and genes, which remain unmethylated and occupy only 10% of the crop genome. GeneThresher™ technology takes advantage of these DNA methylation differences by genetically filtering out methylated DNA and concentrating unmethylated genes into a small fraction, which can then be sequenced. A ten fold savings in cost and time can be achieved.

GeneThresher™ technology is now being employed to sequence five plant genomes including sorghum and corn, and the approach has been demonstrated in all of the major branches of the plant kingdom representing some 500 million years of divergence. Gene enrichment was achieved in all plants tested suggesting that the GeneThresher™ approach will be effective across the entire plant kingdom. Orion Genomics is leading a consortium of SolviGen of St. Louis and NC Plus, funded by the Department of Energy, to employ GeneThresher™ technology in combination with a comprehensive breeding effort to bring to market new varieties of sorghum enhanced for optimal production of bio-energy.

Oral Presentation 6B-02

Directed Molecular Evolution as a Tool to Manipulate Plant Metabolism for Biomass Conversion

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Directed molecular evolution is an in vitro process that mimics plant and animal breeding. It utilizes recursive mutagenesis, recombination, and selection of DNA sequences to engineer proteins with specific characteristics. Typical goals of such projects include evolution of enzymes with improved kinetic properties, evolution of enzymes with altered substrate specificities, evolution of enzymes designed to operate in specific environments, and evolution of enzymes to produce novel products. At Verdia, we have exploited directed molecular evolution to alter plant phenotypes for the benefit of agriculture.

By developing transgenes with increased efficacy, we are endeavoring to improve the economics of using plants to provide feedstocks for fuels and chemicals. Transgenic approaches can impact the production of industrial compounds in plants at two different levels:

- Productivity/production costs – The use of directed molecular evolution may increase productivity and/or reduce costs on existing processes. This translates into decreased cost of goods and increases the practicality of using plant-based feedstocks.
- Creation of novel products/properties – The use of directed molecular evolution could result in the creation of novel products or novel properties in existing products. This translates into the creation of new opportunities for plant-derived materials.

We will present examples of projects demonstrating both of these approaches to improving the production of industrial materials from plants.

Oral Presentation 6B-03

Discovery and Evolution of Enzymes for Modification of Oil Composition in Plants

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Diversa has developed high throughput methods to discover and evolve enzymes for oil biosynthesis and oil processing using a suite of microbial systems. Through the application of these methods, numerous novel enzymes have been discovered with characteristics (activity, specificity, stability, etc.) that make them especially suitable for a variety of oleochemical applications. Currently many of these enzymes are being developed as catalysts for the processing of oils from a variety of sources. Instead of processing oil in a post-harvest reaction, the desired oil can also be produced directly in a plant with a genetically modified oil biosynthesis pathway. Expressing new enzymes in oilseed crops has resulted in transgenic plants with dramatically altered oil composition. Using Diversa's discovery and evolution technologies, mutants of existing enzymes or newly discovered enzymes will be developed for expression in transgenic plants. We are interested in using this approach to generate oilseed crops that produce oil for use as biofuels and biolubricants and to serve as improved feedstocks for post-harvest oil processing applications.

Oral Presentation 6B-04

Transfer of Microbial Polyhydroxybutyrate (PHB) and Cellulase Genes to Maize for Production of Biodegradable Plastic, Fermentable Sugar and Other Chemicals

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Over the last decade, there have been many suggestions to produce biobased industrial chemicals and biofuels in recombinant maize. Model plants have been successfully used and relatively high percentage of heterologous proteins (up to 26% total soluble proteins) has been produced in *Arabidopsis*. Sticklen's laboratory has been intensively involved in system development for an easy and relatively genotype-independent maize transformation. This team expressed three different full-length polyhydroxybutyrate (*phb*) genes in maize while directing the PHB enzymes to maize chloroplasts. Recombinant maize plants accumulated the pHB enzymes inside their chloroplasts.

Also, our team designed and constructed a series of plasmids containing a codon-optimized *Trichoderma reesi cbh1* and wild-type *Acidothermus cellulolyticus e1* genes regulated by rice *rbcS* promoter, and produced over 1,000 maize shootlets. In our plasmid design, we considered the targeting of the enzymes into apoplast, chloroplast or keeping the enzymes for comparison in cytosol. The results of our research on *phb, cbh1* and *e1* gene transfer to maize will be presented at the symposium.

Oral Presentation 6B-05

Near-Infrared Spectroscopy as a Genetic Screening Tool for Corn Stover Cell Wall Chemistry

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Terrestrial plant cell walls are a major sink for photosynthetic carbon fixation, and comprise the feedstock material for lignocellulose conversion processes. Cell walls are the product of the action of many gene products and it is estimated that 15% of plant genes may function in the construction, maintenance and modification of plant cell walls. Yet despite the sequencing of the Arabidopsis and rice genomes the vast majority of these genes have yet to be identified. This project will help identify functions for genes involved in cell wall biogenesis.

Near-infrared (NIR; 350-2500 nm) reflectance spectroscopy was employed as a genetic screening tool to identify individual plants with unusual cell wall composition. Seed for genetically segregating transposon insertion lines were obtained from the Cold Spring Harbor Laboratory maize Mu (Robertson's mutator) collection and grown in Colorado. Spectral data were collected from milled bulk corn stover, detached dried leaf blade segments, and intact live leaves. Multivariate statistical methods were used to process spectral data and identify unusual individuals in the population. The availability of a calibrated PLS1 model for corn stover chemical composition has enabled unambiguous identification of phenotypic patterns consistent with expected 3:1 genetic segregation ratios in individual mutant families.

Oral Presentation 6B-06

Expression of UDP-glucose Dehydrogenase Reduces Cell-wall Polysaccharide Concentration and Increases Xylose Content in Alfalfa Stems

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In plants over 100 genes are involved in cell-wall biosynthesis. Several genes encoding enzymes in the pathways for production of the monosaccharide constituents of the cell-wall matrix have recently been cloned, paving the way for manipulating polysaccharide composition and concentration. Pectin is a complex polysaccharide consisting of galacturonic acid, galactose, arabinose, and rhamnose. With the goal of increasing the amount of pectin in alfalfa stems, we produced transgenic alfalfa plants expressing a soybean UDP-glucose dehydrogenase cDNA under the control of two promoters active in vascular tissues. In initial greenhouse experiments, enzyme activity in transgenic lines was up to 7-fold greater than in untransformed plants; however, field-grown transgenic plants had only up to 1.9-fold more activity than the control. Cell-wall polysaccharide content was lower and Klason lignin content was higher in transgenics compared to the untransformed control. No significant increase in uronic acids was observed in any line. Two neutral sugars, xylose and arabinose, which are downstream of uronic acid synthesis increased 7 to 24% in most transgenic lines compared to the control and mannose concentration decreased slightly in most lines. Increasing pectin content may require over-expression of more than one gene or redirection of glucose from cellulose synthesis.

Poster Abstracts for Session 1A

Feedstock Supply, Logistics, Processing, and Composition

Poster Presentation 1A-07

Perspectives for Bioethanol Production in the Netherlands: Feedstock Selection and Pretreatment Options

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The European market in biofuels is expected to increase considerably in the near future. Current EU policies stipulate a wider use of renewable transportation fuels within the next decade. In the Netherlands for instance, government policies are taking shape that aim at a 10% substitution of fossil fuels by the year 2020. As a result, there is a rapidly growing interest in the use of alternative feedstocks for ethanol production. As part of a broader technical and economic feasibility study, an assessment was made of potential feedstocks and pretreatment methods for large scale (ligno-)cellulose-to-bioethanol production, based on enzymatic hydrolysis combined with heat and power generation from non-fermentable residues. A wide variety of biomass resources is potentially available for ethanol production in the Netherlands. Whereas a substantial fraction of total biomass resources (12 M tons dry matter yr^{-1}) are generated in agriculture, large quantities of agro-industrial residues are produced in the food processing industry. Furthermore, substantial quantities of biomass are available from the maintenance of public areas (roadways, nature parks, etc), where disposal of biomass often leads to problems. Two candidate-feedstocks-i.e. wheat milling residue and grass from roadsides and parks- were identified for further development for enzymatic hydrolysis since they are readily available and costs are relatively low. In the longer term, the feedstock range for biofuel production is expected to broaden to include short-rotation forestry of willow. In addition to feedstock assessment, a qualitative evaluation of pretreatment methods for enzymatic hydrolysis was performed to identify technologies with good development perspectives. Based on a number of criteria two pretreatment processes, including (1) mild alkaline pretreatment at low temperature and (2) weak acid hydrolysis using CO_2 in pressurized hot water, were identified for further development. These two processes are expected to generate high yields of sugars, cause low formation of inhibitors, and have modest investment costs. Following this feasibility study, substantial industrial interest led to the formation of a consortium from industry and R&D to further develop and commercially implement lignocellulosic-to-bioethanol technology in the Netherlands. Results of the feasibility study will be presented, with relevant data of on-going research.

Poster Presentation 1A-08

Hydrodynamic Separation of Grain and Stover Components in Corn Silage

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An efficient way to harvest and store large volumes of whole-plant corn is by chopping with a forage harvester and ensiling in a bunker silo. Advantages with whole-plant corn silage include the availability of high capacity forage harvesters, no need for grain drying, and good quality preservation at low cost in bunker silos. However, the biomass industry requires separation of grain and stover components. Preliminary tests indicate that hydrodynamic separation of corn grain from stover is feasible because of large differences in buoyancy. Grains fall quickly to the bottom while stover components float on water. Hydrodynamic separation could provide a pre-steeping process for the corn wet milling industry while the separated stover components could move into various streams and be processed into multiple products. The paper presents data on gravity, buoyancy and the ease of separation of ensiled whole-plant corn into different components.

Poster Presentation 1A-09

Harvest and Storage of Wet and Dry Corn Stover as a Biomass Feedstock

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Corn stover is an ideal biomass feedstock but the harvest and storage of this material presents many challenges. Information on the physical properties of stover and the operational and functional analysis of various harvesting methods are important to improve the efficiency and economics of corn stover harvest and storage. Original research results will be presented concerning: (1) constituent yield and drydown rate by fraction under Upper Midwest conditions: (2) drying rate of stover treated with a variety of mechanical conditioning systems after grain harvest; (3) productivity, harvesting efficiency, particle size, bulk density and storage characteristics of stover harvested wet (chopped and bagged; baled and wrapped) and dry (large round bales, large square bales and stacks); and (4) soil contamination as affected by harvesting strategy. Suggestions for improvements in harvesting and storing corn stover biomass will be made based on these research results.

Poster Presentation 1A-10

Storage and Characterization of Cotton Gin Waste for Ethanol Production

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Cotton gin waste is the lignocellulosic residue generated after the ginning of cotton fibers and recovery of cottonseeds. This is a potential feedstock for ethanol production since it is rich in cotton cellulose. Cotton gin waste piles were constructed and studied for six months to determine the influence of storage on the feedstock properties for ethanol production. Temperatures in the core of the piles were as high as 70 oC shortly after precipitation events. Pile volumes decreased by 37.8±4.1% after six months storage. The ash content of the feedstocks ranged from 10 to 21% while the 95% ethanol extractives ranged from 7 to 12%. The acid insoluble materials content was very high and ranged from 21 to 27%. The total carbohydrates content (30-50%)of the feedstock was relatively low compared to woody and herbaceous biomass feedstocks. The feedstock was predominantly glucose with minor amounts of xylose, mannose, galactose and mannose. Most of the glucose derived from the cotton cellulose content of the feedstock and ranged from 24 to 37%. The xylose content ranged from 3 to 6%. The major impact of the storage was the loss of cellulose which ranged from 10 to 30% for the piles. These losses were attributed to microbial degradation.

Poster Presentation 1A-14

Biomechanics of Straw and Corn Stover Stem Separation

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A limiting factor for cellulosic feedstock utilization is the lack of equipment and methods to harvest, store, and fractionate biomass for subsequent conversion processes. Multi-component selective harvesting technologies can directly address economic and environmental issues for bioproducts processing of higher value cellulosic stems, while leaving behind the remaining residue for purposes of soil sustainability. To develop such harvesting systems, it is essential to understand the physical and structural properties of the crop residue to be threshed and separated. Additionally, biomass biomechanical properties that differentiate the stem tissues result from functional gene expression that can be identified, and used to rapidly characterize unique biomass characteristics of different crops and crop genotypes that are critical to harvester system performance. To understand and model crop residue fractionation characteristics for the purpose of developing selective harvest systems, we have developed test equipment and methods to quantify biomechanical, structural, and molecular of straw and corn stover biomass.

Poster Presentation 1A-15

Image-Based Flow Characterization and Measurement for Biomass Separation Technologies

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Large-scale displacement of petroleum is expected to come primarily from low-cost crop residue feedstocks. However, a lack of effective equipment and methods for harvesting, storing, and fractionating crop residues for subsequent conversion processes inhibits their utilization. To address these issues, researchers at the INEEL are developing approaches for implementing the unit operations of a combine harvester into a single pass, multi-component harvester for grain and crop residue. This paper describes an image-based measurement system capable of obtaining accurate numeric characterization data on the harvester biomass separation processes. This visualization tool allows computational models to be linked with experimental data in a 3-D visualization system to computationally test performance of various biomass separation techniques and systems for multi-component harvesting. By coupling advanced spatial sensing technologies (i.e., the imaging system) with computational and visualization technologies, researchers will be able to see the results of experimental and computational studies in the context of the combine physical separation environment. This will enable the innate pattern recognition skills of the expert, bringing to light key relationships that have previously been unknown, and result in breakthroughs for understanding biomass separation system performance.

Poster Presentation 1A-16

Understanding the Role of Lignin Synthesis on the Harvestibility of Higher Value Cereal Crop Residues

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To develop better harvesting equipment, it is necessary to understand the molecular composition of the harvested material and how it interacts with the harvesting equipment. By regulating the lignin synthesis pathway in cereal straw, we can better understand how altered lignin synthesis affects the mechanical properties of crop residues and their interactions with harvesting equipment. The straw stem is higher in cellulose relative to other plant parts, and together with lignin, provides much of the biomechanical strength for the plant. The technology to economically harvest only the stems, the most valuable fraction for bioproducts, is still being developed. Lignin biosynthesis has been altered in other plant species to yield healthy plants with less lignin. Our goal is to understand how lignin biosynthesis and its interaction with cellulose affect the harvestability and processability of higher value stem components. To accomplish this, lignin content will be downregulated by attenuating the activity of cinamoyl CoA reductase (CCR) via genetic transformation with antisense copies of the CCR gene. An analysis of the sequence encoding for the CCR gene in stem tissue will be presented as well as how this information will be used to down-regulate lignin in a tissue specific manner.

Poster Presentation 1A-17

A New Class of Plants for a Biofuel Feedstock Energy Crop

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Directly burnable biomass to be used primarily in steam boilers for power production has been researched and demonstrated in a variety of projects in the US. The biomass typically comes from wood wastes, such as tree trimmings or the byproducts of lumber production, or from a cash crop, grown by farmers. Of this later group, the main emphasis has been utilizing corn stover, or a prairiegrass called switchgrass, or using tree seedlings such as willow. This paper proposes an alternative to these energy crops which consist of several different herbaceous plants with the one consistent property that they annually generate an appreciable bulk of dried-down burnable mass. The fact that they are a set of plants (nine are offered as candidates) gives this energy crop a great deal of flexibility as far as growing conditions and annual harvest timeline. Their predicted yield is impressive and leads to speculation that they can be economically feasible.

This research was funded by the Ohio Biomass Energy Program.

Poster Presentation 1A-18

Modeling Soil Carbon and Nitrogen Dynamics Under Various Cropping Systems: Can Corn Residue Removal be Sustainable?

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Corn residue (stover) offers a potentially low cost and abundant feedstock for lignocellulose biomass conversion to fuels and chemicals. However, the long (and short) term effects of stover removal on soil fertility and other agricultural variables are critical questions. Sustainability considerations strongly militate against residue removal for fuels and chemicals if the long term health of the soil is undermined thereby.

Using agroecosystem models (CENTURY and DAYCENT), we have studied the long (100 year) and short term (10 year) impacts of corn residue removal at various levels for typical Midwestern soils and climatic conditions. We simulate the effects of different cropping systems (no till and conventional till, rotation with alfalfa and soybeans, with and without winter cover crops, etc) on soil carbon and nitrogen dynamics. These computer simulations provide some reason for optimism regarding the effects of corn residue removal on soil organic matter. Even with high rates of residue removal, reasonable sets of cropping practices (particularly under no till conditions) exist that provide increasing long term soil organic matter.

The models are less robust as far as soil nitrogen dynamics are concerned. Nonetheless, use of winter cover crops along with corn residue removal under no till conditions seems to provide for both lower greenhouse gas emissions (N_2O) and less nitrogen leaching. Reduced nitrogen leaching would reduce hypoxic conditions in receiving waters. Harvesting winter cover crops would also generate additional biomass for fuels and chemicals.

Poster Presentation 1A-19

Sustainable Feedstocks Strategy for Cargill Dow Polylactide Polymer

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Cargill Dow's Polylactide (PLA) polymer marks a new frontier in the pursuit of truly sustainable materials suitable for a wide range of applications, including fibers, packaging, films and chemical intermediates. Made today from annually renewable resources – corn dextrose – PLA polymer enjoys a significant environmental performance advantage over petroleum-based materials with which it competes in the marketplace. Life cycle analysis and a long-term commitment to sustainability as measured against the "triple bottom line" of economic, social and environmental improvements is driving Cargill Dow toward further improvements. The company is pursuing a sustainable feedstocks strategy that incorporates three key elements. These elements are: (1) improving value derived from currently feedstocks through process improvements, (2) developing a feedstocks platform based on ligno-cellulosic biomass, and (3) developing market-based mechanisms to both directly connect customer preferences concerning feedstocks (e.g., concerns surrounding genetically modified corn) and organic agriculture, as well as to set the stage for improving the sustainability of agriculture practices at farms supplying feedstocks.

Poster Presentation 1A-20

Compositional Variability Among Corn Stover Samples

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Corn stover (stalks+leaves+cobs) is currently employed in the DOE-funded Biofuels Program as a model feedstock for integration of enzymatic cellulose hydrolysis-based biomass conversion technology. The chemical composition of corn stover is quite variable from lot to lot, and there are also indications that different lots of stover exhibit differential reactivity to dilute acid pretreatment, as practiced at NREL. These observations are also likely to apply to other herbaceous feedstocks. Biomass composition and quality have therefore emerged as important issues in the development of integrated conversion technology.

Since input feedstock chemical composition directly influences process yield, process economics can be dramatically impacted by changes in feedstock quality. Our goal is to determine the range of compositional variation as well as the potential causes of variability in mature corn stover. Over 1000 specimens from the 2001 North American crop were obtained from a wide variety of sources, including various commercial grain trials, experimental plots, and germplasm collection seed renewal plots. Samples therefore include a fairly broad cross-section of the maize germplasm, including commercial hybrids, inbreds, open-pollinated varieties, foreign accessions, primitive landraces, and related species. Samples were also obtained that had been produced under a variety of conditions in order to evaluate the relative extent to which genetic and environmental factors impact stover quality. We report here on the statistical analysis of the composition of over 700 stover specimens measured using a calibrated near-infrared spectroscopic method developed at NREL, including the results of an analysis of variance of this data as a function of genetic and environmental variables. Results show that corn stover composition varies over an unexpectedly large range and that both genetic and environmental factors are important influences on stover composition.

Poster Presentation 1A-21

Wet Storage & Transport—The Past is Prologue

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One pass harvest of grain and stover, wet storage and rail transport to the processor appear to be advantageous, with a delivered cost less than $30/dt while returning $35/acre income to the farmer. This system has been successfully used by the non-wood pulping industry for 50 years. Wet storage, 65 to 80% moisture offers significant advantages over bales:

- 2x dry density
- 10% storage area
- Low, 3%, loss of holocellulose
- Removes 70% of solubles
- Nutrient recycled to fields
- Reduces process ash
- More consistent feedstock
- Higher feedstock quality
- Will not burn
- Fits one-pass harvest

Process economics are improved with a wet, more consistent feedstock with less solubles and ash. If the solubles extracted in storage can recycle nutrients removed in the residue, another advantage is gained over bales. Adapting wet storage and rail transport practices now used for bagasse to stover reduces traffic, increases the collection area and the economic plant size. Traffic is cut by 1/3 for 50% solids feedstock, 1/5 for 70% moisture and 1/2 for bales.

Dried, compact feedstock better fits gasification and co-firing. However, compaction increases delivered cost to $50/dt or more, and densification inhibits wet processing. Pellets need to be 'reconstituted' by soaking in water to shorten digestion time for hydrolysis—the Sugar Platform for production of fuels, chemicals and materials.

Feedstock supply area can be economically expanded by locating additional collection sites for rail shipment. Transport costs 200 to 300 miles from the plant are estimated to be about $10/dt compared to $15/dt or more trucking cost. Increasing plant size to 6 million dt lowers the operating cost by 33% or more. Delivering 12 million dt with rail transport helps close the gap between petroleum refineries averaging over 100,000 barrels per day.

Poster Presentation 1A-22

Modeling of Biomass Supply Logistics

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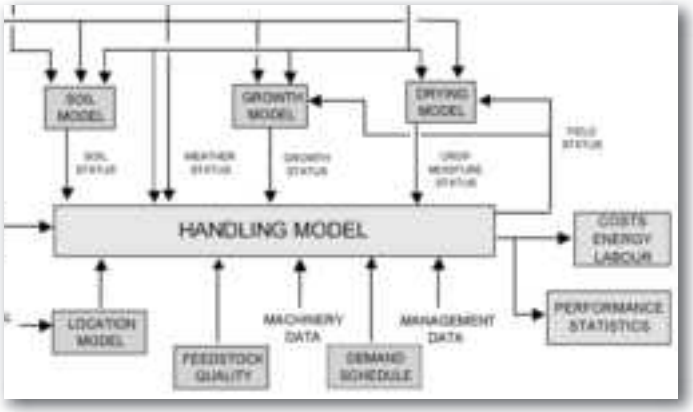

Figure 1. A system model for supply logistics of biomass feedstock

The objective of the research is to develop a modeling tool for analyzing supply of biomass to conversion plants. The model (Figure 1) contains a series of sub models and data that need to be developed and input to the model. The system output is costs ($/ton or $/ha), energy and labor use. The model identifies optimum equipment and infrastructure in dealing with biomass collection and transport to a conversion facility.

The work is being conducted in close collaboration with field operators and systems engineers. Models on plant growth and soil conditions are extracted from literature. Drying data are measured and observed in the field by monitoring mass and moisture content of biomass. Time studies on machinery operations are collected using GPS instruments on mobile equipment. Location model is a multi layer GIS mapping and analysis of roadways and storage locations. Machinery, weather, soil, and management practices form the database. The paper presents the development and validation of the model. Sample input and output from the present model or similar models that have already been applied to biomass collection will be presented.

Poster Presentation 1A-23

The Economics of Energy Crop Production in the United States

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The U.S. agricultural sector could provide a substantial volume of biomass feedstock for the production of biofuels, biobased products, and biopower production. This sector could provide a variety of biomass feedstocks such as grains, oilseeds, sugar, fiber, crop residues, and energy crops. Energy crops have advantages over other biomass feedstocks because of their higher quality, ability to be produced nationally, and homogenous forms. The disadvantage of energy crops production is higher costs relative to crop residues.

More than 100 million acres of excess cropland are available for energy crop production. These cropland areas are idled either by farmers or through different Federal programs. In addition, existing cropland is used for the production of traditional commodities, such as corn, soybeans, wheat, cotton, and alfalfa hay can be replaced by planting energy crops. If the net returns over variable costs per acre of energy crops can favorably compete with the net returns for existing crops. The Federal farm programs have significantly increased the support for traditional crops. The five-year average (1997-2001) net return, including Government payments over variable costs for wheat, corn, soybeans, and cotton, ranged from $67 per acre for wheat to over $150 per acre for cotton. This means that a significant increase in the production of energy crops under the current technology and yield is too costly and economically inefficient. Therefore, in order for the energy crops to be competitive with traditional crops, the only option is to reduce the production costs by introducing new technology to increase yields. Under the current technology, the use of less expensive biomass feedstock, such as crop residues, could have negative implications on soil productivity. In addition, collection and handling issues of crop residues are not solved completely. The prospect for the expansion of energy crops depends on the availability and speed of new technologies. Genetically modified energy crops with higher yield and less production costs per acre could increase the supply of energy crops significantly. Energy crop production could utilize the idled resources, increase farm income, and create jobs in rural areas.

Poster Presentation 1A-24

Detection of Sterol, Stanol, Lipid and Carbohydrate Components in Corn Fiber Products. ^{13}C and ^{1}H NMR and Chromatographic Methods

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Oil extracted from residual corn fiber produced from wet milling contains a variety of high value nutraceutical components. In this work, a variety of authentic sterol and stanol ferulate and coumarate esters and fatty acid sterol esters were synthesized to allow characterization of categories of the high value components by NMR and chromatographic techniques. Diagnostic ^{13}C NMR resonances were identified for characterization of free sterol, free stanol, and sterol/stanol ferulate, coumarate, and fatty acid esters by ^{13}C and ^{1}H NMR spectroscopy in the raw oil, e.g., in the presence of a substantial excess of trigycerides, diglycerides, and other components. Total ferulate content is conveniently measured by observation of aromatic resonances of the ferulate function. The distribution of sterols and stanols in the free form, or as ferulate, fatty acid, or coumarate esters is determined by measuring distinct aliphatic C-O carbons of the sterol ring. Total individual sterol and stanol yields are measured by saponification and ^{13}C NMR and by derivatization-gas chromatographic analysis. The detection of tocopherols and related substances was also examined by NMR and liquid chromatography methods. Results of examination of a series of oil extracts will be presented, along with details of spectroscopic characterization of the sterol, stanol, and tocopherol products.

Poster Presentation 1A-25

Fungal Upgrading of Wheat Straw for Straw-Thermoplastics Production

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Straw thermoplastic composites are not economically competitive with similar wood-based composites because of high feedstock transportation and storage costs, high surface area fines, and poor penetration of the plastic into the lignocellulose matrix. White rot fungi selectively remove hemicellulose and lignin, and could potentially be effective in distributed treatments or while in storage, as an upgrading step for production of improved feedstock for straw composites.

Bench-scale experiments were performed to bracket moisture and degradation time using Pleurotus ostreatus to upgrade unsterile straw stems. Overall degradation was better at higher moisture and inoculum loads. Longer treatments gave progressively smaller gains in xylan removal. Selectivity for xylan degradation, indicating the competitiveness of P. ostreatus, was better at lower moisture, higher inoculum, and shorter duration. Stems were treated with P. ostreatus at 40 mg/g stems and 160% gravimetric moisture for 0, 6, and 12 weeks. Treated and untreated straw stems, high density polyethylene (HDPE), lubricants, and maleated polyethylene blends (MAPE) were varied in composite formulations to evaluate the relative impact of these variables on the extruded product. The composite evaluation included physical and mechanical properties, evaluation of the composite structure, and quantification of the crystallization of HDPE/MAPE.

Poster Presentation 1A-26

Economics of Switchgrass in Iowa: Estimation of Switchgrass Production, Handling, Storage and Transportation Costs and Analysis of Factors Affecting the Costs of Deliverying Switchgrass

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The development of biomass energy is a response to the urgent need to improve the country's energy self-reliance. Switchgrass (*Panicum virgatum*) is one of the promising bioenergy crops currently under study. Its successful development depends on its costs competitiveness and profitability relative to existing fuels and alternative land uses. This paper presents the results of costs estimations for switchgrass production, handling, storage and transportation. It also analyzes the key factors affecting switchgrass production and delivery costs.

Switchgrass total costs range from $58.07 to $79.31 per ton, depending on land quality, storage system, and assuming a 6-ton/acre yield. The major factors affecting switchgrass costs are the expected yield followed by the land charge. Switchgrass costs per ton decrease but at a decreasing rate as yields increase. Converting land from pasture or marginal land produces the lowest production costs.

There is potential for cost reduction especially for harvesting operations, which constitute approximately half of costs in the production year. Potential also exists for decreasing handling, storage and transportation costs that range from 16 up to 36% of the delivered costs. Accounting for environmental externalities in cost estimations will improve the economic competitiveness of switchgrass.

Poster Presentation 1A-27

Examining the Potential Viability of Ethanol Production in California, Using Traditional and Innovative Feedstock Supplies

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California has declared the goal of replacing MTBE in gasoline with ethanol by January, 2004. To achieve that goal, the state will need to import large volumes of ethanol, unless a viable program is developed for producing ethanol within the state's borders.

We examine the firm-level economics and the regional economic impacts of producing ethanol in California. The feedstocks we consider include corn and low-quality fruit that may be available at an affordable price for use in ethanol production. In particular, we consider using low-quality grapes, raisins, and treefruit. California farmers produce large amounts of these crops, each year, and a substantial portion of the produce is sold in secondary markets at reduced prices. We estimate the firm-level costs of using those products to generate ethanol. At current prices, the firm-level economics are not attractive. However, sensitivity analysis with respect to the prices of inputs and outputs reveals the price levels that might motivate firms to consider producing ethanol in California.

The positive regional economic impacts may motivate public sector support of investments in ethanol production capacity, particularly in rural areas where the public benefits of higher rates of economic growth may be substantial. We describe policy recommendations that public officials might consider to encourage investments and production.

Poster Abstracts for Session 1B

Enzyme Catalysis Engineering

Poster Presentation 1B-07

Cellulolytic Mechanism of Thermobifida fusca Cellulases on Bacterial Microcrystalline Cellulose

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Cellulases are key industrial enzymes used to breakdown biomass to fermentable sugars. Understanding the details of the mechanisms governing the process, therefore, is a priority for industries employing these enzymes. To this end, we conducted an extensive study of the mechanism of cellulose hydrolysis by individual and binary mixtures of three *T. fusca* cellulases, Cel5A, Cel6B and Cel9A. The three cellulases represent an endocellulase (Cel5A), an exocellulase (Cel6B) and a processive endocellulase (Cel9A).

A significant contribution of this work is the use of fluorescent labels on the cellulases for direct quantitation of individual components in mixtures. Labeled cellulases retained full activity while allowing concentration measurement accuracies in the range of 7-9%. In the past, bound cellulase concentrations were indirectly determined from free cellulases in the supernatant, either by activity assays (on filter paper and CMC) or HPLC measurements. We have shown that the presence of BMCC does not interfere with fluorescence measurements, therefore, the use of fluorometry now allows direct measurements of bound cellulases. Using this method, we were able to shed some light on the binding behaviors of each component in the mixtures through the course of the hydrolysis reactions. Results showed that where synergism was observed, cooperative binding was also observed. Conversely, where the activity of the mixtures were inhibited, competitive binding was observed. Furthermore, we were able to determine actual binding ratios of the components in reactions for varying input ratios. The use of fluorometry also allowed precise measurements of individual cellulase reactions for building detailed kinetic models based on observed binding and hydrolysis patterns.

Poster Presentation 1B-09

Cellulase Production in *Trichoderma reesei* using Sophorose

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Trichoderma is perhaps the most studied producer of cellulase enzymes.Cellulases are gaining in notoriety with continued attempts to convert biomass to ethanol. Several different inducers have been found for cellulase production in *T. reesei,* but none is a stronger inducer than sophorose. However, the extremely high cost of sophorose has precluded its use for cellulase production. Recently, strides have been made to produce sophorose economically by taking advantage of the β-glucosidase enzyme's trans-glycosylation reactions. Adding small amounts of whole cellulase to high concentrations of glucose produces appreciable amounts of sophorose. This sophorose is enough to overcome glucose repression and induce production of cellulase enzymes when fed to *T. reesei* without purification of the sophorose.

Poster Presentation 1B-10

Cellulase Production by Glucose Grown Cultures of *Trichoderma reesei* RUT C-30 as a Response to Cellulose Feed

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An economic process for the enzymatic hydrolysis of cellulose would allow utilization of cellulosic biomass for the production of easily fermentable low-cost sugars. These sugars could be used for production of fuel ethanol, but also for production of other commodity chemicals. The biologically based route to fuel ethanol production is coupled with the need for a large-scale production of cellulase enzymes. Since the cost of cellulase production currently accounts for a large fraction of the estimated total production costs of bioethanol, a significantly cheaper process for the production of cellulose degrading enzymes is needed.

It will most likely be desirable to obtain cellulase production on different carbon sources – including both polymeric carbohydrates and monosaccharides. The relation between enzyme production and growth profile of the microorganism is the key for designing such processes. In the current work, a careful characterization of growth and cellulase production by the soft root fungus *Trichoderma reesei* - the prime natural cellulase producing organism - was made. *T. reesei* Rut C-30 was grown on Mandels medium with glucose as the carbon and energy source in a laboratory fermenter equipped with on-line gas monitoring. Pulse additions of Solka Floc (purified pine pulp) were made and the response in terms of carbon-dioxide evolution and increased enzyme activity was monitored. The cellulase enzyme production of *T. reesei* is regulated by both glucose repression and induction by intermediate products from the cellulose degradation. At the point of cellulose addition, cellulase activity was relatively low, since enzyme production was not induced. However, there was an immediate and unexpectedly strong carbon dioxide evolution at the point of Solka Floc addition. The time profiles of induction of cellulase activity, cellulose degradation and carbon dioxide evolution are analyzed and discussed in the present work.

Poster Presentation 1B-11

Mono-substituted Phenols Bio-oxidation using *Caldariomyces Fumago* Chloroperoxidase

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Caldariomyces fumago chloroperoxidase (CPO) was used for the degradation of the industrial pollutants 4-chlorophenol (4-CP), 3-chlorophenol (3-CP), 3-methoxyphenol (3-MP), 4-methoxyphenol (4-MP), *para*-cresol (PCR), meta-cresol (MCR), *ortho*-phenylphenol (2-PP), *para*-phenylphenol (4-PP) and *ortho*-aminophenol (OAP).

Reaction conditions were selected from previous experiments carried out for 4-chlorophenol degradation by CPO. As such, reactions at room temperature and pH 6,0, contained 3,5 UI of CPO and phenol and H2O2 concentrations and reagents stoichiometry according to an experimental matrix showing both reagents at concentrations of 0,5; 5,0 and 50,0 mM. Phenol removal was also evaluated in reaction mixtures containing two phenols. Reaction mixtures were analyzed by UV-Vis spectrophotometry and HPLC-RP.

Highest degradation levels, irrespective of the phenol compound studied, occurred in reaction mixtures presenting equimolar phenol and peroxide concentration of 5,0 mM. The enzymatic degradation of OAP, 4-CP, MCR and 3-MP resulted on 95% phenol removal and the formation of soluble yellowish products and a dark precipitate. For 3-CP, PCR and 4-MP degradation levels up to 70% were observed without the formation of the dark precipitate. 4-PP was poorly oxidized (only 10% of phenol removal) and 2-PP was not oxidized by CPO. In reaction mixtures containing two phenols, the presence of 4-CP, MCR or 3-MP increased the removal of 4-PP, 3-CP, PCR and 4-MP around 30%.

Key-words: *Caldariomyces fumago*, chloroperoxidase, bio-oxidation, mono-substituted phenols, insoluble products

Poster Presentation 1B-12

Integration of Computer Modeling and Site-directed Mutagenesis Studies to Improve Cellulase Activity on Cel 9A from *Thermobifida fusca*

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Cellulases are a complex group of enzymes that are fundamental for the degradation of amorphous and crystalline cellulose in lignocellulosic material. Unfortunately cellulases have a low catalytic efficiency on their substrates when compared to similar enzymes like amylases therefore there is a strong interest in improving their activities. *Thermobifida fusca* secretes 6 cellulose degrading enzymes: 2 exo and 3 endocellulases and endo/exocellulase Cel 9A or E4. Cel 9A shows unique properties because of its endo and exocellulase characteristics, strong activity on crystalline cellulose and good synergistic properties. Therefore it is an excellent target for mutagenesis techniques to improve crystalline cellulose degradation.

This poster will describe research conducted to improve E4 catalytic efficiency using rational design and computer modeling. A computer model of E4 was created using the program CHARMM with the PDB structure of E4 plus a cellohexose molecule as a starting model. Initially molecular graphics and energy minimization were used to extend the cellulose chain to 18 glucose residues spanning the catalytic and cellulose binding domains(CBD). The interaction between this cellulose chain and conserved CBD residues was determined in the model and mutations likely to improve the binding properties of the CBD were selected. Site directed mutations were carried out using the pET vector pET26b, *E. coli* DH5-alpha and the QuickChange mutagenesis method. E. coli BL21-DE3 was used for protein production and expression. The purified proteins were assayed for enzymatic activity on filter paper, swollen cellulose, BMCC and CMC. Mutation of the conserved residue F476 to Y476 gave improved activity in all assays (up to 40% in CMC and swollen cellulose).

Poster Presentation 1B-13

Properties of a Recombinant β-Glucosidase from the Polycentric Anaerobic Fungus *Orpinomyces* PC-2 and Its Application for Cellulose Hydrolysis

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A β-glucosidase (BglA, EC 3.2.1.21) gene of the polycentric anaerobic fungus *Orpinomyces* PC-2 was cloned and sequenced. The enzyme containing 657 amino acid residues was homologous to certain animal, plant and bacterial β-glucosidases but lacked significant similarity to those from aerobic fungi. No cellulose (CBD) or protein binding domain (dockerin) was found in BglA. When expressed in *Saccharomyces cerevisiae*, the enzyme was secreted in two forms with mass of about 110 kDa and found also in two forms associated with the yeast. One secreted and two cell-associated forms of BglA were purified and characterized. Analysis of the secreted BglA revealed that a signal peptide of 16 amino acid residues was cleaved off during the secretion. The two cell-associated forms were missing 39 and 48 N-terminal amino acid residues. Treatment of the secreted forms with N-glycosidase F shifted, as revealed on SDS-PAGE, to two sharp bands of 92 and 87 kDa, indicating that BglA secreted by the yeast contained approximately 20% N-glycosylation. K_m and V_{max} values of the glycosylated secreted BglA at 40°C and pH 6.0 were 0.762 mM and 8.20 μmole/min/mg, respectively, with ρ-nitrophenyl glucoside (pNPG) as the substrate and 0.310 mM and 6.45 μmole/min/mg, respectively, for the hydrolysis of cellobiose. Glucose competitively inhibited the hydrolysis of ρNPG with a K_i of 3.6 mM. The enzyme specifically hydrolyzed aryl-β-glucosides, but lacked activity against alkyl-β-glucosides or α-1,4-glucosides. The purified recombinant β-glucosidase significantly enhanced the conversion of cellulosic materials into glucose by *Trichoderma reesei* cellulase preparations, demonstrating its potential in use for biofuel and feed-stock chemical production.

Poster Presentation 1B-14

Development and Application of an Integrated System for Ethanol Monitoring

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An automated Flow Injection Analysis system using enzymes alcohol oxidase and horseradish peroxidase was applied for quantifying ethanol reacting with 4-aminophenazone and phenol. A colorimetric detection method was developed using two different methodologies of analysis, with free and immobilized enzymes. The system with free enzymes permitted analysis of standard ethanol solution in a linear range of 0,05-1 g ethanol/l without external dilution, a sampling frequency of 15 analyses per hour, and relative standard deviation of 3,5%. A new system was designed consisting of a microreactor with 0,91 ml internal volume filled with alcohol oxidase immobilized on glass beads and addition of free peroxidase, adapted in a FIA line, for continued reuse. This integrated biosensor – FIA system is being used for quality control of biofuels, gasohol and hydrated ethanol. The FIA system integrated with the microreactor showed a logarithmic calibration curve in the range of 0.005 a 0.05 g ethanol/l and good results were obtained compared with the ethanol content measured by HPLC and CG standard methods.

Poster Presentation 1B-15

Model Based Soft-Sensor for On-Line Determination of Substrate

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A software sensor for on-line determination of substrate was developed based in a model for fed-batch alcoholic fermentation process and on-line measured signals of ethanol, biomass and feed flow. The ethanol signal was obtained with a colorimetric biosensor, using an enzymatic reaction of ethanol integrated to a FIA system of analysis, which reached the detection range 0-40 g/l. For biomass analysis, an optical sensor, developed in previous works, was also adapted to a continuous sampling line of the bioreactor, which permitted a direct monitoring of the biomass in the range of 0-60 g/l. The volume in the bioreactor could be continuously calculated using the totalization of the feed flow measured signal. Results obtained show that the model used is adequate for the proposed software sensor and determines continuously the substrate concentration with efficiency and security during the fermentation process. This will permit the implementation of control strategies to improve process productivity and yield.

Poster Presentation 1B-16

Screening of Dowex^{R} Anionic Exchange Resins for Invertase Immobilization

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Commercial yeast invertase (Bioinvert^{R}) was immobilized by adsorption on anion exchange resins, collectively named Dowex^{R} (1X8:50-400; 1X4: 50-400 and 1X2:100-400) The optimal binding was obtained in pH 5.5 at 32^{o}C. Among different polystyrene beads, the complex Dowex-1X4-200/ Invertase has shown a yield coupling and an immobilization coefficient equal to 100%. The thermodynamic and kinetic parameters for sucrose hydrolysis for both soluble and insoluble enzyme were evaluated. The complex Dowex/Invertase was stable without any desorption of enzyme from the support during the reaction and having the thermodynamic parameters equal to the soluble form. The stability against pH presented by the soluble invertase was between 4.0 and 5.0, whereas for insoluble one was between 5.0 and 6.0. In both cases, the optimal pH values were found in the range of the stability interval. The K_M and V_{max} for the immobilized invertase were 38.2 mM and 0.0489 U/mL, whereas for the soluble one they were 40.3 mM and 0.0320 U/mL.

Poster Presentation 1B-17

4-Chlorophenol Degradation by Chloroperoxidase: Isolation, Purification and Identification of Oxidized Products

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The enzymatic oxidation of the pollutant 4-chlorophenol was performed at room temperature, in optimized reaction mixtures containing 3.5 UI of chloroperoxidase, 5.0 mM 4-CP, 5.0 mM hydrogen peroxide in potassium phosphate buffer 100 mM pH 6.0.

The soluble yellowish and dark precipitate products were separated by filtration. Soluble products were submitted to ether liquid:liquid extraction followed by concentration. Ether extracted and precipitate fractions were chromatographed in a open column of silica-gel (35-70 mesh ASTM) using a chloroform:methanol gradient. The obtained fractions were analyzed by TLC on aluminum sheets of silica-gel (60 mesh) which were eluted using a chloroform:methanol (7%) mixture. They were also analyzed by HPLC-RP using an isocratic methanol:water (60:40) separation. Structure analysis of main purified fractions were performed by IR spectrometry and GC-mass spectrometry.

Analytical profiles indicated that 4-CP enzymatic oxidation results in the formation of quinones, biphenyl-like compounds and oligomeric ether-like products up to five aromatic rings. They also suggest that chloroperoxidase did not catalysed 4-CP dechlorination. IR spectrometry and GC-mass spectrometry of insoluble products were not consistent, probably due to its complexity degree and size.

Further NMR analysis of all purified products will be performed aiming at to elucidate their complete structures.

Key-words: chloroperoxidase, 4-chlorophenol, phenol enzymatic degradation, quinones, phenol polymeric products

Poster Presentation 1B-18

Characterization and Performance of Immobilized Amylase and Cellulase

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Studies were performed to determine the efficacy of immobilized cellulases and amylases for hydrolysis of cellulose and starch. Enzymes were immobilized onto a siliceous support, and enzyme uptake was determined from the soluble enzyme activity prior to and following immobilization. For amylase, the uptake ranged between 20 and 60%, depending upon the enzyme source and immobilization conditions. With cellulase, the uptake was 7 to 10%.

Immobilized amylase performance was assessed by incubating the immobilized enzyme in 10-30% (100-300g/L) corn flour at 65°C in a batch reactor, and measuring sugar production. Depending upon the substrate loading and immobilization conditions, between 40 and 60% starch conversion could be achieved using immobilized amylase.

The effect of immobilization on the thermal stability of amylase was also assessed. An enzyme sample (either soluble or immobilized) was pre-incubated in a water bath at either 85°C or 105°C, then tested for activity as described above. Soluble amylase lost 60% and 80% of its activity when incubated at 85°C and 105°C, respectively. By comparison, immobilized amylase lost only 20% of its activity after incubation at these temperatures, confirming that the immobilized amylase was significantly more stable.

The performance of immobilized cellulase was evaluated by incubating the enzyme in 1% shredded waste paper at 55°C, and measuring sugar production. Significant hydrolysis of the waste paper was observed in trials with immobilized cellulase, indicating that immobilization does not preclude access to and hydrolysis of insoluble cellulose, in spite of the apparent mass-transfer issues that could be imposed by such an approach.

Poster Presentation 1B-19

Oligosacharides Production by Free and Immobilized Enzyme in Organic Media

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Oligossacharides are a class of compounds used as functional foods. Their importance consists of the incentive of the bifidobacteria production, in a function of what they are denominated prebiotics. The aim of this work was to study the synthesis of fructooligosaccharides from sucrose by free and immobilized inulinase in organic media. The enzyme was produced by fermentation by *Kluyveromyces marxianus var. bulgaricus* ATCC 16045. The synthesis products were identified and measured by ion chromatography. Factorial design and response surface methods were used to optimize the synthesis of fructooligosaccharides. Using butyl acetate as solvent, a 2^{6-2} fractional experimental design was used to study the following variables: pH, temperature, sucrose concentration, enzymatic activity, aqueous-organic solvent and PEG. Four variables were shown to be significant and were studied in a 2^{4-1} factorial design: pH, sucrose concentration, enzymatic activity, aqueous-organic solvent. The production of fructooligosaccharides ranged from 13.9% with free enzyme to 19,5% with immobilized enzyme. The conditions for a production of 13.9 and 19.5%, were temperature 45 and 40°C, pH 5.75 and 4, organic-aqueous solvent ratio of 25/75 and 75/25%, sucrose concentration 55 and 80%, and enzymatic activity of 4 IU/mL, for free and immobilized enzyme, respectively.

Poster Presentation 1B-20

Studies Of Alcohol Oxidase (AO) Expression In YR-1 Strain Of *Mucor circinelloides*

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As a result of improper disposal processes or spills of petroleum or petroleum-derived products, there are many contamination problems in soils produced by hydrocarbon compounds. In this sense, many microorganisms have the capability to metabolize those compounds and this represents a biotechnological importance in the field of bioremediation. 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 some regulatory aspects on alcohol oxidase expression in function of the presence of different carbon sources in the culture media. The results strongly suggest the existence of an inductive mechanism for alcohol oxidase expression in function of the presence of a hydrocarbon as sole carbon source.

First of all, we made experiments using different carbon sources, including glucose at different concentrations, decane, hexadecane and glycerol as sole carbon sources. Also we made some experiments of transference of mycelium grown in glucose to a fresh medium containing glucose at different concentrations, decane, hexadecane or glycerol. In all cases, the expression of AO activity in cell free extracts was detected only when hydrocarbon was present in the culture media.

In cases when glucose was present in any of the different concentrations used, no AO activity could be detected. In glycerol, only a very low AO activity was detected. Also we prove the effect of the presence of different concentrations of glucose to explore if its presence could produce a mechanism of catabolite repression or inhibition on AO activity. In this point, the results suggest that the glucose at any concentration produces catabolite repression of AO expression, but has not an inhibitory mechanism.

Poster Presentation 1B-21

Effect of *Trichoderma* Endoglucanases on Secondary Fiber Properties

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Since the 1970`s pulp and paper industry has been using several biotechnological processes in bio-mechanical pulping, modification of fiber properties, deinking and improving drainage. During paper recycling pulp properties decline. The poor pulp quality could be improved in an environmentally friendly way applying cellulases. Although the enzymatic mechanism is not quite clear it seems that endoglucanases have a main role in the quality improvement. (Endoglucanases randomly attack the amorphous regions of cellulose substrate, yielding mainly higher oligomers.)

Cellulose is not a homogenous material, there are crystalline and less structured, so-called amorphous regions as well. In secondary fibers the fines and fibrils that cause lower rate of drainage consist decisively of amorphous cellulose. The aim of enzymatic treatment is to hydrolyse fines and thus improve the drainage. Since amorphous cellulose is more accessible it is not necessary to hydrolyse with the whole cellulase system, endoglucanases may be effective.

Cellulases from fungal sources have a general structure, including a catalytic domain and a cellulose-binding domain (CBD), which connects into each other via a linker peptide region that is often heavily glycosylated. The role of CBD is not well explored in cellulose hydrolysis whereas in the hydrolysis the catalytic domain must also be adsorbed. Some experiments confirmed the supposition that the role of this domain is to bind to and loosen up crystalline cellulose.

In present work the effect of *Trichoderma reesei* endoglucanase components were compared on the drainage of secondary fiber pulp. In addition the significance of substrate binding domain was investigated in case of EG I. The aim of the enzymatic treatment was to improve drainage by 10-15% based on control without considerable deterioration in strength properties. Commercial (EG III core, EG V core) and own produced (EG I and EG I core) endoglucanases were investigated. The enzymatic treatments were carried out under industrial conditions concerning temperature, pH and reaction time. The drainage of pulp, air permeability and the strength properties (tensile strength, tear strength, burst strength) of the paper were determined.

Poster Presentation 1B-22

Overexpression of Xylanase B of A. Niger in *Pichia pastoris*

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The interest in xylanases has increased due to their potential application in biomass fuel, biobleaching, animal feed and in food industry. Our objective was to overexpress the *Aspergillus niger* xylanase B gene (xynB) in *Pichia pastoris* and to optimize the xylanase expression conditions in recombinant strains. The xynB gene was amplified by PCR method from the genomic DNA of *A. niger* Z11. The xynB cDNA was obtained by a second PCR with a pair of partial complementary primers to delete the intron. It contained a 678 bp open reading frame that encoded a propeptide with 219 amino acid residues. The cDNA fragment for the mature protein containing 188 amino acid residues was fused in frame with the sequence for -factor signal peptide of *P. pastoris* integration vector pPIC9K to construct recombinant vector pPICH6. The *xynB* cDNA in pPICH6 was successfully expressed in *P. pastoris* under the control of AOX1 promoter and terminator. Three recombinant P. pastoris strains, ZH2, ZH3 and ZH7, could overexpress extracellular xylanase B protein (XynB). The recombinant XynB activity produced by ZH3 could reach 1, 600 IU/ml, which was 16-fold higher than that of the original xylanase from *A. niger* Z11. SDS-PAGE results showed that the molecular weight of the recombinant XynB was 21KD, and the recombinant XynB consisted of over 80% in total extracellular proteins. The recombinant XynB showed optimum activity at 50 C, at pH 5.0 and maximum stability at 50 C, these were consistent with the properties of the original xylanase from *A. niger* Z11. The recombinant XynB was effective in hydrolyzing xylan from corn or birch *in vitro*. In conclusion, the xynB gene was overexpressed as an active, extracellular xylanase in *P. pastoris*, and the activity was higher than that of the original xylanase from A. niger Z11.

Key words: xylanaseB *Pichia pastoris* integration expression

Poster Presentation 1B-23

Evaluation of Lipase Production by Solid State Fermentation by *P. simplicissimum* using Soy Cake

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Lipases have been proposed for application in wastewater treatment of food industry. Nevertheless, for such applications low cost enzyme preparations must be available. Lipase production by solid-state fermentation (SSF) may be suitable for this purpose, since SSF offers many advantages over submerged fermentation (SmF), which include high productivity, higher product concentration, simpler equipments and the use of low cost substrates as agroindustry byproducts. In this study the production of lipases by SSF was evaluated, using *Penicillium simplicissimum* and soy cake as substrate. A factorial experimental plan was used to investigate the effect of temperature, water content of the medium and supplementation of the substrate with olive oil. Fermentation temperatures were in the range of 27 to 33 °C, initial water content was between 50 and 70 wt% and the cake was supplemented with up to 4 wt% of commercial olive oil. The lipase production kinetics was followed for each experiment, as well as the water content and pH of the medium. A statistical analysis of the results was performed and used to optimize the experimental conditions to maximize lipase production. The results show that lipase activity was maximum around 48 h of fermentation and that high enzyme activity could be achieved even with non-supplemented substrate.

Poster Presentation 1B-24

Production of Thermostable Pectinases from Thermophilic *Thermoascus aurantiacus* by Soli-State Fermentation of Sugar Cane and Orange Bagasses

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Solid-state fermentation (SSF) is an attractive one because it presents higher productivity per reactor volume, lower capital and operating costs, lower space requirements, simpler equipment and easier downstream processing compared to that of submerged fermentation (SmF). This work present the polygalacturonase production by thermophilic *Thermoascus aurantiacus* by SSF using as substrate a mixture of sugar cane bagasse and orange bagasse (1:1). Fermentative parameters as pH (5.0; 5,5 and 6,0), temperature (50 and 55°C) and substrate moisture (70 and 80%) were evaluated in a factorial assay. The highest production of enzyme was obtained at pH 5.0, substrate moisture 70% and 50°C. The results indicated that is feasible to use agroindustrial residues for polygalacturonase production by SSF under conditions determined.

Poster Presentation 1B-25

Quantification and Reactivity of Cellulose Reducing Ends

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This study is aimed at developing methods for the characterization of CHB-accessible reducing ends on cellulose substrates. These measurements are important because of the exo-acting nature, and in some cases reducing-end specificity, of these enzymes. For CBH I, for example, the measurement of enzyme-accessible reducing ends is likely the most direct measure of substrate concentration. The number of reducing ends per gram representative celluloses was determined using colorimetric assays (Dinitrosalicyclic acid (DNS) and Bicinchoninic acid assay (BCA)) and a radioisotope assay (NaB^3H_4 labeling). Estimates of the number of reducing ends per unit mass cellulose were found to be dependent on the assay system (i.e. the DNS and BCA assays gave strikingly different results). DNS-based values were several-fold higher than those obtained using the BCA assay. Results from the tritium labeling experiments suggest that the colorimetric assays provide an indication of the total reducing ends associated with the substrate. Sodium borohydride treatments, cold or radiolabelled, provide a reasonable approach for estimating numbers of solvent-accessible reducing ends. It appears that 30 to 40% of the reducing ends on a typical cellulose substrate are not solvent accessible, i.e. they are buried in the interior of cellulose structures and thus not available to exo-acting enzymes, at least in the initial phase of cellulose saccharification.

Poster Presentation 1B-26

Production of Bacterial Cellulose Using Hydrolysate Obtained from Paper Recycling Mill Sludge

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Bacterial cellulose (BC) presents significant advantages over plant-derived cellulose with its structural and mechanical properties. However its commercial use is limited by the costs of production. Then, it will be desirable to investigate the effectiveness of using cheaper culture media for BC production. Lignocellulosic materials constitute an attractive feedstock for this purpose because of their availability at low costs and large amounts.

The rapid accumulation of sludge from paper recycling mill effluents derived from the increase of the recycling rates poses an environmental problem and a strong interest is emerging in finding novel value-added uses for this waste. Following process optimisation, we have completely saccharified a single stock of this sludge using a mixture of commercial enzymes, obtaining a liquor consisting of glucose and xylose in absence of biologically inhibitory compounds. The hydrolysate sugars were used for supplementation of suitable culture media of acetic acid bacterium for cellulose production. The bacterial strain used in this work was isolated in our laboratory and has previously demonstrated to be able to produce BC using glucose in the culture medium.

Poster Session 1B-27

Enzymatic Analysis of Biomass-Derived Producer Gas Fermentation

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In an alternative approach to produce liquid fuel and chemicals from biomass, native Oklahoma switchgrass has been gasified in a pilot-scale fluidized bed reactor. The "producer gas" consists of carbon monoxide, carbon dioxide, nitrogen, and hydrogen. Other organic and inorganic species have been found in trace amounts. The gas was fed to a bubble-column bioreactor containing a novel clostridium species (P7). The anaerobe produces ethanol and butanol and its corresponding acids. Since essentially all of the biomass is gasified, this process can potentially utilize more of the carbon for products than competing saccharification/fermentation processes.

To realize the economic benefits, the metabolic stoichiometry must be fully understood and optimized. Ethanol synthesis follows the acetyl-CoA pathway. Three key enzymes of ethanol formation include carbon monoxide dehydrogenase (CODH), formate dehydrogenase (FDH), and hydrogenase.

The activities of these enzymes are dependent upon many factors including pH, redox potential, temperature, media, and inhibiting impurities. It has been observed that P7 ceases to utilize hydrogen, accompanied by a drastic change in pH when fed with producer gas. This points to a shift in metabolism. To this end, enzyme analyses will be presented to aid in assessing the metabolic changes between producer gas and clean bottled gases.

Poster Presentation 1B-28

Asparaginase II Fermentation Kinetics of *Saccharomyces cerevisiae ure2dal80* Mutant: Effect of Nitrogen Source and pH

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Previous results showed that the levels of the periplasmic asparaginase II produced by the nitrogen de-repressed *Saccharomyces cerevisiae ure2dal80* mutant were 4-fold higher when cells were grown in ammonium and 16-fold higher when cells were grown in proline, in comparison to the enzyme levels in the corresponding wild-type cells. However the use of the non-preferred nitrogen source proline is not feasible due to its high cost.

This work studied the kinetics of cell growth, substrate consumption, asparaginase II accumulation and pH change in *Saccharomyces cerevisiae ure2dal80* fermentations containing glucose as carbon source and ammonium, urea or proline as nitrogen sources. All growth media showed a C/N ratio around 30. The effect of pH was also evaluated in a first round of proline fermentations using un-buffered and phosphate buffered medium in the pH range 4.5 to 6.5. Best results were obtained in buffered medium pH 6.5 and this condition was subsequently used in all experiments. It was also observed higher enzyme stability during the fermentation process. Proline and urea fermentations resulted in peak enzyme levels of 269 IU/L and 270 IU/L respectively, which were 2.5-fold higher in comparison to un-buffered initial pH 4.5 fermentations. Using ammonium, a preferred N source, maximum asparaginase II levels were of 157 IU/L. As cell growth and glucose consumption were not affected by differences in pH and nitrogen source, this lower enzyme level could be related to the effect of the GATA factor Nil2p, that repress nitrogen regulated genes, such as *ASP3* that codes for asparaginase II, in ammonium grown cells.

As urea, a low-cost, non-preferred nitrogen source, successfully substituted proline in the asparaginase fermentations, this substrate could be preferentially evaluated for the production of other nitrogen repressed enzymes and metabolites of industrial interest. Asparaginase itself is used in the treatment of acute lymphoblastic leukemia.

Poster Presentation 1B-29

The Effect of Temperature, Pressure and Depressurization Rates on Lipase Activity in $SCCO_2$

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Bioconversion of vegetable oils through the use of enzymes as catalysts in supercritical medium is a matter of great scientific and technological interest nowadays. The possibility of using a much less pollutant fuel when compared to diesel from petroleum has motivated the biotransformation of vegetable oils to result in reduction in environment investments. To conduct such reactions at high pressures, the enzyme behavior in $SCCO_2$ is of primary importance as the loss of enzyme activity may lead to undesirable poor reaction rates and reduction of desired products production. In this context, this work investigates the influence of temperature and pressure on lipase activity in high-pressure CO_2 medium. The experiments were performed in a high-pressure variable-volume view cell, varying the temperature (30-70°C), pressure (70-250bar), using some high-pressure exposure times and adopting several decompression rates. An experimental design with two levels was built so as to identify the main and cross interaction variables effects on the lipase activity loss. Enzyme activity was determined as the initial rates in esterification reactions between lauric acid and propanol. Results show that an increase in exposure time leads to an enhancement of enzyme activity losses while a remarkable activity decay is verified at high decompression rates.

Poster Presentation 1B-30

Immobilized Enzyme Studies in a Micro-Scale Bioreactor

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The focus of the work is to examine the performance of an immobilized enzyme micro-reactor constructed by microfabrication techniques. Enzymes are immobilized on channel walls with cross dimensions on the order of tens to hundreds of micrometers.

Micro-scale bioreactors are constructed as systems of polydimethylsiloxane (PDMS) micro-channels fabricated on silicon wafer molds upon which a three dimensional SU-8 photoresist surface has been cast using lithographic techniques. Reactors employing channels with micro-scale cross dimensions minimize reactant diffusion path lengths to the catalyst-coated walls. The extremely high surface area to volume ratio creates a highly efficient continuous flow reaction system. It is also an excellent material forming the reactor body as it is tough, light, flexible, and relatively inexpensive.

Experimental results will be presented for several immobilized enzyme microreactor configurations and dimensions. The microreactor system has been demonstrated with three enzyme systems—urease, catalase and amyloglucosidase. System performance is presented as a function of operating conditions and microchannel geometry.

Poster Presentation 1B-31

Studies on Lipase Immobilization in Hydrophobic Sol-Gel Matrix

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The technique based on sol-gel approach was used to generate silica matrices by hydrolysis of silane compounds. The present work evaluates the matrix obtained with tetraethoxysilane (TEOS) on the immobilization yield of lipase from Candida rugosa by three methods: physical adsorption, covalent binding and gel entrapment in presence and absence of polyethyeleneglycol (PEG-1450). Silica and their derivatives were fully characterized with regard to particle distribution specific surface area, pore size distribution (B.E.T. method), graffeted yield (TGA) and chemical composition (FTIR). Immobilization yields based on the lipase activity recovered vary from 3.0 to 32.0% and the highest efficiency was attained when lipase was encapsulated in the presence of PEG. Investigations are being carried out to optimize the procedure for enzyme encapsulation and characterize the lipase containing gels for application in vegetable oils modifications.

Poster Presentation 1B-32

Indigo and Indirubin Production by Recombinant Bacterial Flavin-containing Monooxygenase of a Marine Methylotrophic Bacterium, *Methylophaga thalassica* SK-1

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A plasmid clone pBlue that produced blue pigment in *Escherichia coli* was obtained from a DNA library of *Methylophaga thalassica* SK-1. The bacterial pigment produced from an *E. coli* strain harboring the plasmid pBlue consisted of major blue (indigo) and minor pink (indirubin, a structural isomer of indigo) components. The plasmid, pBlue, carried a single flavin-containing monooxygenase (FMO). A deduced amino acid sequence of the open reading frame (ORF) showed similarities to eukaryotic flavin-containing monooxygenase. The ORF contained FAD, NADPH, and FMO identifying motif (FXGXXXHXXX(Y/F)). This suggests indigo production be mediated by the monooxygenase cloned in the plasmid pBlue. An *E. coli* strain containing the plasmid pBlue produced 330 mg of indigo and 35 mg of indirubin per liter. The monooxygenase gene isolated in this study has a potential to be applied in microbial indigo and antileukemia indirubin production.

Poster Presentation 1B-33

CMCase and Xylanase Production by *Thermoascus aurantiacus* in Solid State Fermentation in Different Residues

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Waste and raw material represent and alternative source for the growth of microorganisms aiming the production of biomass and metabolites. The purpose of this work was to study the production of CMCase and xylanase of a Brazilian strain of Thermoascus aurantiacus in solid state fermentation (SSF) using different agricultural residues as substrates and characterize the crude enzymes. The microorganism quickly grew in stationary, simple and of low cost medium and secreted the enzymes extracellularly. The study of the set of extracellular enzymes of the T. arantiacus showed that the fungus is more xilanolitic than cellulolitic. It produced high level of enzymes in corncob, grasses and straw corn. All the enzymes were stable over a broad pH range (3.0-9.0) and temperature (60ºC). The optimum pH and temperature for xylanase and CMCase were 5.0-5.5 and 5.0 and 75ºC, respectively.

Poster Presentation 1B-34

The Effect of pH on the Cellulase Production of *Trichoderma reesei* RUT C30

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Due to environmental considerations during the past decades, biomass originated alternative fuels has gained significant attention. For the near future, ethanol, due to it advantageous physical properties, seems to be a potential substitute of gasoline in internal combustion engines. The obvious choice of raw material, which could support large-scale fuel ethanol production, would be lignocellulosics because of availability. Sensitivity analysis of a lignocellulosic biomass to ethanol process has showed that the key factor influencing the production cost is the overall ethanol yield. One of the process alternatives suggested for the production of ethanol from lignocellulosics is based on the enzymatic saccharification of the cellulose. However, for efficient conversion of the cellulose fraction large enzyme dosage per unit of raw material has to be applied, which due to the high market price of cellulases increases the production cost significantly.

Trichoderma reesei RUT C30 is known to be one of the best hyper-cellulolytic fungi. Several factors, including carbon-source amount and quality, temperature and pH of the cultivation, aeration etc., influence the enzyme production of this strain. It has been indicated in previous studies that the pH and the pH controlling strategy have a great effect on the amount of cellulase produced. In the present study three controlling strategies at different pH levels were examined and compared using various puffers in shake flask cultures. Detailed results will be presented during the conference.

Poster Presentation 1B-35

Production of Galactooligossaccharides by β-galactosidase from *Scopulariopsis sp.*

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A strain of *Scopulariopsis sp* which produced high activity of extracellular β-galactosidase was isolated from soil. This enzyme is used industrially for the hydrolysis of lactose from milk and milk whey for several applications. In high lactose concentrations the β-galactosidase has galactosyltransferase activity, producing a galactooligosaccharides (GOS) mixture. The benefits of galactooligosaccharides ingestion arise from the population of bifidobacteria in the colon that suppress the activity of putrefactive bacteria and reduce the formation of toxic fermentation products. *Scopulariopsis* strains showed good productivity of β-galactosidase when grown on semi-solid fermentation medium. The optimal temperature for galactosyltransferase activity was in the range of 35 to 45ºC and the pH effect was minimal at high lactose concentration; the enzyme concentration for GOS synthesis was 4-6 U/mL. When 40% (w/v) lactose solution (in 0.1M sodium acetate buffer, pH 4.5, 45ºC) was incubated with 6 U/mL of β-galactosidase, the enzyme converted 30% of the lactose in oligosaccharides (224.92 mg/mL of 4' galactosyl-lactose). This product shows potentiality as an food ingredient with high prebiotic value.

Poster Presentation 1B-36

Characterization and Purification of Cyclodextrin Glycosyltransferase (CGTase) Produced by Thermophilic Strain H69-3

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A new alkalophilic thermotolerant strain of *Bacillus sp* H69-3 which produces thermostable cyclodextrin glycosyltransferase (CGTase) was isolated from soil. CGTase production was investigated in submerged fermentation at 45°C, using soluble starch as substrate. The bacterium produced maximum CGTase activity between 48 and 60 hours of fermentation. The temperature and pH for optimum CGTase activity were in range of 70-75°C and at pH 5.5, respectively. The crude enzyme was stable at 55°C by 1 hour and at pH 4.5-11.0 by 24 hour. The culture medium was concentrated by ammonium sulfate and CGTase was purified to homogeneity by using Q-Sepharose chromatography and gel filtration. The cyclodextrin (CD) pattern demonstrated that crude and purified enzymes produced mainly beta-CD.

Poster Presentation 1B-37

Enhanced Production of CGTase by Optimization of Culture Medium Using Response Surface Methodology

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Response surface methodology was employed aiming to investigate the combined effect of concentrations of soluble starch, peptone, yeast extract, and the initial pH of culture medium on cyclodextrin glycosyltransferase (CGTase) production by *Bacillus sp* subgroup *alkalophilus* E16 in shaker flasks. The optimum concentrations of the components determined by a 24 full-factorial central composite design were found 13.4 g/l soluble starch, 4.9 g/l peptone, 5.9 g/l yeast extract and initial pH 10.10. Under the optimized conditions the maximum CGTase activity was 5. 9 U/ml in 48 hours of cultivation. This yield was 65.4% higher than that obtained when the microorganism was cultivated in a not optimized culture medium. Also, it was observed that in 24 hours of cultivation, using the optimized medium, the CGTase production was equal to that obtained in 48 hours of cultivation by using the not optimized medium.

Poster Presentation 1B-38

Heterologous Expression, Purification, and Characterization of a Cellobiohydrolase from *Penicillium funiculosum*

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Ascomycete and Basidiomycete fungi are recognized for their role in the biodegradation and recycling of organic matter in nature. Their ability to digest cellulosic biomass (e.g. leaf litter and wood), and in the case of many Basidiomycetes both cellulose and lignin, is of great interest to the emerging bioenergy industry, since biomass represents an enormous renewable resource for the production of fuels and chemicals. Among the notable genera of industrially important fungi that produce Glycosyl Hydrolase family 7 cellobiohydrolases are Trichoderma and Penicillium. The cellobiohydrolases from this structural family are generally recognized as being the principal enzyme in the construction of engineered component cellulase systems designed for hydrolysis of microcrystalline cellulose. In this study, we report the heterologous expression of an active and stable full-length cellobiohydrolase from *Penicillium funiculosum* in transformed *Aspergillus awamori*. We compare the kinetics and biochemical properties of the recombinant form of the enzyme engineered and expressed using different signal sequences compared to the wild type enzyme.

Poster Presentation 1B-39

Towards the Discovery of New Enzymes Involved in Biomass Degradation: Combination of SSH and Microarray Technologies to Identify *Trichoderma reesei* Biomass-induced Genes

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The concerted action of many enzymes is required for the hydrolysis of cellulosic biomass. However, only a few have been identified and characterized in detail. Mixtures of selected cellulase components have been shown to be inefficient for the conversion of biomass to glucose. This may suggest that additional components are required for complete saccharification of biomass. *Trichoderma reesei* is the best-studied cellulolytic fungus, however its genome has not been sequenced. To identify new *T. reesei* genes involved in biomass conversion, we have employed the high throughput analysis of expression of subtractive cDNA libraries by DNA microarray technology. The cDNA libraries have been generated by suppression subtractive hybridization (SSH), which allows not only the selection of differentially expressed mRNAs, but also the enrichment for rare mRNAs and equalization of cDNA in a pool. Messenger RNA from *T. reesei* cells grown on glucose, cellulose, or acid-pretreated corn stover was isolated and used for construction of the SSH cDNA libraries. Three SSH libraries representing cellulose-induced, corn stover-induced and stover minus cellulose-induced transcripts were constructed. Successful subtraction and normalization was confirmed by Southern blot hybridization of subtracted and unsubtracted cDNA pools and by DNA sequence analysis of randomly selected cDNA clones. Approximately 4000 cDNAs from three SSH libraries were amplified by high throughput rolling circle amplification (RCA) to produce DNA for microarray printing. Microarray hybridization with tester and driver probes identified 708 cDNA clones corresponding to a likely smaller number of up regulated transcripts. To this end we have sequenced more than 200 of these cDNAs and found several new genes likely to be involved in biomass degradation. Thus, the combination of SSH and cDNA microarray technologies has proven to be a powerful tool for discovery of new differentially expressed genes involved in biomass utilization.

Poster Presentation 1B-40

The Effect of Lignin Modifying Enzymes on the Molecular Weight Distribution of Kraft Lignin

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Lignin, second only to cellulose as a source of fixed carbon in the biosphere, is generally considered to be highly resistant to rapid biological degradation. White rot fungi, the primary lignin decomposers, produce various extracellular redox enzymes and electron transfer mediators, which are thought to facilitate degradation of polymeric lignin. Although the enzymes, such as lignin peroxidase, manganese-dependent peroxidase, and laccase are extracellular, confirmation of direct enzyme-lignin interaction is unclear. Enzyme-mediated electron transfer from lignin molecules to oxidized cofactors such as Mn^{+3} and H_2O_2 is thought to be enhanced by the ability of these low molecular weight compounds to penetrate the wood ultrastructure and carry out oxidation of lignin *in situ*, where steric hindrances make direct enzyme interaction unlikely. Laccase in particular has been implicated in the mediation of both depolymerization of high molecular weight lignin and polymerization of lignin and lignin precursors. In this study, we use high-pressure size exclusion chromatography (HPSEC) coupled to multi angle laser light scattering (MALLS) detection to determine the effect of several enzymes and cofactors on the molecular weight distribution of treated kraft lignin

Poster Presentation 1B-41

Secretion Signal Swapping for Improved β-glucosidase Expression in *Trichoderma reesei* and *Saccharomyces cerevisiae*

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We have found that by utilizing a heterologous secretion signal, high yields of *Aspergillus oryzae* β-glucosidase were obtained in *Trichoderma reesei* under the control of a *T. reesei cbh1* promoter. Humicola insolens EG5 signal swapping was also shown to considerably improve *A. oyzae* β-glucosidase expression in *Saccharomyces cerevisiae*. Increasing the amount of β-glucosidase in *T. reesei* enzyme mixture resulted in a 2-fold improvement in the conversion of cellulose to glucose.

Poster Presentation 1B-42

Insights from Quantitative Modeling into the Mode of Action of Fungal Cellulases

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A new mathematical model was developed to analyze the mechanism of cellulose hydrolysis based on information from the literature. The model incorporates three main fungal cellulase components (CBH 1, CBH 2 and EG 1) and two physiochemical properties of cellulosic substrates (the fraction of _-glucosidic bonds accessible to cellulase, Z, and degree of polymerization, DP). Typical values of Z and DP of phosphorous acid swollen cellulose, bacterial microcrystalline cellulose and Avicel are about 0.03, 0.06, and 0.003 and 40, 300 and 3000, respectively. The model provides insights into the basis for previously unexplained observations concerning the extent of synergy under various conditions. For example, model results indicate that the degree of synergism between exoglucanase and endoglucanase is impacted by DP and Z, exo/endo ratio, enzyme loading, and incubation time. The model predicts that tightly adsorbed endoglucanase has a great positive impact on the rate of hydrolysis of cellulose with high DP and low Z but limited contribution to amorphous cellulose. Improved endoglucanase adsorption affinity would increase the gross cellulose hydrolysis rate significantly only in the case of low Z substrate and high DP. Good agreement between experimental and predicted results is obtained in several instances for which data are available. Modeling results are used to suggest new hypotheses, experiments to test such hypothesis, and directions for improving hydrolysis rates.

Poster Presentation 1B-43

A Pichia pastoris Vector for the Expression of a Xylanase Gene Isolated from *L. edodes*

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Before lignocellulosic biomass can be utilized to produce fuel and other chemical feedstocks, it must usually be hydrolyzed to simpler constituents. In an effort to reduce the costs associated with processing this biomass, we are cloning genes encoding new lignocellulolytic enzymes from organisms that grow on lignocellulose substrates. These genes are then modified to improve the activities of the encoded enzymes. In this poster we describe the cloning of a xylanase gene (Xyn11A) isolated from the white rot fungi *Lentinula edodes*. The Xyn11A gene encodes a 283-amino acid enzyme that is active when produced in *Pichia pastoris* using a commercially available expression vector. To achieve efficient, high throughput screening of mutant Xyn11A gene libraries, a new *P. pastoris* expression vector was designed that allows for rapid recovery of clones.

Poster Presentation 1B-44

Synthesis of Esters Catalyzed by Experimental Preparations of Immobilized Lipases in Solvent Free Medium

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Synthesis of esters catalyzed by lipases has been already described for more than 50 flavor compounds. In principle, the process can be performed in a reaction system consisting of appropriate amounts of alcohol and carboxylic acid in the presence of organic non-polar solvents. However, the elimination of solvents is technically feasible and offers significant cost savings. In addition, solvent free systems facilitate downstream processing, avoid solvent toxicity and are particularly interesting in the food industries, where severe regulations exist for solvent uses.

The objective of this work is to evaluate the performance of experimental preparations of immobilized lipase for the synthesis of flavor esters in solvent free systems. Reaction mixtures of alcohol (butanol) and organic acids (C4 to C12) were incubated with experimental preparations of immobilized lipase onto styrene-divinylbenzene copolymer (STY-DVB) and controlled pore silica (CPS), for a maximum period of 72 hours at 40°C and shaking. For each immobilized preparation, a full two-factorial design was used to study the influence of the carbon chain length (C4 to C12) and the biocatalyst concentration (10 to 30%) on the esterification yield and productivity. The use of molecular sieves was found to be essential for removal of the water formed during the reaction. Data were analyzed by Statgraphics software version 6.0 and mathematical models were proposed that represent the ester yield in the experimental region studied.

In the case of the lipase immobilized on STY-DVB, the ester formation was strongly influenced by both variables. The reaction was maximized (63%) for acyl donors with long carbon chain C12 (lauric acid) and enzyme concentration of 10%, corresponding to a productivity of 1.4 grams of butyl laurate/ mL.h. For the lipase immobilized on CPS, only the biocatalyst concentration was statistically significant ($p < 0.01$). Higher yields were obtained for the system butanol and octanoic acid and biocatalyst concentration of 20%, which gave productivity in the order of 1.2 grams of butyl octanate/ mL.h.

Poster Presentation 1B-45

Ester Synthesis Catalyzed by *Mucor miehei* Lipase Immobilized onto Polysiloxane-Polyvinyl Alcohol Magnetic Particles

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Mucor miehei lipase was immobilized onto polysiloxane-polyvinyl alcohol magnetic particles (POS-PVA) with high activity. The performance of the resulted immobilized biocatalyst was evaluated on the synthesis of flavor esters using heptane as a solvent. The reaction was conducted by mixing an immobilized lipase with appropriate concentrations of the starting materials (alcohols and organic acids) in a 20-mL batch reactor with shaking at 37°C, and the reactants consumption and/or product formed were monitored by gas chromatography analysis. The effects of organic acid/alcohol molar ratio, enzyme concentration, reactants carbon-chain length and the alcohol structure were determined on the reaction rate.

Ester synthesis was maximized for substrates containing organic acid in excess and biocatalyst concentration greater than 15% (w/v) of total weight reactants. The biocatalyst selectivity for the carbon chain length was found to be different concerning the organic acids and alcohols. High reaction rates were achieved for organic acids that had 8 or 10 carbons whereas increasing in length of the alcohol carbon-chain from 4 to 8 carbons gave much lower esterification yields. Optimal reaction rate was determined for the synthesis of butyl octanate (12 carbons). The rate of synthesis was also dependent on the alcohol structure, with maximum activity occurring for primarily alcohol. Secondary and tertiary alcohols decreased the reaction rates in more than 60%.

Poster Presentation 1B-47

Chloroperoxidase Stabilization by Covalent Binding in Mesoporous Sol-gel Glass

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A unique mesoporous sol-gel glass possessing a highly ordered porous structure (with a pore size of about 100 Å in diameter) was used as a support material for enzyme immobilization. Prior work has shown stabilization of a hydrolase, α-chymotrypsin. Here an oxidase, chloroperoxidase (CPO), was bound onto the glass via a bifunctional ligand, trimethoxysilylpropanal. The glass-bound CPO exhibited greatly enhanced thermostability in aqueous solution. The half-life of the glass-bound CPO was over 10-fold higher than that of the native enzyme at 40ºC. At 50ºC, the native CPO is unstable but retained some activity when bound on the sol-gel. From these results, it appears that the glass-enzyme complex developed through the present work can be used as high performance biocatalysts for various chemical processing applications, particularly in harsh conditions.

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Poster Presentation 1B-49

Discovery of Novel Alkaline and Thermophilic Cellulases and Their Industrial Applications

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Diversa utilizes proprietary genomic technologies to discover and optimize novel enzymes from diverse environmental sources. Small samples are collected that contain heterogeneous populations of microorganisms, from highly diverse and extreme environments. DNA is extracted from these samples and used to construct genomic libraries. Utilizing our high-throughput screening capabilities we are able to rapidly screen our libraries for novel enzyme activities and gene sequences. These technologies have enabled the discovery of a large number of enzymes, across all enzyme classes, with unique physical and biochemical properties. This presentation will address enzyme discovery and the distinct biochemical and extreme physical properties of discovered novel cellulases. The possible applications of these enzymes as industrial biocatalysts will also be presented.

Poster Presentation 1B-50

Coriolopsis SP Ligninases Production and Characterization in Different Substrates

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Plant cell walls store a large quantity of energy which is displayed in its components: celluloses, hemicelluloses and lignins. Some fungi, to use this energy, may degrade such components by employing ligninases, among other enzymes. In this study, *Coriolopsis sp* basidiomycete ligninases have been studied and isolated from decaying wood. The fungus has been cultivated in an Erlenmeyer flask at 28° C containing different lignocellulosic substrates: wheat bran, sugar cane bagasse and sawdust, until large substrate colonization. The enzymes were extracted by adding 8 ml of water to 1 g of substrate. Lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase activities have been detected due to veratryl alcohol, MnS04, and ABTS oxidation, respectively. Enzymatic reactions have been carried out to determine temperature and optimal pH levels from 20 to 60° C in 10° C intervals as well as 3,5 to 8,5 pH in 0,5 intervals. Wheat bran fungal growing was faster than the other substrates. It has been observed a greater LiP production in wheat bran and sawdust, which are more lignified substrates, whereas laccase has only been produced in wheat bran. MnP production has been minimum in all of the substrates. The highest activity of laccase and MnP has been detected at 20° C and there hasn´t been temperature influence on LiP activity. Optimal pH was 4,5 to laccase activity produced in wheat bran.

Financial Support: FAPESP

Poster Presentation 1B-51

Cloning, Expression and Characterization of a Novel Family 74 Xyloglucanase from *Trichoderma reesei*

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Trichoderma reesei possesses a number of enzymes that are involved in the solubilization of plant cell walls. Recently, we have identified a novel gene encoding a family 74 catalytic domain and fungal cellulose binding domain. Comparative alignment with other family 74 glycosyl hydrolases shows that the deduced amino acid sequence of this novel gene shares 47% identity with the deduced amino acid sequence of *Aspergillus aculeatus* Avicelase III (SPTREMBL:O74170). Microarray data suggests that this gene is not strongly induced in the presence of cellulose.

Upon expression of the gene product in *Aspergillus oryzae* and characterization of the recombinant purified protein, the enzyme was demonstrated to have high specific activity on xyloglucan.

Poster Presentation 1B-52

Enzymatic Activities of *Ceriporiopsis subvermispora* Acting on Sugarcane Bagasse

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Sugarcane bagasse was pre-treated with the white-rot fungus *Ceriporiopsis subvermispora* from 7 to 60 days. The aim of the work was the production of pulps or modified fibers from the pre-treated bagasse. Enzymatic activities for lignin peroxidase (LiP), manganese peroxidase (MnP), laccase (Lac) and xylanase (Xyl) were determined. The table shows the preliminary results for Xyl and MnP production in experiments in which the inoculum charge was varied. Xyl increased during the fermentation and also by the increasing of inoculum charge. On the other hand, MnP has a maximum of production until 30 days and seems to be not influenced by the inoculum charge. These parameters are useful to determine the better fermentation conditions. Additional results on Lac and LiP production will be presented.

Inoculum charge (mL)	7 days	15 days	30 days	45 days	60 days
			Xylanase		
100	51 ± 1	118 ± 5	4787 ± 269	3542 ± 195	4847 ± 446
250	1065 ± 30	1209 ± 90	4384 ± 189	4161 ± 480	6678 ± 479
500	526 ± 25	1661 ± 93	4691± 190	5504 ± 316	6709 ± 447
750	1094 ± 20	5273 ± 94	4931± 295	2314 ±196	5960 ± 514
			MnP		
100	-	-	310 ± 29	82 ± 3	19 ± 2
250	294 ± 12	6 ± 1	215 ± 20	23 ± 2	28 ± 1
500	339 ± 13	101 ± 5	30 ± 3	97 ± 3	6 ± 1
750	294 ± 12	112 ± 9	118 ± 12	30 ± 2	27 ± 2

[Financial support from FAPESP and CNPq]

Poster Presentation 1B-53

Bleaching of Ethanol/Water Pulp of Sugarcane Bagasse with Xylanase and Classification by FTIR-PCA

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Pulps obtained from the organosolv cooking (ethanol/water) of sugarcane bagasse were bleached with xylanase enzyme obtained from the fungus *Thermomyces lanuginosus* IOC-4145 and with commercial enzyme Cartazyme HS from Sandoz. The pulps were classified by using Fourier transform infrared (FTIR) spectroscopy and principal component analysis (PCA). Increasing the enzyme dosage from 4.3 to 36 IU/g of pulp, kappa number and viscosity were maintained when the xylanase from T. lanuginosus was used. On the other hand, by using Cartazyme, kappa number decreased 4%. FTIR - PCA showed that the first three principal components (PCs) explained more than 90% of the total variance of the pulp spectra. [Financial support from FAPESP and CNPq]

Poster Presentation 1B-54

Increased Thermal Tolerance of *T. fusca* β-Glucosidase *via* Directed Evolution

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The use of heat-tolerant enzymes can improve turnover rates and tolerance to the stresses of large-scale processes. Directed evolution with high-throughput screening is a logical methodology for pursuing such enzyme process improvements. We have used such an approach to increase the thermal tolerance of a β-D-glucosidase encoded by the *bglC* gene of the cellulolytic actinomycete *Thermobifida fusca*. The coding sequence for the *bglC* gene (provided by David Wilson, Cornell University) was mutagenized by error-prone PCR. In the first campaign, more than 22,000 mutagenized clones were picked using an Autogen Autogenesys colony picker and screened *via* direct temperature challenge in 384-well plates. This resulted in about two dozen candidates, which were subsequently screened using a novel temperature gradient plate assay. At least nine candidates showed enhanced thermal tolerance and were further characterized. Pair-wise combinations were then made of some of the most promising mutations. Recombinant mutant and wild type BglC proteins were purified from *E. coli*, and differential scanning microcalorimetry and temperature challenge experiments were performed. One of the combinations generated a BglC protein with an increase in thermal stability of at least 5°C over wild type. This protein was demonstrated to have a nine-fold increased half-life at temperatures of 62-64°C. Through successive rounds of mutation and screening, and additional combinations of mutations, it should be possible to further increase the thermal tolerance of this β-glucosidase.

Poster Presentation 1B-55

Using HPLC/ELSD Methods to Quantify Oligosaccharides and Determined Cellulose Molecular Weight Distribution During Enzymatic Hydrolysis of Cellulose

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A high performance liquid chromatography (HPLC) method with evaporative light scattering detector (ELSD) was developed to determine the mono- and oligosaccharides obtained from lignocellulosic materials. A Prevail Carbohydrate ES column (5µ, 250 x 4.6mm) with a water/acetonitrile gradient was used to elute analytes. This method was applied to determine the release of glucose and its oligomers during both enzymatic and acid hydrolysis of model celluloses (Avicel and Sigmacell). The production of sugar oligomers, particularly the cellobiose and cellotriose, during both celluclast and endoglucanase hydrolysis of model celluloses was monitored during a 96-hour incubation time. An HPLC/ELSD method was also developed to measure the molecule weight distribution of cellulose during model cellulose hydrolysis by different enzyme fractions. Previous GPC methods required derivatization of the analyte prior to determine the molecule weight distribution of cellulose. In this isocratic method, tetrahydrofuran (THF) was used as delivering solvent and minimum sample preparation was required. The potential of using both methods to investigate the mechanism of enzymatic depolymerization of cellulose present in different substrates was described.

Poster Presentation 1B-56

Immobilization of β-glucosidase on Eupergit C for Cellulose Hydrolysis

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Typically, β-glucosidase has been used in excessive amounts during the enzyme hydrolysis of cellulose, in order to minimize end-product-inhibition from cellobiose. However, the high cost associated with the high enzyme loading has significantly added to the operational cost of the whole bioconversion process. It is apparently that a cost effective enzyme recovery strategy needs to be implemented before an enzyme-based hydrolysis process can be considered to be efficient. While membrane filtration has proven to be tedious and impractical, immobilization of β-glucosidase has shown promise as a means of reusing this type enzyme, as both enzyme and its substrate (cellubiose) are present in a "dissolved" form in the reaction system. Different carriers, including Eupergit C, silica and Dulite resin, were examined in this study for their potential to retain β-glucosidase (Novozyme® 188). The catalytic activities as well as other enzyme properties such as protein-binding capacity, immobilization yield, pH profile, stability and kinetic constants were assessed and compared with free β-glucosidase.

The immobilized β-glucosidase were added to Celluclast and then used in the hydrolysis of different substrates including organosolv pretreated substrate, steam exploded corn fiber and Avicel. The efficiency and stability of immobilized β-glucosidase during batch repeated hydrolysis of these substrates was evaluated. An enzyme recycling strategy using immobilized β-glucosidase will be described.

Poster Presentation 1B-57

Proteome Expression Analysis of the Consequences of Metabolic Engineering in *Zymomonas mobilis*

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In recent years it has become increasingly important to develop fuels from renewable energy sources such as lignocellulosics. One bottleneck associated with the fermentation of lignocellulosics is the co-utilization of xylose in the hemicellulose fraction along with glucose as a fermentable sugar. This work utilizes proteome-wide expression profiling through two dimensional gel electrophoresis (2-DE) to gain a more defined understanding of physiological responses in the proteome of metabolically engineered *Z. mobilis* C25 to stresses associated with the utilization of the recombinant pentose phosphate (PP) pathway.

This work was able to tentatively assign identifications for three categories of proteins, including: 8 glycolytic/fermentative enzymes, 4 PP enzymes, and 4 stress proteins. Hierarchical cluster analysis was performed on time course protein profiles of relative protein abundance for mixed substrate growth. Through the cluster analysis, it was determined that functionally related proteins also show similarity in their protein expression profiles. The general trends that these clusters of similarly expressed proteins show are decreases in the relative abundance of PP enzymes, a peak in the relative abundance of stress proteins after glucose consumption is complete, and a continual increase in several glycolytic/fermentative enzymes throughout the course of the fermentation.

Poster Presentation 1B-58

Review of Enhanced Production of *R*-Phenylacetylcarbinol (*R*-PAC) Through Enzymatic Biotransformation

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The pharmaceutical intermediate *R*-Phenylacetylcarbinol (*R*-PAC), used in the manufacture of the decongestants ephedrine and pseudo-ephedrine can be produced enzymatically by the condensation of benzaldehyde and decarboxylated pyruvate using partially purified pyruvate decarboxylase (PDC). The traditional yeast-based fermentation method of production involves concentrations of 10-22 g/l *R*-PAC and yields of 55-65% on benzaldehyde have been reported with significant added benzaldehyde converted to benzyl alcohol due to the presence of alcohol dehydrogenase (ADH) or other oxido-reductase activity in the yeast (Rogers et al, 1997).

An enzymatic process based on partially purified PDC overcomes this problem of benzyl alcohol production. Up to 28 g/l R-PAC with improved yields have been reported by our group for an enzyme-based process using partially purified Candida utilis PDC (Shin and Rogers, 1996).

Screening of PDC from 14 ethanol producing filamentous fungi has also demonstrated their potential for *R*-PAC production(Rosche et al, 2001). Under optimised conditions of improved buffering and enzyme stabilization, partially purified *Rhizopus javanicus* and *C. utilis* PDC both produced up to 50 g/l *R*-PAC with good yields (Rosche et al, 2002a).

Based on knowledge of optimised biotransformation conditions and inactivating and inhibitory influences of substrates and products, an aqueous/organic two-phase reaction system was designed to maximize *R*-PAC production. The toxic substrate benzaldehyde which partitioned away from the enzyme into the organic phase (octanol) was delivered continuously in soluble concentrations to the aqueous phase containing pyruvate and PDC. *R*-PAC and by-products acetoin and acetaldehyde were continuously extracted into the organic phase. Under optimised conditions 141 g/l *R*-PAC was achieved in the organic phase with additional 19 g/l formed in the aqueous phase in 50 hours using *Candida utilis* PDC. Molar yields on consumed benzaldehyde and pyruvate were 90% and 73% respectively. The results demonstrate the potential of two-phase enzymatic biotransformation processes to increase product concentrations and enzyme efficiencies (product / U enzyme) (Rosche et al, 2002b).

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Poster Presentation 1B-59

Enzymatic Hydrolysis of Wheat Arabinoxylan in an Industrial Bioethanol Byproduct Stream

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Hydrolysis of arabinoxylan is an important prerequisite for improved utilization of wheat hemicellulose in the ethanol fermentation industry. This study investigated the individual and combined efficiencies of four commercial, cellulytic and hemicellulytic enzyme preparations, Celluclast 1.5 L, Ultraflo L, Finizym and Viscozyme L, in catalyzing the liberation of arabinose and xylose from an industrially derived water-soluble wheat arabinoxylan fraction remaining after ethanol fermentation. The content of arabinose and xylose together constitute approximately 80% of the total monomeric sugar composition in the industrial substrate. Ultraflo™ L was the best enzyme preparation in releasing arabinose, liberating 46 wt% of the theoretical maximum after 6 hours of reaction. Celluclast®1.5 L was superior to the other enzyme preparations in releasing xylose, liberating 25 wt% of the theoretical maximum after 6 hours of reaction. Mixtures of the enzyme preparations showed no synergistic cooperation in arabinose and xylose release after 6 hours of reaction, but a synergistic cooperation in xylose release was found between Ultraflo L and Celluclast1.5 L as a 50:50 mixture of the two enzyme preparations liberated 62 wt% of the theoretical maximum after 24 hours of reaction (10 wt% enzyme/substrate ratio, 50°C, pH 5). The observed synergism between Celluclast1.5 L and Ultraflo™ L is proposed to be a result of positive interaction between -L-arabinofuranosidase and endo-1,4-β-xylanase activities present in Ultraflo™ L, and -xylosidase activities in Celluclast1.5 L.

Poster Abstracts for Session 2

Microbial Catalysis and Engineering

Poster Presentation 2-07

Platform Organisms for the Biorefinery

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The next generation of microbes for the biorefinery should be versatile, highly productive and user friendly. Within this context, the limitations of current platform microbes will be examined. The ideal organism would be able to convert a variety of sugars and complex biomass to value added products. The ideal process parameters would include extracellular production of enzymes to hydrolyze substrates and production of products at low pH. The process should be compatible with existing bioprocess facilities, produce minimal waste with high conversion rates and high product yield. For the biorefinery of the future, platform organisms capable of growth on a variety of substrates to produce multiple products is the ideal goal. Some examples of fungal processes already in place will be evaluated against this goal. We will describe a strategy, and the progress made, toward discovery and construction of the next generation of microbes for the biorefinery.

Poster Presentation 2-08

Directed Evolution of a Thermostable A. *oryzae* β-Glucosidase Utilizing a *Saccharomyces cerevisiae* Expression System and Robotic Screening

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A directed evolution approach combined with automated screening techniques was successfully used to obtain a significantly more thermostable β-glucosidase (BG).

The degradation of cellulose (a polymer of -1,4-linked glucose) is due to a complex interaction of several cellulolytic enzymes. The action of cellobiohydrolyases produces the disaccharide cellobiose; two glucose residues linked by a -1,4-glycoside bond. BG hydrolyses this -1,4-glycosyl bond to produce glucose during the saccharification process. Breaking this bond not only produces glucose, but also decreases product inhibition of the cellobiohydrolases by cellobiose. A more thermostable BG will enable the saccharification process to be performed at higher temperatures. The *Aspergillus oryzae* BG was mutagenized by error-prone PCR. These mutagenized products were subcloned by gap repair in a linearized yeast expression vector for expression in yeast (*S. cerevisiae*). The generated primary libraries were plated onto minimal media containing the substrate 5-Bromo-4-Chloro-3-Indolyl-β-D-Glucopyranoside (X-Glc), which becomes blue if cleaved by active BG. These blue colonies were automatically picked with a Genetix Q-Pix colony picker. The rate of mutagenicity of the libraries was indicated by the ratio of white versus blue colonies obtained. Automated high throughput screening for thermostable BG mutants was been accomplished through the use of a robotic system. Improved variants were chosen based on comparison before and after heat challenge. The BG coding sequence of the thermostable mutants obtained from screening were amplified using non-mutagenic conditions and transformed into yeast competent cells with the linearized yeast expression vector. Due to the ability of yeast to randomly recombine homologous regions of DNA, the mutations in the selected BG mutants were recombined, generating BG shuffled libraries. Thus far screening has identified a BG mutant with greater than 10-fold better thermo stability than the original BG. These results and other improvements in the BG screening and expression procedures will be discussed.

Poster Presentation 2-09

Isolation and Performance Optimization of Cultures Capable of Converting Syngas to Ethanol

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Several microbial cultures capable of converting syngas into ethanol have been isolated from a variety of conventional and exotic environments using techniques and media developed at Mississippi State University. These cultures have demonstrated the ability to produce ethanol at rates comparable to or better than those reported for *Clostridium ljungdahlii*. Numerous media formulations have been tested for the growth of these cultures and/or production of ethanol from these cells. The results thus far have shown that greater amounts of cells can be produced on media formulations that are non-traditional in composition and targeted toward an economically viable growth technique for cells. Another series of experiments have been oriented toward optimizing ethanol production. This leads to the development of a dual-fermentation system. By separating growth from ethanol production; the optimization of ethanol production is obtained. Various fermentation configurations are being theorized from this activity including the immobilization of the cells within alginate-based beads or using a dual-fermentation system for cell growth and ethanol production. Finally, a methanogenic conversation step for utilizing some of the waste products generated is under development as a novel energy recovery step.

Poster Presentation 2-10

Microarray-Analysis of Xylose-Growing Recombinant *Saccharomyces cerevisiae* Strains

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Saccharomyces cerevisiae can efficiently convert the hexoses present in lignocellulosic hydrolysates to fuel ethanol. To enhance the ethanol yield from this raw material efficient recombinant *S. cerevisiae* strains capable of xylose utilisation need to be constructed.

Introduction of the *Pichia stipitis* genes encoding xylose reductase and xylitol dehydrogenase, as well as overexpression of endogeneous xylulokinase, results in a stable xylose-fermenting *S. cerevisiae* strain (Eliasson *et al.* 2000). However even aerobically this strain grows very slowly on xylose.

Strains with improved aerobic xylose growth have been generated through adaptation or mutation. To pinpoint variations that can be responsible for the enhanced growth on xylose, microarray-analysis has been performed on strains fermented in continuous mode. Results from this investigation will be presented.

Eliasson, A., C. Christensson, C. F. Wahlbom and B. Hahn-Hägerdal. (2000). Anaerobic xylose fermentation by recombinant *Saccharomyces cerevisiae* carrying *XYL1, XYL2,* and *XKS1* in mineral medium chemostat cultures. Appl Environ Microbiol 66(8): 3381-6.

Poster Presentation 2-11

Polykaryon Formation Using a Swollen Conidium of *Trichoderma reesei*

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The cellulolytic fungus, *Trichoderma* has oval and mononucleate conidia. When these conidia are incubated in a liquid medium, conidia begins to swell and the shape becomes spherical followed by increasing its inner space. In such a swollen conidium, it is possible to produce a larger autopolyploid nucleus using a mitotic arrester compared with the case of the original conidium. In this report, polykaryon formation was attempted using these swollen conidia.

Dried green mature conidia of *Trichoderma reesei* QM6a (IFO 31326) were incubated in Mandels' medium in order to swell. These swollen conidia were treated with a mitotic arrester for autopolyploidization. Such swollen conidia containing autopolyploid nuclei were treated with a reagent that can cause micronucleation. After the treatment, multiple smaller nuclei were produced from an autopolyploid nucleus in a swollen conidium. The conidia generated on the colonies derived from these treated swollen conidia have multinucleate conidia. Such multinucleate conidia could be also selected using our double layer selection medium. This indicated that the cellulase productivity of such multinucleate conidia was positively changed.

Poster Presentation 2-12

Minimizing the Toxicity of Sugarcane Bagasse Hemicellulosic Hydrolysate by Controling the Cultivation Conditions

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Sugarcane bagasse is a by-product generated in large amounts by sugar-alcohol industries during the ethanol production season in Brazil. Its hemicellulosic fraction could be profitably hydrolysed and the resulting sugars used as substrates for bioconversion processes. A major problem in the use of these hydrolysates as fermentation media is the presence of a wide range of compounds, like weak acids, furan derivatives and phenolics, which are inhibitory to microorganisms. These toxic compounds hinder the bioconversion of the sugars present in the hydrolysates, leading to poor fermentability. Several strategies have been developed in order to minimize the concentrations of inhibitory by-products in the hydrolysates before the fermentations, including neutralization and adittion of active charcoal.

In the present study, a sugarcane bagasse hemicellulosic hydrolysate, previously treated by neutralization and addition of active charcoal, was used as the fermentation medium for xylitol production by Ca-alginate entrapped *Candida guilliermondi*i cells in a STR reactor. A 2^{5-1} fractional factorial design was employed to determine whether variations in the cultivation conditions, namely hydrolysate concentration factor, air flowrate, agitation speed, initial concentration of immobilized cells and initial pH of the fermentation medium, would have any effects on the fermentability of the hydrolysate.

According to the results, although the detoxification strategy by neutralization and addition of active charcoal has promoted a good reduction in the levels of phenolics and the elimination of furan derivatives, the inability to remove acetic acid led to poor hydrolysate fermentability in the presence of the remaining phenolics. By adjusting the cultivation conditions, it was possible to overcome these toxic effects and attain a good yield (0.79 g/g) and productivity (0.39 g/l.h).

Acknowledgements: Fapesp,CNPq/Brazil

Poster Presentation 2-13

Effect of Organic Acids and Aldehydes on the Growth and Fermentation of *K. marxianus*

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The inhibitory effects of various lignocellulose degradation products on glucose fermentation by thermotolerant yeast *K. marxianus* were studied in batch cultures. The toxicity of binary combination of the aromatic alcohol cathecol and two aromatic aldehydes (4-hydroxybenzaldehyde and vanillin), which have been identified as the most toxic compounds in a previous work (1), was tested. The aldehyde furfural, that usually is presented in relatively high concentration in hydrolyzates was also tested in combination experiments.

Experiments were conducted by combining agents at concentrations that individually caused 25% inhibition of growth. Compared to the relative toxicity of the individual compounds, all binary combinations showed synergistic effect on toxicity, and cause a 60 to 90% decrease in cell mass. The combination of vanillin was roughly additive for all compounds tested and caused a 90% reduction in cell growth and fermentation.

Poster Presentation 2-14

Corn Fiber Hydrolysis and Fermentation to Butanol Using *Clostridium beijerinckii* BA101

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Corn fiber was hydrolyzed by dilute sulfuric acid treatment and enzymes by a method reported by Grohmann and Bothast (1997). Sulfuric acid concentration of 0.3-0.5 % resulted in better hydrolysis than 0.1-0.2 %. Sixty three g/L corn fiber (16 % moisture content) was treated with sulfuric acid [0.3-0.5% (v/v)] at 121 ^{o}C for 1 h followed by cooling to room temperature, and adjusting pH to 4.5 with 10 M NaOH. The hydrolysate so obtained contained approximately 30 g/L total sugar. Subsequent hydrolysis with enzymes [cellulase and cellubiase, both at 1 mL/100 g corn fiber] increased sugar concentration to approximately 52 g/L. There was no effect of reduced particle size on the hydrolysis and final sugar content in the hydrolysate. The hydrolysate was used to measure cell growth of *Clostridium beijerinckii* BA101 in anticipation for producing butanol. Compared to the control reduced cell growth was observed, possibly due to growth inhibitory components present in the hydrolysate. The results on hydrolysis and butanol fermentation will be reported during the 25th Biotechnology Conference on Fuels and Chemicals.

Reference: Grohmann K, Bothast RJ (1997) Saccharification of corn fiber by combined treatment with dilute sulphuric acid and enzymes. Proc. Biochem. 32: 405-415

Poster Presentation 2-15

Metabolic Engineering of *Escherichia coli* for the Production of Succinic Acid

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Succinic acid is a member of C_4-dicarboxylic acid family and can be used as a precursor of numerous products including pharmaceuticals, fine chemicals and biodegradable polymers. Recently, several metabolic engineering strategies were developed for the production of succinic acid by *Escherichia coli*.

In this study, we used a metabolically engineered *E. coli* strain for the production of succinic acid from various substrates. We also carried out metabolic flux analysis and metabolic control analysis to examine the degree of engagement of various pathways in overall metabolic pathways.

This work was supported by the National Research Lab program of the Ministry of Science and Technology.

Poster Presentation 2-16

Production of Short-Chain-Length and Medium-Chain-Length Polyhydroxyalkanoate Copolymers by Metabolically Engineered *E. coli*

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Escherichia coli was metabolically engineered to produce novel short-chain-length (SCL) and medium-chain-length (MCL) polyhydroxyallkanoate (PHA) copolymers. Metabolic pathways involved in the biosynthesis and degradation of fatty acids were engineered so that the precursors for PHAs are available for the PHA synthase as substrates. Detailed strategies employed for the engineering of metabolic pathways, and the results on the production of SCL-MCL PHA copolymers will be reported.

This work was supported by the National Research Lab program and by the BK21 project from the Ministry of Education.

Poster Presentation 2-18

Effect of Carbon:Nitrogen Ratio and Substrate Source on Glucose-6-Phosphate Dehydrogenase Production by *Saccharomyces cerevisiae* W 303 181

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Glucose-6-phosphate dehydrogenase (G6PDH) is an important enzyme in biochemical researches, and can be produced by a strain of genetically modified *S. cerevisiae*. The medium used to grow this yeast is of high cost. This work studied the use of new media components to reduce these costs. The experiments were performed in shaker at 30ºC, 100 rpm, 26 h, initial cell concentration of $1 g.L^{-1}$. The enzyme production was evaluated in media containing molasses, yeast extract, and glucose. Three C:N ratios were tested: 7, 10 e 14. Cell concentration, activity, glucose, nitrogen and total protein concentrations were evaluated. The results showed that molasses are feasible as carbon source to produce the enzyme. Besides this substrate is of low cost, and provided quite good results. The C:N ratio did not affect the enzymatic activity. However, the cultivation with molasses provided maximum activity ($7534 U.L^{-1}$), 5.1 times higher than that provided by the conventional medium ($1467U.L^{-1}$).

Acknowledgments: FAPESP, CNPq

Poster Presentation 2-19

The Effect of Viscosity Change on the Rate and Extent of *Zymomonas mobilis* Cellulose Fermentation

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Corn stover, a lignocellulosic biomass, possesses strong potential as a renewable feed for bioethanol production. Dilute sulfuric acid pretreatment removes the shielding network of hemicellulose, making the cellulose more susceptible to bacteria digestion. In this study, pretreated corn stover was fermented by a recombinant bacterium *Zm* 39676:pZB4L that can produce ethanol from xylose-rich hemicellulose. The ethanol-production efficiency of this organism is substantially dependent upon rheological suspension characteristics.

In addition, fermentation broth viscosity is required when using computational fluid simulation for design and scale-up of production system. In systems with suspended solids, rheological measurements are difficult to perform due to settling in the measurement devices. In this study viscosities of the fermentation broth were measured using a helical ribbon impeller viscometer. A calibration procedure is required for the impeller method in order to obtain the shear rate constant, k, which is dependent on the geometry of the measurement system. The measurement of rheological characteristics were made every two hours for 2 days. This study characterized the growth performance of *Z. mobilis* 39676:pZB4L as a function of mixed sugar and acetic acid concentration using a synthetic acid pretreated corn stover hydrolysate containing 1 %(w/v) yeast extract, 0.2 % KH_2PO_4, 5 % (w/v) xylose, and 0.1 % (w/v) glucose with varying amounts of acetic acid. The relationship between rheological characteristics and ethanol yield was determined.

Poster Presentation 2-20

Construction of Recombinant *Escherichia coli* Strains for Poly-(3-hydroxybutyrate-co-3-Hydroxyvalerate) Production

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Plastic wastes are considered to be a worldwide environmental problem and the demand for biodegradable plastics has become a highly visible issue. One of the most important characteristics of microbial polyesters is that they are thermoplastic with environmentally degradable properties. In this experiment, pUC19/PHA was cloned and transformed into two different *E. coli* strains. Both strains were successfully expressed in the production of PHA but *E. coli* HMS174 was superior for the production of Poly-(3-hydroxybutyrate-co-3-Hydroxyvalerate) [P(HB-HV)] which reached as high as 45% in shake flask culture with relatively high cell density. The cell dry weight and PHA content of recombinant HMS174 reached as high as 10.27 g/L and 43% (wt/wt) respectively in fed-batch fermenter culture. P(HB-HV) was accumulated in the cells and the biopolymers accumulated were identified and analyzed by GC, ^{1}H NMR and DSC.

Index Entries: *Escherichia coli*; polyhydroalkanoates; fed-batch fermentation; nuclear magnetic resonance; differential scanning calorimetry.

Poster Presentation 2-21

Conversion of Corn Fibrous Material into Ethanol

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Over 1.7 billion gallons of fuel ethanol were produced in the U.S. (2001), over 90% of which was produced from corn. Corn is prepared for ethanol fermentation by either wet milling or dry grinding in approximately equal volumes. In both processes, the fibrous components of the kernel are folded into animal feed co-products. U.S. ethanol production is expected to continue to increase, which may saturate these feed markets. We have investigated the possibility of converting the fibers to ethanol as an alternative use for the byproduct. Corn fiber (wet milling) and distillers wet grains (dry grind) were both analyzed for carbohydrate composition and determined to each have a high fraction of fermentable carbohydrates. Each fiber product was converted to ethanol by pretreating with dilute sulfuric acid and fermenting the sugars using either a recombinant ethanologenic *Escherichia coli* (FBR5) or *Saccharomyces cerevisiae*. For the *S. cerevisiae* fermentations, cellulase was also added to hydrolyze the cellulose. Strain FBR5 has the potential for higher ethanol yields than *S. cerevisiae* because it ferments both pentoses and hexoses. FBR5 fermentations were completed typically in 30-48 hr and gave ethanol yields 90-96% of theoretical, based upon fermentable sugars. Yeast fermentations gave yields 92% of theoretical, based upon glucan content.

Poster Presentation 2-23

Xylitol Production by Flocculating Yeast, *Candida* sp. HY200

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A novel strain isolated from the soil of Korean rice fields, *Candida* sp. HY200, was used to produce xylitol from xylose. There is a general trend that the xylitol production was improved by using organic nitrogen sources. Maximum xylitol production was obtained with soytone, while maximum cell mass was obtained with liquid starch waste. Most of the inorganic nitrogen sources could not show a sufficient cell growth, and also not adequately support for a considerable production of xylitol. As an interesting result, however, flocculation ability of strain, *Candida* sp. HY200, was better than other nitrogen sources, when ammonium phosphate was used. Therefore, these results indicated that organic nitrogen sources, such as light steep water, corn steep powder and soytone, could be used for effective production and the optimization of xylitol production by *Candida* sp. HY200. *Candida* sp. HY200 produced xylitol in a batch culture containing 300-g/L xylose medium with 72.4% of conversion yield and 1.68-g/L/hr of xylitol productivity. As an effective way to obtain a high yield and productivity, a fermentation strategy was adopt by using a two-stage fed-batch process where the first stage was operated for cell growth and the second stage for xylitol production. When the total xylose concentration was adjusted to 260-g/L in the second stage, 64.6% of conversion yield and 2.34-g/L/hr of xylitol productivity were achieved.

Poster Presentation 2-24

Biosynthesis of (*R*)-(-)-3-Hydroxybutyric Acid by Metabolically Engineered *Escherichia coli*

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Polyhydroxyalkanoates (PHAs) consist of optically pure (*R*)-3-hydroxyalkanoic acids (RHAs). RHAs have the potentials for the applications for the synthesis of various chiral compounds including antibiotics, vitamines, perfumes and pheromones. Recently, we have reported an efficient production of RHAs by regulating cyclic nature of PHA biosynthesis and degradation in natural PHA producing bacteria, *Alcaligenes latus* and *Pseudomonas* sp. In this study, we report metabolic engineering strategies for the production of (*R*)-3-hydroxybutyric acid (R3HB) in recombinant *Escherichia coli*. Heterologous expression of PHA biosynthesis genes and degradation genes is established in *E. coli* by introducing the *Ralstonia eutropha* PHA biosynthesis operon along with the *R. eutropha* intracellular PHA depolymerase gene. By employing this metabolically engineered *E. coli*, R3HB could be efficiently produced from glucose. [This work was supported by the Ministry of Commerce, Industry and Energy.]

Poster Presentation 2-25

Synthesis of Polyhydroxyalkanoate (PHA) by Microorganisms from Activated Sludge

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Numerous microorganisms in activated sludge synthesize and accumulate polyhydroxyalkanoates (PHAs). Therefore, the PHA-accumulating behavior of these microorganisms in their native habitat will provide valuable information.

In this study, screening of the PHA-producing bacteria from different municipal sewage treatment works in different season was performed. This work compared the PHA-accumulating ability of single culture and mixed culture of isolated bacteria. Varies types of co-polymer were produced by different bacteria community. The media used were activated sludge and synthetic wastewater. BOD, COD, TOC, TKN and phosphorus were determined during the cell growth. The experiment indicated that activated sludge could accumulate PHAs for not more than 5% of cell dry weight. However, the mixed culture produced up to 50% of PHAs and the amount of excess sludge can be reduced by 33%.

Poster Presentation 2-26

Molecular Weight Distribution Study of Microbial-produced Polyhydroxyalkanoates by Improved Viscosity Method

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Many reports indicated that the magnitude of the molecular weight and the molecular weight distribution are some of the most important determinants of the properties of a polymer. However, little effort has been made to characterize the size of the molecular weight of polyhydroxyalkanoates, which is an important parameter characterizing the physical properties of the polymer. Measuring average molecular weight by viscosity which is an easier and a faster method and suitable for polymers with wide range of molecular weight has been utilized largely in industry. In general, however, a traditional intrinsic viscosity measurement by extrapolation with five different concentrations of samples takes more time and more samples, so that there is less used in production of PHAs in bench experiments or industry. More rapidly and more economically modified intrinsic viscosity method with only one concentration of a sample, called the one-point method, to measure the molecular weight of PHAs for industrial application was built in this study. With this modified method, we suggest three better formulae for PHA molecular calculation:

$[\eta] = \ln\eta / C$ is for calculation of PHB and P(3HB-co-3HHx)

$[\eta] = \eta_{sp} / C$ or $[\eta] = (\eta_{sp} + 3 \ln\eta_{r}) / 4C$ is for calculation of P(3HB-co-3HV)

Poster Presentation 2-27 Student

Real Time Estimation and Neuro-Fuzzy Control of Fed-Batch Baker's Yeast Cultivation

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Baker's yeast (*Saccharomyces cerevisiae* biomass) is still one of the most important biotechnological products for human food use. In order to produce baker's yeast economically, a proper environment must be sustained in the bioreactor, to maximize efficiency and productivity of yeast growth. The process should be carried out in fully aerobic conditions. Extracellular concentrations of glucose profoundly affect the metabolism of baker's yeast (when the glucose concentration exceeds a critical value, part of sugar is metabolized via anaerobic pathway, resulting in ethanol, the Pasteur effect). To overcome this effect, the process is carried out under sugar-limiting conditions in fed-batch cultures, which in turn must be optimized to enhance the overall productivity. This may be done manipulating sugar feed rates in order to minimize ethanol formation.

This work presents experimental results obtained from *S. cerevisiae* (commercial Baker's yeast) cultivations carried out in 5-L automated fed-batch stirred tank bioreactor operated in fed-batch mode. An artificial neural network was used to estimate biomass concentration from on-line measurements. Secondary variables, such as air flow rate, pH, carbon dioxide and oxygen molar fraction in the effluent gas, were used for this purpose. Oxygen uptake rate (OUR), carbon production rate (CPR) and respiratory quotient (RQ) were calculated on-line and a neuro-fuzzy controller was implemented to manipulate glucose feed rates. The neuro-fuzzy algorithm was implemented in a microcomputer connected via TCP-IP to the supervisory system, hosted in a second computer.

Poster Presentation 2-29

Genetic Analysis of the CO-Shift Reaction in *Rubrivivax gelatinosus*

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When photosynthetic bacterium *Rubrivivax gelatinosus* CBS is cultured in an atmosphere containing CO, a unique CO-oxidation pathway is induced whereby CO and H_2O are converted into H_2 and CO_2. When light is present, *Rx. gelatinosus* CBS-2 can assimilate CO as the sole source of carbon and exhibit a doubling time near 10 h. CO was shown to be the compound inducing the CO-oxidation pathway at the transcriptional level. The CO-oxidation reaction is mediated by the enzymes CO dehydrogenase (CODH) and an evolving hydrogenase. Recently two CBS transposon mutants unable to produce H_2 were isolated, and preliminary biochemical analysis suggests that the mutations are non-allelic. The mutated genes were cloned and appear to encode CODH and hydrogenase proteins, respectively. Utilizing the aforementioned genes as probes a genomic library was screened and has led to the identification of five additional genes involved in either the oxidation of carbon monoxide or in the production of hydrogen. Preliminary analysis suggests that the *Rubrivivax gelatinosus* CBS CO dehydrogenase is similar to known proteins of the same function, while the hydrogenase appears novel when compared to known CO-shift related hydrogenases.

Poster Presentation 2-30

Gata Factors Regulation of *Saccharomyces cerevisiae* Invertase: Role of Ure2P and the Gln3P and Nil1P Transcriptional Factors

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Nitrogen regulation is a global control mechanism, involving GATA family transcription factors, that tunes cell physiology in accordance to nitrogen nutrition. When *Saccharomyces cerevisiae* grows in a non-preferred nitrogen source such as proline, the dephosphorylated Gln3p and Gat1/Nil1p migrate into the nucleus and binds to GATA sequences of their target promoters activating transcription of nitrogen regulated genes. The Dal80p GATA repressor competes with Gln3p and Gat1/Nil1p. When cells are grown in preferred sources such as ammonium, the transcription of nitrogen regulated genes is hindered by the GATA repressor Deh1p. Moreover, under these conditions the phosphorylated Gln3p and Gat1/Nil1p are located in the cytoplasm.

This work studied nitrogen regulation of invertase in *Saccharomyces cerevisiae* mutant cells lacking the Ure2p and the transcriptional activators Gln3p and Gat1p/Nil1p. Activity was measured in fresh mid-log cells grown in either ammonium or proline. Although equivalent enzyme levels (150 U/g cell d.w.) were observed in both the wild type and the ure2 mutant, the single nil1 and gln3 mutations increased invertase activity two - to four-fold, respectively in comparison to their wild type counterparts. In the double *gln3nil1* mutant activity increased twenty four-fold (3600 U/g cell d.w.). In proline grown cells it was observed an overall 1.5 to 4.0 fold invertase activity decrease.

These findings relating nitrogen regulators to invertase levels substantiate the existence of a cross-regulatory signalling pathway between carbon and nitrogen metabolism. Indeed it has been shown a convergence of TOR-nitrogen and Snf1-glucose signalling pathways onto Gln3p phosphorylation/ dephosphorylation mechanism. Gln3p phosphorylation mediated by TOR kinase proteins occurs in the presence of preferred nitrogen sources and via Snf1 AMPK pathway when glucose levels are low. Analysis of the nucleotide sequences 5' of the start point of translation of the major *SUC2* genes repressors such Mig1p, Cyc8p and Tup1p shows putative binding sites for GATA factors like Nit-2p which is homologous to Gln3p. Therefore there is a possibility that gln3p regulates *SUC2* repressors. In conditions of high glucose and low nitrogen availability the dephosphorylated Gln3p would simultaneously activate nitrogen regulated genes and the *SUC2* repressors preventing high invertase levels.

Poster Presentation 2-31

The Biological Water-Gas Shift Reaction in the Photosynthetic Bacterium *Rubrivivax gelatinosus*

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Upon feeding CO to the culture gas phase, the purple non-sulfur photosynthetic bacterium *Rubrivivax gelatinosus* exhibits a water-gas shift (CO shift) reaction according to the equation: $CO + H_2O \leftrightarrow H_2 + CO_2$. Carbon monoxide was demonstrated to induce the proteins necessary for the overall shift reaction. At least 10% CO is required during culturing to ensure maximal induction. Although a phototroph, once induced, this bacterium carries out the CO shift reaction equally well both in light and in darkness. At ambient temperature, the equilibrium of the reaction favors the direction of H_2 production. Consequently, biological CO shift reaction is an ideal process to produce additional H_2 from synthesis gas, and the latter can be produced from the gasification of waste biomass. Bacterial CO shift pathway involves a series of intricate enzymatic reactions, initiated by the enzyme CO dehydrogenase (CODH) catalyzing the CO oxidation step, and the reducing equivalents from which are then transferred, via a ferredoxin-like iron-sulfur protein, to a terminal hydrogenase yielding H_2. Our effort in biochemistry has resulted in the partial purification of the CODH enzyme. Our effort in molecular biology has led to the identification of the partial sequences of seven putative genes involved in CO shift, three of which encode CODH, ferredoxin and the hydrogenase. Their amino acid sequences show high level of homology with those from *Rhodospirillum rubrum*. More work is underway to decipher genes in the flanking region and to predict their function with the intent to genetically engineer microbes to produce more H_2, constitutively.

Poster Presentation 2-32

Proteomic Analysis of Physiological Properties Between *Candida magnoliae* Wild and its Mutant Strain by Two-Dimensional Electrophoresis and Microsequencing

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Erythritol is a non-cariogenic, non-caloric (0.3 kcal/g) sweetener and safe for diabetics. The four-carbon sugar alcohol has about 70-80% of the sweetness of sucrose and is found in fruits, mushrooms and some fermented foods.

The objective of this research includes identification and analysis of the expression patterns of proteins between *Candida magnoliae* wild and its mutant strain.

Two-dimensional gel electrophoresis was performed for quantitative and qualitative analysis of total intracellular proteins. Most of the proteins were distributed in the range of pI 4-7 and MW 29-97 kD.

Twenty four proteins were identified by MALDI-TOF and ten proteins overexpressed were analyzed by scanning, digital image analysis tools and ESI-MS/MS.

NCBI blast database and diploid nblast were used to search the proteins which have sequence homology with those of *Saccharomyces cerevisiae* and *Candida albicans*.

Poster Presentation 2-33

Fed Batch Fermentation For Xylitol Production By *Candida guilliermondii* and its Relationship with Aeration Rate

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Xylitol is an anticariogenic and insulin-independent polyol, produced industrially by chemical reduction of D-xylose derived from agro-industrial wastes. The limiting step in this process is the purification of xylitol from other polyols and sugars. Biotechnological production of xylitol is attractive due to the enzymes specificity involved in D-xylose metabolism, which can lead to high yields and easier purification steps.

The aim of this work was to evaluate the fed batch process for xylitol production by *Candida guilliermondii* and its relationship with aeration rate, on experiments carried out in a bench scale bioreactor. Different feeding strategies (pulsed and continuous flow rate) and oxygen supply (constant and variable aeration rate) have been investigated. Their performance was also compared to the results obtained in a simple batch apparatus.

In all experiments, high values of xylitol concentration, between 150 and 160 g/L, were achieved. Comparing to the simple batch results, for the same substrate uptake (460 g), the pulsed fed batch, with differential aeration rate, resulted in an increase of 6.7 % of the xylitol yield factor ($Y_{XOL/S}$), and the extended fed batch, with constant aeration rate, led to an 14.4 % increase of volumetric productivity (Q_P).

Poster Presentation 2-34

Overcoming Nation's Roadblocks to Photosynthetic H_2 Production

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Algal photosynthetic hydrogen (H_2) production is a potentially clean energy resource. However, there are a number of technical issues that must be addressed before algal H_2 production can become practical. In this paper, we will discuss the following six physiological problems that currently challenge researchers and investors in the field of photosynthetic H_2 production. These problems are: (1) restriction of photosynthetic H_2 production by accumulation of a proton gradient, (2) competitive inhibition of photosynthetic H_2 production by CO_2, (3) requirement for bicarbonate binding at photosystem II (PSII) for efficient photosynthetic activity, (4) competitive inhibition by O_2, (5) classic O_2 sensitivity of the hydrogenase enzyme, and (6) light-saturation phenomenon due to large antenna size. Potential solutions to overcome these roadblocks to photosynthetic H_2 production will also be presented. The solutions are based on a novel approach that has recently been developed at Oak Ridge National Laboratory (2001 ORNL Invention Disclosure). In this approach, a "designer alga" for efficient and robust H_2 production will be created by genetic insertion of hydrogenase promoter–programmed polypeptide proton channels in photosynthetic thylakoid membranes. This designer alga will be integrated also with the benefits of an O_2-tolerant hydrogenase that will be created by NREL and a smaller chlorophyll antenna size that will be created by UC Berkeley. Therefore, this ORNL effort is complementary with those of NREL and UC Berkeley, and will contribute jointly with the sister NREL and UC projects to achieving a common goal of effective photobiological H_2 production. By use of this approach, we will be able to simultaneously solve the six physiological problems for efficient and robust production of H_2 through photosynthetic water splitting.

Poster Presentation 2-35

Production of Biosurfactant by *Bacillus subtilis* Using a Cassava-Processing Effluent

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Some microrganisms are known to produce surface-active compounds. Surfactin is a well-studied lipopeptide biosurfactant obtained by *Bacillus subtilis* cultures. In this study, a cassava flour- processing effluent was evaluated as substrate for surfactant production by two *Bacillus subtilis* strains. *B.subtilis* ATCC 21332 reduced the surface tension of medium to 25,9 mN/m producing a crude biosurfactant concentration of 2,2 g/L . The wild-type strain *B.subtilis* LB5a reduced the surface tension of medium to 26,6 mN/m showing a crude biosurfactant concentration of 3,0 g/L. After 48 hours of cultivation a strong decrease on surface activity and surfactant concentration levels was observed for *B. subtilis* ATCC 21332, whereas for wild- type strain the levels were maintained approximately constant. An increase in proteolitic activity of culture medium was also observed after 48 hours for *B.subtilis* ATCC 21332. These findings suggested that the product degradation at late cultivation times was probably caused by the enzymatic activity on the peptide moiety of surfactant. The biosurfactant produced on cassava effluent medium by *B. subtilis* LB5a was similar to surfactin.

Poster Presentation 2-36

Comparative Statistical Metabolic Control Analysis of Ethanol Production in *E. coli* and *S. cerevisiae*

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One of the most important problems in metabolic engineering of biochemical reactions networks considers the identification of the enzymes that one should manipulate towards the achievement of a desirable performance. However, the lack of information about the kinetic characteristics of the enzymes involved in the pathway of interest makes such identification almost impossible. On the other hand, extensive research within metabolic engineering has made the estimation of metabolic fluxes feasible. Such information while it provides significant understanding about the function of the metabolic pathways and, some times, guidance for metabolic engineering, it does not allow a quantitative prediction of the metabolic pathway responses to metabolic engineering actions, such as changes in enzyme activities.

We have recently developed a method that overcomes these limitations. The framework employs knowledge about the stoichiometry of biochemical networks and the estimated values of the associated metabolic fluxes, modeling concepts from metabolic control analysis, computational methods, and nonparametric statistics. The method has been applied to the central carbon pathways in *E.coli* and *S. cerevisiae* for the identification of the metabolic engineering strategies (i.e., changes in enzyme activities) with the highest probability of success in optimizing conversion of glucose to ethanol.

Poster Presentation 2-37

Development and Application of Genetic Systems for Anaerobic, Thermophilic, Ethanol-Producing Bacteria

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A substantial group of thermophilic anaerobic bacteria rapidly ferment biomass components (e.g. cellulose, cellodextrins, xylan, xylose) to a mixture of ethanol and organic acids. Metabolic engineering in order to increase ethanol yields is of interest but requires a genetic system, which is not available for many species of interest. We report here results obtained with two thermophiles: *Thermoanaerobacterium saccharolyticum* strain YS485, which utilized xylose but not cellulose; and *Clostridium thermocellum* (several strains considered), which utilize cellulose but not xylose. Cloning of catabolic genes associated with organic acid formation (hydrogenase, acetate kinase, phosphotransacetylase, lactate dehydrogenase) will be briefly described. For *T. saccharolyticum*, for which a genetic system is available (Mai et al., 1999), development of knockout vectors and attempts to obtain knockouts will be described. For *C. thermocellum* we present a new electrotransformation procedure using a custom pulse generator, shuttle vector pIKM1, and selection based on resistance conferred by an *mls* antibiotic resistance gene. Readily repeated transformation of several strains of *C. thermocellum* has been observed with confirmation based on both retransformation of *E. coli* using plasmid recovered from presumptive transformants as well as PCR using primers specific to the *mls* gene. Transformation efficiency has been shown to be highly sensitive to the conditions used, with a 3000-fold improvement having been obtained at the time of submittal. Factors impacting transformation frequency will be described, as will efforts to optimize and apply the procedure.

Poster Presentation 2-38

Cell immobilization on the Production and Utilization of Lactic Acid Probiotic Bacteria in the Dairy Industry

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Cell immobilization by gel entrapment allows a rapid microbial development, also it allows high biomass concentration in well limited spaces. During the biomass growth, the microbial colonies brake the gel and they are leaked from the gel support due to the high biomass concentration. This characteristic is inherent to the cell immobilization by gel entrapment and was used for the continuous inoculation of sterile skim milk with acid lactic probiotic bacteria for yogurt and acid milk production. We have immobilized *Lactobacillus delbrueckii* subsp. *bulgaricus* in α-carrageenan and we have used the immobilized particles containing the acid lactic probiotic bacteria in a column reactor. Thus, we have inoculated skim milk with a probiotic bacterial concentration of 2×10^8 UFC/ml conntinously for almost a week. The lactic fermentation proposed represent a real application of the cell immobilization on the dairy industry.

Poster Presentation 2-39

Cell Immobilization Application on the Maintenance of Viability of Lactic Bacteria

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One of the main problems related to the lactic starters market is the loss of bacteria's viability during the processes used for the microbial preservation. The lactic acid bacteria are too sensitive to freeze-drying process. In this work, we study the role of the cell immobilization on the maintenance of viability of lactic acid bacteria during the freeze-drying process in order to preserve the biological activity. Thus, we have freeze-dried *Lactobacillus delbrueckii* subsp. *bulgaricus* free and entrapped in α-carrageenan. We observed that the free cells lost 99.5% of viabilitya after the freeze-drying process, compared with the immobilized cells that maintained 45% of their viability. The results obtained confirm that cell immobilization allows the preservation of bacteria viability during the freeze-drying process.

Poster Presentation 2-40

Cell Immobilization on the Gibberellic Acid Production

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The gibberellic acid represents one the most important plant growth regulator produced from microorganisms due to its extensive practical uses in agriculture. Nowadays, gibberellic acid is produced by submerged fermentation with the filamentous fungi *Gibberella fujikuroi*. The gibberellic acid production is effective on nitrogen limitation. Cell immobilization allows high biomass concentration and gradients formation inside the immobilization support by diffusional limitations. Thus, that kind of phenomena can help to create different micro-environments inside the carrier were fungi cells can develop under nitrogen limitations and produce gibberellic acid.

In this work we present the production of gibberellic acid by immobilized cells compared to that of free cells.

Poster Presentation 2-41 Student

Cellulase and Hemicellulase Production in *Penicillium brasilianum* When Changing the Substrate Composition

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Ethanol is an alternative to oil and gas, and it can be produced by fermentation of sugar monomers. Ethanol is a "high volume low cost" product and this highlights the necessity of cheap substrates for the fermentation in which ethanol is produced. Biomass and different waste streams are relatively cheap carbon sources. Prior to fermentation the sugar monomers need to be hydrolysed from the sugar polymers. An enzymatic hydrolysis with cellulases and hemicellulases is an environmentally friendly process. Today, the enzymatic hydrolysis is a costly process, which calls for the development of more efficient enzyme systems.

After screening *Penicillium* species for their ability to produce cellulases and hemicellulases three species was chosen for further investigation in the production and regulation of these enzymes. These filamentous fungi were cultivated under well defined conditions on Mandels and Weber medium with either cellulose or hemicellulose as carbon source. During cultivations, pulses with different monomeric carbon sources were performed to investigate how the cellulase and hemicellulase production were regulated. For samples taken throughout the cultivations the amounts of cellulases and hemicellulases were measured by capillary electrophoresis. In addition the activity of the single cellulytic and hemicellulytic enzymes were determined in enzymatic assays. The ability of the enzyme mixtures - produced under different conditions - to hydrolyse lignocellulosic materials was evaluated.

Poster Presentation 2-42

A Miracle "Chimeric" Gene Enabling the *Saccharomyces* Yeast to Convert Cellulosic Biomass to Ethanol, Lactic Acid, and …

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Cellulosic biomass, which contains large amounts of glucose and xylose, is the new ideal feedstock for the production of ethanol to substitute or replace gasoline. It has been found that the naturally occurring *Saccharomyces* yeasts used for industrial production of ethanol are unable to ferment xylose. We have successfully developed genetically engineered *Saccharomyces* yeasts that can effectively co-ferment both glucose and xylose simultaneously to ethanol. We accomplished this by the cloning and over-expression in the yeast of three xylose-metabolizing genes, the xylose reductase gene (XR), xylitol dehydrogenase gene (XD), and xylulokinase gene (XK). In comparison with the recombinant yeast developed by others, the cloning and over-expression of XK was the key that made our yeast capable of fermenting xylose. Only recently, we realized that the most extraordinary event happened during our cloning of the xylulokinase gene more than ten years ago. Without this "miraculous" development, we would never have been able to develop the ideal glucose/xylose co-fermenting yeast that we designed. I will share with you the rationales that led to our design of this yeast as well as the exciting and unexpected "miracle" that made our yeast the one and only *Saccharomyces* able to effectively co-ferment glucose and xylose from domestically available renewable cellulosic biomass to ethanol, lactic acid, as well as many other wonderful new products to be developed.

Poster Presentation 2-43

Kinetic Modeling to Investigate the Interactions of the Engineered Pentose Pathway with the Glycolytic (ED) Pathway in *Zymomonas mobilis*

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Zymomonas mobilis has engineered several years ago with 4 new enzymes to ferment xylose along with glucose and a network of pentose pathway enzymatic reactions interacting with the native glycolytic Entner Doudoroff pathway has been hypothesized. We are currently investigating this proposed reaction network by developing a kinetic model for all the enzymatic reactions of the pentose phosphate and glycolytic pathways. Kinetic data on different sugar metabolism rates and enzymatic activity data will be used to refine the model parameters available in the literature and validate the proposed reaction network. In addition to the well characterized glucose and xylose fermentation data, striking differences in fructose fermentation rates for the host and engineered strains of *Z. mobilis* provide additional insights into the reaction network under investigation. New ^{31}P NMR spectroscopy results from intact cells and cell extracts provide dynamic information on the concentrations of various intracellular phosphorylated intermediates. These experimental data will be compared with model simulations for the hypothesized reaction network in this presentation.

Poster Presentation 2-44

The Production of Ethanol from Cellulosic Biomass Hydrolysates Using Genetically Engineered Saccharomyces Yeast Capable of Co-Fermenting Glucose and Xylose

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Recent studies have proven ethanol to be the ideal liquid fuel for transportation and renewable cellulosic biomass to be the attractive feed-stocks for ethanol-fuel production by fermentation. The major fermentable sugars from hydrolysis of cellulosic biomass (such as rice stow, sugarcane bagasse, corn fiber, softwoods, hardwoods, and grasses) are D-glucose and D-xylose. The efficient fermentation of both glucose and xylose present in cellulosic biomass to ethanol is essential for these renewable resources to be used as feed-stocks for bio-fuel production. The naturally occurring *Saccharomyces* yeasts have proven to be safe, effective, and user-friendly microorganisms for the large-scale production of industrial ethanol from glucose-based feedstocks. However, these yeasts cannot metabolize xylose. Our group at Purdue University succeeded in the development of the genetically engineered *Saccharomyces* yeasts that can effectively co-ferment glucose and xylose to ethanol. This was accomplished by the cloning and over-expression of three major xylose-metabolizing genes; xylose reductase, xylitol dehydrogenase, and xylulokinase genes in yeast. In this presentation, we demonstrate that our stable recombinant *Saccharomyces* yeast can efficiently ferment glucose and xylose present in hydrolysates from different cellulosic biomass to ethanol.

Poster Presentation 2-45

^{31}P Nuclear Magnetic Resonance Studies of Sugar Metabolismin *Zymomonas mobilis*

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^{31}P-Nuclear magnetic resonance (NMR) spectroscopy is a valuable tool for continuous observation of the metabolic and energy status of the metabolically active cells. It enables to monitor the uptake rates of substrates as well as the formation rates of the various intracellular and extra-cellular phosphorylated metabolites. Glucose and xylose catabolism in *Zymomonas mobilis* strains were studied using ^{31}P-NMR spectroscopy in *vivo* and of perchloric acid extracts. In *vivo* measurements revealed the noninvasive information about the kinetics of sugar utilization by *Z. mobilis* as well as the energetics of sugar metabolism of cells grown on glucose and xylose. Analysis of cell extracts provides more accurate information about metabolism through the pentose phosphate and Entner-Doudoroff pathways via the levels of individual phosphorylated metabolites.

Poster Presentation 2-46

Screening Ethanologens on Corn Fiber Hydrolysate

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The demand for ethanol as a renewable source of fuel is increasing and with this increased demand comes a need to optimize fermentations. Corn wet milling produces byproducts that until now have been ignored as fermentation substrates. The present study was undertaken as an effort to further optimize ethanol production from one of these byproducts, corn fiber.

Nine species of microorganisms, including bacteria, yeasts, and molds, were screened for their ability to utilize concentrations of 20% and 50% corn fiber hydrolysate in ethanol fermentations. Carbohydrate usage as well as ethanol production was used as a measure of fermentation efficiency and the results obtained were used to select an organism for use in pilot plant-scale fermentations. The organism ultimately selected was a recombinant engineered for pentose fermentation.

Poster Presentation 2-47

Measurement of Xylose Transport in Xylose-fermenting *Zymomonas mobilis*

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Recombinant strains of *Zymomonas mobilis* efficiently convert glucose, xylose and arabinose to ethanol however, glucose is fermented more quickly and completely than the pentose sugars. Conversion of pentose sugars to ethanol requires transport into the cell followed by metabolism through the pentose phosphate pathway. In *Z. mobilis* all sugars enter the cell by facilitated diffusion through a single transport protein called the glucose facilitator, Glf. Rapid filtration assays measuring uptake for 3 seconds indicate that glucose is transported with a V_{max} of 200-300 nmol/min/mg protein and a K_m of 15 to 20 mM. Improving pentose fermentation requires knowledge of which step is rate limiting. We are examining the kinetics of glucose and xylose transport in a variety of strains of *Z. mobilis* with different genetic backgrounds and fermentation profiles to determine whether transport limits ethanol production.

Poster Presentation 2-48

Bridging Between Global Gene Expression and Metabolic Phenotype of Recombinant *Saccharomyces cerevisiae* During Xylose Fermentation

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Metabolic engineering of xylose fermentation has progressed though the expression of xylulokinase genes (*XYL3* or *XKS1*) within an engineered genetic background of *XYL1* and *XYL2*, which code for xylose reductase and xylitol dehydrogenase, respectively. However, xylose fermentation by recombinant *S. cerevisiae* is not as efficient as that of the xylose-fermenting yeast, *Pichia stipitis*. Low ethanol yield and accumulation of xylitol are the main problems to be tackled. With a hypothesis that the metabolic phenotype is a manifestation of gene expression, we used DNA microarray and quantitative real time PCR to characterize patterns of gene expression at the genomic level following genetic and environmental perturbations. Expression patterns of *HXK1*, *KGD1*, *SDH1*, *MDH1*, and *QCR2* in response to changes in carbon source and oxygen availability revealed that xylose is not recognized as a fermentable carbon source. The mRNA levels of genes involved in NAD^+/NADH shuttle systems (*NDI1*, *NDE1*, *GPD1*, and *GUT2*) increased significantly in cells grown on xylose, which suggests that cells have to cope with redox imbalance during xylose metabolism. Based on these observations, we further engineered recombinant *S. cerevisiae* to force cells to use xylose in a non-oxidative manner by disrupting mitochondria function. As expected, the respiration deficient mutant showed a different gene expression pattern, which reflects a different metabolic phenotype of this mutant.

Poster Presentation 2-49

Cellulase Production on Pure Cellulose Using *Trichoderma reesei* Strains L27, RL-P37, Rut C-30, and MTC-a-13

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Cellulase, which is produced by many *Trichoderma reesei* strains, can be used to enzymatically hydrolyze cellulosic biomass to glucose. Glucose can then subsequently be fermented to a variety of products including ethanol for use as a transportation fuel. The objective of this study was to assess the relative performance of cellulases produced from several *T. reesei* strains. The enzyme was produced in 7-L fermentors on a pure cellulose source (Solka-floc) using *T. reesei* strains L27, RL-P37, Rut C-30, and MTC-a-13. The performance of the cultivation was tracked from measurements of cellulose, cell mass, and protein concentrations as well as by filter paper and β-glucosidase activity. The efficacy of the cellulase preparations was also evaluated in a simultaneous saccharification and fermentation (SSF) process. Although, the same level of filter paper activity and yield was produced by L27 and RL-P37, a significantly greater ethanol yield from SSF (76% compared to 31%) was achieved by the L27-produced cellulase because of a higher level of β-glucosidase activity expressed by this microorganism.

Poster Presentation 2-50

Utilization of Dynamic Secondary Membranes for Flux Optimization in Membrane Microfiltration

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Fouling is one of the major problems encountered during membrane separations. The present work concerns crossflow microfiltration of protein-cell mixtures. The external, cake layer of deposited yeast on the membrane is used to shield the primary microfiltration membrane from internal fouling by protein filtration. Reverse filtration is used periodically, to remove the external cake layer. At the start of next cycle, a new layer of yeast is deposited which acts a fresh dynamic secondary membrane.

Yeast was deposited for times ranging from 5-30 s with concentrations of 0.034-1.68 g/L. Transmembrane pressures (TMP) 5-10 psi for forward and reverse filtration, with cellulose acetate 0.2 micron membrane filters. Normalized average permeate flux after 4000 s of filtration is reduced to 5 ± 0.3 % of the clean membrane flux. With dynamic secondary membranes of yeast, an increase up to 4 fold in this flux has been achieved.

Poster Presentation 2-51

Xylanase Production and Oxygen Limited Xylose Utilization of *Neurospora crassa*

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Neurospora crassa could produce cellulase, xylanase and convert cellulose and semi-cellulose to ethanol directly, as a result of which, much interests were paid on this microbial-organism in the recent years. In this study, xylanase production and xylose utilization of *N. crassa* were studied. It was found that xylanase could be induced by many kinds of cellulosic materials. The xylanase activity induced by the powder of corn core could reach 25.02 IU/mL. Cellulose-xylan could co-induce xylanases production. Glucose and xylose repressed xylanases production strongly. Xylose utilization under various oxygen limitation conditions was investigated. *N. crassa* had considerable ability on xylose utilization producing ethanol and xylitol. The degree of oxygen limitation had great influence on the ratio of ethanol to xylitol produced. The maximum ethanol conversion yield was 61.6 % under semi-aerobic conditions. The maximum xylitol conversion yield was 31.4 % obtained under much severe oxygen limitation conditions than that for ethanol production. Xylitol accumulation was always behind the accumulation of ethanol.

Poster Presentation 2-52

Micro Pilot Anaerobic Reactor with Axial Flow

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This work describes the results obtained in the construction of a 18 liter anaerobic column reactor, which consists of four phases or differentiated zones. The the reactor zones are: sedimentation zone, treatment zone, clarifier and a biogas capturer.

The reactor uses the flow input to produce an axial movement which mixes and keeps the system homogeneous. This innovation lets the system work in anaerobic conditions avoiding the inconveniences of a mechanical anaerobic system, in which the mechanical pieces are used up letting air entrance mainly at the beginning of the process.

The system was made of acrylic material to make it suitable for teaching purposes enabling us to monitor the components and the functioning of the system. In addition, it contains some mobile components like the dragging tube and the upper reflector which slides to different levels along the rails. The system has the advantage of making possible to change the system conditions for the sake of teaching and research purposes.

At present, the reactor has been completely constructed and data about basic mixing and circulation times as well as flowing behavior has been obtained.

Poster Presentation 2-53

Bioenergetics of Microbial Cellulose Utilization of *Clostridium thermocellum*

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Continuous cultures of *C. thermocellum* ATCC 27405 were grown at various dilution rates on ~ 5 g/L Avicel and cellobiose. A new cellulase-specific ELISA (Zhang and Lynd, *Analytical Chemistry* in press) was used to determine cell and cellulase mass concentrations. As compared to the traditional Pirt model, the following additional processes appeared to be significant factors in the bioenergetics of microbial cellulose utilization by C. thermocellum: cellulase synthesis, phosphorolytic cleavage of β-glucosidic bonds, and substrate transport. Our results showed that the assimilated longer cellulose segments are cleaved preferentially by phosphorolytic (e.g. cellobiose and cellodextrin phosphorolases) instead of hydrolytic (e.g. β-glucosidase) enzyme activities, resulting in a significant bioenergetic benefit during growth on cellulose. Bioenergetic analysis suggested that cellodextrins with chain length $\geq$ 4 were taken up directly by the cells before further degradation to cellobiose and glucose. More energy was required for cellulase synthesis but less energy was spent on sugar transport for growth on Avicel as compared to cellobiose. The metabolic cost of cellulase synthesis appeared to be less than the bioenergetic benefits associated with phosphorolytic cleavage of cellodextrins and cellobiose.

Poster Presentation 2-54

Optimization from the Inside Out: Multivariant Metabolic Engineering for Biocatalyst Development

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Process optimization conventionally has examined multiple external variables to maximize product formation. Temperature, pH, aeration rate, substrate concentration, trace elements, nitrogen and carbon sources are altered to shift the flow of metabolites from one state to another. These environmental variables affect cellular responses by increasing or decreasing metabolic activities, providing energy for growth or inducing specific pathways. Mutagenesis and strain selection traditionally have been coupled to process optimization for yield improvement. Metabolic pathway engineering offers a more rational alternative than random mutagenesis for genetic modification, but it does not readily address the issue of pathway optimization. Because metabolic networks are closely integrated and highly interdependent, it is very difficult to alter flux through one pathway without affecting others. Enzymes for each reaction step can be present at many different levels, and expression profiles can change dramatically with growth conditions. Over expression of genes for one pathway can adversely affect another, thereby limiting cell growth or viability. The need to account for diverse unknown interactions creates a multivariant optimization problem similar to the manipulation of environmental factors. Our research seeks optimal expression of up to 15 enzymes in a pathway. A technique for the rapid construction of cassettes that will allow the simultaneous evaluation of many different enzymes at various levels has enabled us to bypass the tedious and labor intensive step-wise alteration of individual genes. When coupled to genome wide expression analysis, we can rapidly determine which steps are rate limiting and what sorts of concerted changes are required to increase flux. Genome-wide expression studies on engineered organisms cultivated under various environmental conditions reveals how they adapt to the heterologous pathways and which activities are most likely to be rate-limiting. They also reveal how to alter expression to fit desired patterns and expression levels. This presentation will use pathway engineering of xylose metabolism in *Saccharomyces cerevisiae* as a model for the rapid optimization of metabolite flux.

Poster Presentation 2-55

Enzymatic Synthesis of Monolaurin

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Monolaurin, the monoglyceride studied in this work, is an anionic surfactant with important applications in cosmetic, pharmaceutical and food industries. The antimicrobial activities of monolaurin have also been reported, especially as intravaginal microbiocides for protection against sexually transmitted diseases. The synthesis of monolaurin was carried out by esterification of lauric acid with glycerol in a solvent-free system. A commercial immobilized lipase (Lipozyme IM 20, Novo Nordisk) was mixed with the organic reagents, in an open batch reactor with constant stirring. The effects of fatty acid/glycerol molar ratio (0.65 to 1.35), temperature (50 to 60°C) and Lipozyme concentration (1 to 5 %w/w) on the selectivity of monolaurin were determined using an experimental design technique. Reactions were carried out for 6 h and the concentration of lauric acid, mono-, di- and trilaurin were determined by capillary gas chromatography (GC). Conversions close to 70 % were attained. Higher selectivities were obtained under the following experimental conditions: lauric acid/glycerol molar ratio of 1, temperature of 55°C, and enzyme concentration of 3 %w/w. In most of the experiments, the concentration of monolaurin was about twice that of dilaurin, and only trace amounts of trilaurin were found.

Poster Presentation 2-56

Strain Development for the Complete Utilization of Mixed Carbohydrates in Lignocellulosic Biomass

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There is intense interest in microorganisms and their fermentation processes that produce lactic acid from plant polysaccharides since lactic acid can be used as a platform chemical in the manufacture of chemicals, solvents, fibers, and packaging materials. Presently, poly-lactate and ethyl lactate are produced from glucose derived from cornstarch but the potential exists in the future to use all carbohydrates present in lignocellulosic biomass.

Maximum utilization of carbohydrates is required for economically processing of lignocellulosic biomass to lactic acid, but most naturally occurring microorganisms are subject to carbon catabolite repression and do not use all carbohydrates simultaneously, if at all. Our studies focused on screening lactic acid bacteria for the industrial lactate production. Lactobacillus brevis was found to be a non-carbon catabolite repressed strain that is able to use the mixed carbohydrates from a rice straw hydrolysate simultaneously. In the presence of xylose, arabinose, and glucose, *L. brevis* did not show any suppression for consumption of these carbohydrates and maintained a maximum utilization rate. A series of experiments revealed that the *L. brevis* strain was not inhibited by the rice straw hydrolyzate and was a good candidate strain for simultaneous saccharification and fermentation. Recently, we isolated a mutant of *Lactobacilli* sp. that showed the same simultaneous carbohydrate utilization characteristics as *L. brevis*, albeit at a higher lactate yield from hexose sugars. In fermentation studies, *L. brevis* showed less than 1.0 (mM/mM) product yield, however, the newly developed strain showed 1.8 (mM/mM) of lactate yield from glucose.

This research has been supported by a grant from the U.S. Department of Energy Office of Biomass Program.

Poster Presentation 2-57

Evaluation of Newly Developed Integrants of Recumbinant *Zymomonas mobilis* for Ethanol Production Process with Corn Stover Hydrolysate

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Cofermentation of glucose and xylose is critical for an economic bioconversion of lignocellulosic biomass to ethanol. The bacterium *Zymomonas mobilis* is an efficient ethanologen, but wild type strains of this bacterium are not able to coferment glucose and xylose produced during the pretreatment of lignocellulosic biomass. We have recently developed several integrants based on *Z. mobilis* ZM4 capable of cofermentation of glucose and xylose. The intent of this work was to evaluate the performance of these newly developed integrants on corn stover hydrolysate. The integrants were tested first for stability by repeated transfers in non-selective medium containing glucose only. They were stable up to 160 generations tested. The effects of temperature, pH, and acetic acid concentration on ethanol production of the integrants were investigated in a sugar containing laboratory medium. One of the top performers was selected for further evaluation on corn stover hydrolysate. Several process parameters such as hydrolysate conditioning requirements, dilution, and concentration of initial sugar loadings were evaluated. The strain fermented overlimed corn stover hydrolysate with sugar mixture of glucose (8%) and xylose (5%) to ethanol at 84% yield.

Poster Presentation 2-58 Student

Kinetic Studies of a Metabolically Engineered *Zymomonas mobilis* Fermenting Glucose and Xylose Mixtures

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Developing a cost-effective fermentation process for ethanol production from lignocellulosic materials requires a microorganism that is capable of efficiently converting both hexose and pentose sugars to ethanol. In the present study, a metabolically-engineered strain of *Zymomonas mobilis* capable of fermenting both glucose and xylose to ethanol was characterized in batch culture studies. Experiments were carried out at temperatures of 30 and 40°C over a pH range of 5.0-6.0, and in the presence of varying initial amounts of acetic acid, using 10% w/v total sugar concentration (pure glucose, pure xylose, or glucose/xylose mixtures). The concentrations of the following components were measured: (i) glucose, (ii) xylose, (iii) ethanol, (iv) intracellular adenosine triphosphate (ATP), (v) dry cell mass, (vi) xylitol, and (vii) acetic acid. Specific sugar uptake and product formation rates were determined, as well as the yields of cell mass, ATP and ethanol. In order to improve the consistency and accuracy of the kinetic data, an autosampler system was employed on the fermentors so that more samples could be taken over the course of each run. Results demonstrate that this *Z. mobilis* strain can ferment moderately high concentrations of biomass sugars (up to 100 g/L) to ethanol at yields greater than 85% of theoretical over a range of pH, temperature, and acetic acid conditions. Fermentation pH strongly influences the inhibitory effect of acetic acid on strain performance. The implications of these and other results on applying *Z. mobilis* to convert biomass-derived sugars to ethanol will be discussed.

Keywords: Xylose fermentation; inhibition; cofermentation; multiple substrates; acetic acid; autosampler system; batch cultivation; ATP determination; ethanol; byproducts

Poster Presentation 2-59

A Novel Ethanologenic Yeast for the Fermentation of Lignocellulosic Hexose Sugars

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A novel, genetically unmodified ethanologenic yeast, integrated into the pre-hydrolysate fermentation stage of the softwood-to-ethanol bioconversion process, was identified as being capable of rapid assimilation and catabolism of all wood-derived hexose sugars (galactose, glucose, and mannose). This yeast strain was shown not to be subject to glucose-mediated catabolite repression, and employed a unique enzymatic mechanism to rapidly and concomitantly convert galactose, glucose, and mannose to ethanol.

Regardless of substrate conditions, the selected yeast strain immediately initiated galactose metabolism at the onset of fermentation, demonstrating complete consumption of the sugar alongside that of glucose and mannose. The conventional sequential sugar utilization observed in most ethanologenic yeasts, consistent with preferential cell membrane transport and catabolism, was notably absent, as was the conventional slow rate of galactose consumption.

Ethanol yields were comparable with those achieved by current industrially utilized yeast strains.

Poster Abstracts for Session 3

Bioprocessing, Including Separations

Poster Presentation 3-08

The Effect of Process Parameters in the Production of a Biopolymer by *Rhizobium sp.*

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Bacterial extracellular polysaccharide production has attracted strong interest, due to its wide application in different fields. These biopolymers have been widely studied so as to obtain new polymers with high industrial skills. The objective of the research was examine the production potentialities of extracellular polysaccharide by *Rhizobium sp.*. The assays were carried out in a fermenter (New Brunswick Scientific, BioFlow IV model), using mannitol-yeast extract broth (YM) enriched with manganese ions (Mg^{+2}). The pH, agitation and dissolved oxygen were controlled on line and the software *Statistica*™99 5.5 for Windows was used to evaluate the results. It was used 2^3 factorial experimental planning with central point; mannitol concentration, aeration and agitation were input variables and the substratum conversion factor to exopolysaccharides ($Y_{P/S}$) was the output reply. Results have shown that pH did not vary during the process, revealing that the product had a neutral feature. A better performance of the process, was observed with agitation of 200 rpm, and 10g/L mannitol concentration. The absolute viscosity of the polymer solution at 1%, measured in a Brookfield Viscosimeter, at 30ºC and shear rate 0.204 s^{-1}, was equal to 30,500 cP.

Poster Presentation 3-09

Design of Nanostructured Catalysts for the Conversion of Biorenewable Feedstocks

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Bioprocessing of biorenewable feedstocks will likely require the development of new chemical processes as well as biological processes for economical manufacture of chemicals and/or fuels. However, biorenewable feedstock conversion with heterogeneous catalysts provides new challenges in inorganic catalyst research and development relative to the voluminous historical work with petrochemical feedstocks. These unique challenges include the need to convert selectively highly functionalized molecules and to develop catalytic liquid-solid interfaces in which the liquid phase is commonly aqueous. The implications of these requirements on catalyst design will be discussed as motivation for the utilization of newly synthesized nanostructured materials. These catalyst structures with high surface areas and regular arrays of uniformly sized mesopores hold promise for precise control of the reaction domain at the molecular level. Presented will be results for the application of acid functionalized mesoporous catalysts for the conversion of lipids and carbohydrates. The influence on catalyst performance due to the method of catalytic site incorporation into the porous framework (grafting versus co-condensation), the modification of site acidity, and the control of the pore diameter size will be shown. Finally, the potential use of nanostructured catalysts in achieving the elusive goal of biomimetic catalysts will be discussed.

Poster Presentation 3-10

Production of Butanol from Concentrated Lactose/Whey Permeate Using *Clostridium acetobutylicum* and Removal by Perstraction

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Acetone-butanol-ethanol (ABE) were produced from whey permeate medium, supplemented with lactose, in a batch reactor using *Clostridium acetobutylicum* P262, coupled with ABE removal by perstraction. ABE (87.2 g/L) were produced from lactose (277 g/L) at a yield of 0.43 and productivity of 0.22 g/L.h. These results are superior to ABE batch fermentation where a maximum ABE concentration of 20 g/L is achieved. Silicone tubing (membrane area 0.1130 m^2 based on inside diameter) was used as the perstraction membrane while oleyl alcohol was used as the perstraction solvent. Removal of ABE by perstraction was faster than their production in the reactor, and the maximum concentration of ABE in the oleyl alcohol was 7.5 g/L. However, the fermentation ceased prior to complete lactose utilization, probably due to back-diffusion of oleyl alcohol into the fermentation medium. Hence, perstraction compares unfavourably with gas stripping (Maddox *et al*. 1995) as the product removal technique in integrated batch fermentation/product removal processes utilizing high sugar concentrations.

Reference:

Maddox IS, Qureshi N, Roberts-Thomson K (1995) Production of acetone-butanol-ethanol from concentrated substrates using *Clostridium acetobutylicum* in an integrated fermentation-product removal process. Process Biochem. 30: 209-215

Poster Presentation 3-11

Effect of Pretreatment on the Extraction of Silymarins from Milk Thistle

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The silymarins silychristin and silbinin show therapeutic benefits including an ability to reduce biliary cholesterol levels, an ability to intervene in hormone refractory human prostate cancer and an ability to decrease the nephritic effects of chemical injury. The traditional method for extracting silymarin compounds from milk thistle includes a 24 hour defatting step with petroleum ether followed by a 4 hour Soxhlet extraction with methanol. There is room for significant optimization in the extraction process to more economically and efficiently recover the silymarin compounds

This presentation concentrates on examining alternative defatting protocols which would render the biomass more susceptible to compound extraction. Results from a variety of pretreatment/defatting techniques are presented including treatment with dilute acid, dilute base, $NaHCO_3$ and NaCl, and enzyme preparations. The pretreated solids were subsequently extracted in ethanol to yield silymarin compounds, with the standard procedure described above used as the control. This comparison of pretreatment protocols can most likely be applied to other phytochemical extraction systems.

Poster Presentation 3-12

Extraction of High Value Co-products from Energy Crops

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Energy crops offer enormous opportunities for increasing the sustainability of agriculture and energy production in the U.S. This is particularly true in the Southeast, where 1.59 quads of biomass are used in energy production, or 56% of the total used nationally. To date, energy crops have not been economically competitive with fossil fuels, but provisions in the pending Energy Bill should help to launch their commercialization. Nevertheless, opportunities for sustaining biomass energy production may well hinge on producing energy **and** extracting high value products from the same crop.

This presentation centers on the extraction of antioxidants from mimosa and sericea, two potential energy crops that show both high forage yields, and high total phenolics and oxygen radical absorbance capacity (ORAC) values in crude water and methanol/water extracts. Results are presented on the fractionation of the crude biomass extracts by HPLC and the identification of antioxidant compounds by LC/MS.

Poster Presentation 3-13

Silymarin Extraction from Milk Thistle Using Hot/Liquid Water

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Hot liquid water is attracting attention as an extraction solvent in the recovery of compounds from plant material as the search for milder and "greener" solvents intensifies. Previous work by the authors showed that water at 85-100°C was effective in extracting flavanolignans and the dihydroquercitin taxifolin from milk thistle without prior defatting with petroleum ether, a procedure outlined in the traditional extraction protocol. Milk thistle is an interesting system because the flavanolignans display hepatoxic protection properties. This work also showed that the more polar taxifolin was preferentially extracted at lower temperatures, while the more nonpolar silybinin compounds were preferentially extracted at increased temperature.

This presentation seeks to explore the use of hot/liquid water as an extraction solvent for milk thistle at temperatures above 100°C and as high as 250°C, a temperature where significant compound degradation is expected. Yields of taxifolin, silychristin, silybinin A and silybinin B are presented as a function of operating conditions, as are kinetic data on compound degradation presented as a function of temperature. It is fully expected that the hot/liquid water system could be applied to the extraction of other polar and nonpolar compounds.

Poster Presentation 3-14

High Productivity Continuous Biofilm Reactor for Butanol Production: Effect of Acetic and Butyric Acids and CSL on Bioreactor Performance

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A continuous biofilm reactor was used to study the effect of acetic and butyric acids and corn steep liquor (CSL) on reactor performance. In batch reactors supplementation of acetic acid to the medium has a dramatic effect on the culture stability and production of high concentration of acetone butanol ethanol (ABE or solvents) (Chen & Blaschek, 1999). In double series reactors it has been observed that acids produced in the first reactor are converted into solvents in the second reactor (Qureshi & Maddox, 1988). It has also been demonstrated in ABE recovery experiments that under appropriate conditions acids are converted into solvents (Qureshi & Maddox 1991; Ezeji et al. 2002). Conversion of acids into solvents enhances ABE yield. Hence, it was our objective to study the effect of supplementing fermentation medium with acetic and butyric acids. Additionally, experiments were conducted to investigate the effect of CSL on reactor stability, and solvent production.

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Qureshi N, Maddox IS (1991) Integration of continuous production and recovery of solvents from whey permeate: use of immobilized cells of *Clostridium acetobutylicum* in a fluidized bed reactor coupled with gas stripping. Bioprocess Engineering **6**: 63-69

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Poster Presentation 3-15

Optimization of an Integrated Process for the Production of Inulinase by *Kluyveromyces marxianus*

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In the modern biotechnological industry, process development is regarded as an integrated operation so that upstream, downstream and fermentation steps should be designed and optimized simultaneously. Therefore, industrial fermentation broths that usually are complex media, containing for example molasses and corn steep liquor, should be pretreated before fermentation, leading to clarified media. However, this pretreatment should not jeopardize fermentation yield and productivity, as well as give a fermented broth as clear as possible to facilitate downstream processes, mainly when chromatographic purification steps are involved, as in the case of enzyme purification. In this work, an integrated optimization study was accomplished, using experimental design techniques, concerning the pretreatment of the fermentation broth ingredients (molasses and corn steep liquor) with activated charcoal, followed by the optimization of the fermentation medium composition, and finally studying the effects of the different media on the enzyme purification in an expanded bed column containing ion exchange resin. The mainly results were an improved fermentation step with an increase of 30% in the enzyme production and an improved purification process with higher purification factors and recovery yields, similar to the ones obtained with synthetic medium.

Poster Presentation 3-16

Influence of Culture Conditions on the Expression of Green Fluorescent Protein (*GFPuv*) by *Escherichia coli*

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The recombinant green fluorescent protein (*GFPuv*, excitation/emission maxima at 394/509nm), was expressed by transformed cells of *Escherichia coli* DH5-α. The starting cultures were grown in LB/amp broth up to 10^4-10^5 CFU/mL. One mL inoculum was transferred to 25 mL of LB/amp broth and the suspensions were stored at 4°C prior to further incubation on a rotatory shaker at 37°C for 8h and 24h. To evaluate the effectiveness of different parameters to improve the expression of *GFPuv* by *E. coli*, eight culture conditions were set up by a fractional factorial (2^{4-1}) design at two levels: (i) the effect of storing the starting culture in LB/amp broth at 4°C for 24h and 48h ("pre-storage") prior to incubation at 37°C; (ii) the effect of agitation speed (100, 150 and 200 rpm); (iii) the concentration of IPTG (isopropyl–β-D-thiogalactopyranoside, final concentrations of 0.05, 0.275 and 0.5 mM); (iv) the addition of IPTG at set cell densities (OD_{660} between 0.01 and 0.8) corresponding to cultures at 10^3-10^7 CFU/mL. The expressed *GFPuv* was released by direct extraction from the cells by the three phase partitioning method (TPP). Protein extracts were eluted through a methyl HIC column and the fluorescence intensity of the samples were related to µg *GFPuv*/mL. The constant exponential rate for culture growth, varied from 0.99 h^{-1} to 2.10 h^{-1}. The culture conditions (37°C/ 24h incubation) which provided the highest yields of GFPuv were: (i) pre-storage at 4°C for 24h; (ii) agitation speed at 100 rpm; (iii) the concentration of IPTG at 0.5 mM; (iv) the addition of IPTG at cell densities at OD_{660} = 0.8. Agitation speed and IPTG concentration showed, respectively, greater negative and positive influences on *GFPuv* expression at the end of the logarithmic phase.

[Acknowledgement: CAPES, CNPq, FAPESP]

Poster Presentation 3-17

Thermal Stability of Recombinant Green Fluorescent Protein *(GFPuv)* at Various pH Conditions

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The kinetic thermal stability parameters of the recombinant green fluorescent protein (GFPuv), expressed by transformed cells of *Escherichia coli* DH5-α and isolated by TPP extraction with HIC, were analyzed at various pH values and temperatures (75°C – 95°C). Samples of *GFPuv*(between 5.0 and 10 µg *GFPuv*/mL) were exposed (24h /4°C) to pH from 4.0 to 8.0 in the buffers: (i) 10 mM acetate (pH 4.0 to 7.0), (ii) 10mM phosphate (pH 5.5 to 8.0) and (iii) 10 mM Tris-HCl (pH 7.0 to 9.0) before heating. For pH> 6.0, the fluorescence intensity (excitation/emission maxima at 394/509nm) of *GFPuv* was shown to be quite stable, and the soluble protein concentrations varied around 20%, independent of the type of buffer used. The extent of protein denaturation by moist heat in the various buffers, as a measured in the loss of fluorescence intensity, was expressed in decimal reduction time (D-value, at reference temperature), the interval of time required to reduce one decimal logarithm of the initial fluorescence intensity of *GFPuv*. For the range of temperatures and pH studied (7.0 to 8.0), the thermal stability of *GFPuv* was slightly greater in phosphate buffer than in Tris-HCl, even though Tris buffers are recommended for the maintenance of the *GFPuv* structure at pH ≥8.0. The decrease in fluorescence intensity was unique when heating at 75°C and 80°C. These temperatures generate two distinct linear portions for the *GFPuv* inactivation curves, the slopes are used to calculate close mean D-values for the similar heat conditions. At 85°C, the mean D-value at pH 7.0 ranged from 8.33 min (acetate) to 13.88 min (phosphate) and at pH> 8.50 ranged between 18.5 min and 27.7 min, confirming higher thermal stability for *GFPuv* in phosphate buffer. The thermal stability for *GFPuv* in Tris-HCl (pH=8.0-8.9) was constant at 90°C and 95°C, the mean D-values were 7.93 min (pH=8.38 – 8.92) and 6.0 min (pH=8.05 – 8.97), respectively. The thermal stability of *GFPuv* provides the basis for its potential utility as a fluorescent biological indicator to assay the efficacy of processes at temperatures lower than 100°C.

[Acknowledgement: CAPES, CNPq, FAPESP]

Poster Presentation 3-18

Recovery of Acetic Acid from Byproduct Streams

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This research focused on the development of a low-cost and efficient method of recovering acetic acid from byproduct or waste streams of the agricultural processing industry. Such streams arise from the production of furfural (C_4H_3OCHO) or from the anaerobic digestion of wastewater or animal manure. Lime and sulfur dioxide were the major materials used for separating acetic acid from dilute water solution. This process, recently demonstrated in our laboratory, involved the use of lime and either sulfur dioxide or sulfur trioxide for neutralizing and regenerating acetic acid. The lime and sulfur oxide can be recovered and recycled in the overall acetic acid separation process. Therefore, this acetic acid separation process should be cost-effective and nonpolluting.

Key words: acetic acid, separation, recovery, waste stream and anaerobic digestion.

Poster Presentation 3-19

Two-stage Membrane Bioreactor System to Ethanol Production from Lignocellulosic Biomass

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Ethanol production from lignocellulogic materials comprises the enzyme hydrolysis process and the ethanol fermentation. The enzyme recycling hydrolysis with ultrafiltration was also investigated. When the enzymes were recycled through the membrane system, FPAase was not deactivated but the activity of -glucosidase was decreased with an increase in operating temperature and transmembrane pressure. The enzyme recycling hydrolysis with the addition of -glucosidase could get higher yield in reducing sugar production up to 2.23 times compared to that of batch enzyme hydrolysis. For the enhancement of ethanol production, the concentrated hydrolyzate was used as a substrate. High sugar concentration of wood hydrolyzate could not convert to ethanol because it has toxic compounds. Therefore, yeast strain was developed through the selection of hyperfermentable mutant by U.V irradiation of *Brettanomyces custersii* H1-39 at high sugar concentration of wood hydrolyzate. *B. custersii* H1-603 yielded 89.5% of the theoretical ethanol yield from 10% (w/v) hydrolyzate. Total retention culture was investigated with ultrafiltration and pervaporation module. Two-stage membrane bioreactor coupled with two membrane modules could be the continuous process and this system is unique in the literatures. For the pervaporation process, PTMSP (poly[1-trimethylsilyl)-1-propyne]) was used and down-stream pressure reduced to 10mmHg at 40C. Ethanol concentration in reactor was 44.5 g/L and in the trap by pervaporation was 166 to 212 g/L. Above two step membrane bioreactor system was maintained up to 94 hrs at steady-state condition and the productivity of this system was 7.79 g/L hr. The productivity is the highest than any other bioreactor system reported.

Poster Presentation 3-20

Characterization of Xylose Reductase Extracted by CTAB-reversed Micelles from *Candida guilliermondii* Homogenate

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Xylosereductase (XR) (E.C.1.1.1.21), produced by *Candida guilliermondii* grown in sugar cane bagasse hydrolysate, was separated directly from the cell homogenate by reversed micelles of Cetyl Trimethyl Ammonium Bromide (CTAB), attaining a recovery yield of 100% and enrichment factor of 5.6 fold. The extraction conditions were: pH=7.0, electrical conductivity= 14mS/cm, T=5°C, 5% (w/w) of hexanol, 22% (w/w) of butanol and 0.15M CTAB. The XR after extraction was stable in pH interval of 5.9-6.7 and its heat inactivation constant was about 6.5 fold higher than that before extraction. The V_{max} values against both xylose and NADPH for XR before and after extraction by reversed-micelles differed about 6%, whereas the difference on K_M values were more pronounced. The $(K_M)_{xylose}$ for XR after extraction was about 35% higher than before extraction, meanwhile $(K_M)_{NADPH}$ was about 30% lower after extraction than before one. As the K_M variations indirectly signaled, the XR affinity simultaneously diminishes for xylose and increases for NADPH. Thereby, this could probably explain why the V_{max} values for XR before and after extraction were quite similar.

Poster Presentation 3-21 Student

Determination of the Rheological Properties of Distillers' Grains Slurries Using a Helical Impeller Viscometer

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Power ethanol facilities are coming on-line in a number of midwestern locations. Consequently, the quantity of distillers' grains is increasing. The National Renewable Energy Laboratory (NREL) and others are interested in developing a process to convert the cellulose and hemicellulose in distillers' grains to fermentable sugars, thus increasing ethanol yield and enhancing the protein content in the remaining solid product (distillers dried grains with solubles). To assist in the design and construction of such facilities, the rheological properties of distillers' grains slurries have been studied over a range of concentrations.

Distillers' grains slurries are non-Newtonian, heterogeneous fluids subject to particle settling. Traditional methods of viscosity measurement, such as cone and plate and concentric cylinder, are not adequate in determining rheological properties of these fluids. A helical impeller viscometer was employed to accurately measure impeller torque over a range of rotational speeds. Newtonian and non-Newtonian calibration fluids were utilized to obtain constants that relate shear stresses and shear rates to the experimental data. Newtonian calibration fluids are used to determine the impeller constant, c, while non-Newtonian calibration fluids are used to calculate the shear rate constant, k. Rheological properties were modeled empirically using the Herschel-Bulkley model.

Poster Presentation 3-22 Student

The Effect of Toxic Products on Ethanol Production from Concentrated Corn Stover Hydrolysates in a Three-Liter Bench Scale Bioreactor

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Zymomonas mobilis, strain 39676:pZB4L, is a recombinant bacterium that can produce ethanol from xylose-rich hemicellulose. D-xylose, a pentose sugar, is the major carbohydrate component present in hydrolysates generated by dilute acid pretreatment of a corn stover. The efficient fermentation of the xylose-rich hydrolysates represents a significant opportunity to improve the economics of large-scale fuel ethanol production from corn stover.

The ethanol yield obtained during fermentation of lignocellulosic hydrolysates is decreased due to the presence of inhibiting compounds. Acetic acid and furfural (a pentose degradation product) are highly toxic to Zm 39676:pZB4L at levels envisioned for a pretreated corn stover liquid hydrolyastes. Acetic acid 1 % (w/v) and furfural 0.3 % (w/v) interact antagonistically causing decreased specific cell growth rate. In order to obtain high ethanol yields and productivities, reduction of these inhibiting products to appropriate levels prior to fermentation is desired. The hydrolysates are then converted via simultaneous saccharification and cofermentation. This study characterized the growth performance of *Z. mobilis* 39676:pZB4L as a function of mixed sugar and acetic acid concentration using a synthetic, acid-pretreated corn stover hydrolysate containing 1 % (w/v) yeast extract, 0.2 % KH_2PO_4, 5 % (w/v) xylose, and 0.1 % (w/v) glucose with varying amounts of acetic acid. The objective of this study was to establish the effects of toxic byproducts on the process parameters for ethanol production in a three-liter bench scale reactor.

Poster Presentation 3-23

Separation of Xylose Reductase and Xylitol Dehydrogenase from *Candida guilliermondii* Homogenate by BDBAC Reversed Micelles

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The intracellular enzymes xylose reductase (XR, EC 1.1.1.21) and xylitol dehydrogenase (XD, EC 1.1.1.9) from *Candida guilliermondii*, grown in sugar cane bagasse hydrolysate, were separated by reversed micelles of BDBAC [N-Benzyl-N-Dodecyl-N-bis (2-hydroxyethyl) ammonium chloride] cationic surfactant. An experimental design was employed to select the best conditions (temperature=11.4°C, co-solvent hexanol=9% v/v, surfactant concentration = 0.14M, pH = 7.0, and electrical conductivity = 14 mS/cm) to separate and purify both enzymes. The separation yielded total XD and XR recovery of about 100%. The purity of XD increased 2.3-fold and XR increased 4.8 fold. This study demonstrated that liquid-liquid extraction by reversed micelles is a process able to, efficiently, separate the enzyme XD from XR present in the cell homogenate, and simultaneously increase the enzymatic activity and the purity of both enzymes produced by *C. guilliermondii*.

Poster Presentation 3-25 Student

Techno-Economic Evaluation of Ethanol from Softwood Potential of Energy Savings in an SSF Process

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In a previous study it was shown that one of the most important factor for the economic outcome in the ethanol production from softwood is the income from the solid fuel co-product. Lignin, which constitute a large portion of the raw material, cannot be utilized for ethanol production but is an excellent solid fuel with a high heating value and low ash content.

The steam needed in the ethanol production process is produced by incineration of the solid residue not utilized for ethanol production. Therefore, the production of the solid fuel co-product is dependant on the energy consumption in the process i.e. the need for fresh steam in the various process steps. The energy consumption can be reduced significantly by reducing the addition of fresh water for instance by recycle process streams. Other options, which are considered in this study, are optimisation of the process itself for instance by integration of the different process steps such as distillation and evaporation or employing other energy saving techniques such as mechanical vapour recompression.

The process simulations are carried out using the commercial flowsheeting program Aspen Plus and models for the various process steps are based on experimental data. The results from the simulations are used in the economic evaluation which are performed using the commercial program Icarus Process Evaluator. Results from this study will be presented.

Poster Presentation 3-26

CFD Simulation and Redesign of a Vertical Screw Conveyor Reactor

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A shrinking-bed reactor was designed by NREL to maintain a constant bulk packing density of cellulosic biomass. This high solid-to-liquid ratio in the pretreatment process would allow a high sugar yield to be obtained, thereby avoiding the need to flush large volumes of solution through the reactor. A bench-scale shrinking-bed reactor was used in the hardwood yellow poplar sawdust pretreatment process and over 90% sugar yield was achieved.

To scale up the shrinking-bed reactor and make it more feasible for future industrial application, NREL had adapted the shrinking-bed reactor into the screw conveyor reactor where solid biomass was conveyed by a series of interrupted screws, with an interrupted flight employed to mimic the “shrinking bed” effect. Initial studies of yellow poplar hardwood pretreatment with a pilot scale screw conveyor reactor resulted in a xylose yield of almost 90%. Glucose yields from cellulose was slightly less than predicted from bench work at just under a normalized 25% yield.

In this study, computational fluid dynamics (CFD) was utilized to analyze the flow regime inside the screw conveyor reactor. The Porous Media Model and the Rotating Reference Frame were used to simulate the flow pattern, velocity, pressure and other flow properties. Based on simulation results, the screw conveyor reactor was redesigned to produce an even flow distribution resembling plug-flow. The redesigned screw conveyor reactor was also simulated with CFD and compared with the original design. Results of this study can be used to guide future screw conveyor reactor design and aid in commercialization.

Poster Presentation 3-27

Production of Biodiesel Fuel by Transesterification of Rapeseed Oil

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Fatty acid methyl esters (FAMEs) show large potential applications as diesel substitutes, and they are known as biodiesel fuel. Biodiesel fuel as a renewable energy is an alternative that can reduce energy dependence on petroleum and air pollution. Several processes for the production of biodiesel fuel have been developed. Transesterification process under alkali-catalysis and short-chain alcohol gives high level yield of methyl esters in short reaction times.

In this research, transesterification of rapeseed oil was investigated to produce the FAMEs. Experimental reaction conditions included molar ratio of oil to alcohol (1:3 to 1:10), concentration of catalyst (0.5 to 1.5%), types of catalysts (sodium methoxide, NaOH and KOH), reaction time (within 1 hr), and reaction temperature (40 to 70 °C). The conversion ratio of rapeseed oil enhanced with the alcohol-oil mixing ratio and with the reaction temperature.

Poster Presentation 3-28

Recovery of Tocopherols Through Molecular Distillation Process

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Molecular distillation process represents a special type of vaporization at low pressures, and, therefore, low temperatures, finding great usefulness in the separation and purification of materials with molecules of high molecular weight, as well as for those thermally sensitive, as the vitamins, minimizing losses by thermal decomposition.

Another important characteristic of molecular distillation is the reduced time in which the material is submitted to the process temperature, usually from 1 second to 1 minute. This reduces, considerably, possible effects of thermal decomposition. In a general way, these peculiarities show the high potential of this process in the separation, purification and/or concentration of natural products, usually constituted by complex and thermally sensitive molecules.

Taking all of these in consideration, in this work, the molecular distillation process for tocopherols recovering (for obtaining Vitamin E), using the Deodorizer Distillate of Soya Oil (DDSO) with the conditions and the quality demanded by the market, is being proposed. Modeling and simulations (with the DISMOL simulator) of the process were, then, developed for recovering tocopherols (vitamin E) from DDSO, in order to determine the feasibility of the process and the best experimental conditions.

Poster Presentation 3-29

Optimization of an Extractive Alcoholic Fermentation Process

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In this work the optimization of a continuous alcoholic fermentation process combined with a flash column under vacuum is studied. The objective is to maximize yield and productivity in the fermentor. The results using surface response analysis combined with modeling and simulation are compared to that obtained when the problem is written as a nonlinear programming (NLP) problem and solved with a successive quadratic programming (SQP) technique.

Two process models are evaluated when the process is optimized using the SQP technique. The first one is a deterministic model, whose kinetic parameters were experimentally determined as functions of the temperature, and the second is an statistical model obtained using the factorial design technique combined with simulation.

The optimization results using response surface analysis, SQP + deterministic model and SQP + statistical model are compared and it is shown that the approach using SQP + statistical model can be a good choice when the deterministic model is complex and/or the computation effort to solve the optimization problem using the deterministic model is too high, as can be the case for real time optimization problems.

Poster Presentation 3-30

Controlled Fed-batch Fermentations on Dilute Acid Hydrolysate in PDU-scale

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Inhibitors formed during wood hydrolysis constitute a major problem in fermenting dilute acid hydrolysates to produce ethanol. High levels of aldehydes, organic acids and phenolic compounds drastically lower the ethanol productivity. However, by applying a fed-batch technique the levels of inhibitory compounds can be held low enabling high ethanol productivity.

In the present work a previously developed fed-batch strategy was implemented in a PDU-scale reactor. In previous lab-scale experiments, the control algorithm was based on the CO_2 content in the exhaust gas. However, since no nitrogen was flushed through the reactor in the PDU-scale experiments, the total gas evolution, measured with a mass flow meter, was used as input variable in the control algorithm in the PDU-scale. The results from the lab-scale fermentations were well reproduced in larger scale. Importantly, the feed rate in the PDU-scale fed-batch experiments could be properly controlled based on the total gas evolution from the reactor. In experiments made with TMB 3000, an inhibitor tolerant strain of *S. cerevisiae* isolated from spent sulphite liquor, close to 100 % of the glucose and mannose present in the hydrolysate was fermented. In comparison, less than 20 % of these sugars were fermented in a batch process.

Poster Presentation 3-31

The Modeling of Alcoholic Fermentation at High Glucose Concentrations

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The vehicles in Brazil, a large ethanol-producing derived from sugar cane, have long used a gasoline blend containing ethanol, a effective fighter of air pollution. The expansion of this clean-air technology depends of the optimization and control of ethanol plants. The mathematical modeling is an important tool to predict the dynamic composition profiles in the batch fermentation process and to reduce the number of lab on-site assays that tests each batch of ethanol. The dynamic model of batch fermentation by *Saccharomyces cerevisiae* using glucose at concentration from 60 to 300 g/L is presented. The specific growth rate decreases at high substrate and product concentrations due to the toxic effect on the biomass. The reaction rate is written according to Levenspiel & Han model. The ethanol and glucose concentrations of 115 and 450 g/L respectively inhibited the cellular growth. The maximum specific rate of cellular growth was 0.28 h^{-1}. The simulated and experimental dynamic profiles of concentration of ethanol, cells and substrate showed a good fitting in the studied interval, in spite of the gap between the predicted and experimental end values due to the observed low nitrogen concentration in the medium.

Poster Presentation 3-32

Production of Lactic Acid in a pH-Controlled Separative Bioreactor

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A robust bacterial strain was constructed that produces lactic acid efficiently in minimal medium. However, the lactic acid produced results in a rapid decrease in pH that inhibits further metabolism unless base is used to neutralize the acid. An electrodeionization (EDI) stack incorporating a mixed ion-exchange resin wafer was constructed to determine if rapid removal of lactic acid as it was produced could be used to control the pH of the fermentation reaction. When the microbial cells were circulated through the EDI reactor, the lactic acid was rapidly removed from the reaction compartment and concentrated in the product compartment. The separation of lactic acid was nearly complete. The productivity of the reactor was 0.3 g/L/hour during a 24 hour run. During the fermentation, the pH was maintained between 5.8 and 7.0. No salts or nutrients were used in the fermentation and the cells could be stored in the cold for eight days and reused. The product of the fermentation was isolated as concentrated lactic acid. The pH-controlled EDI separative bioreactor thus permits the efficient production of lactic acid without the need to neutralize the fermentation with base and without the generation of a lactic acid salt.

Poster Presentation 3-33

Use of Deterministic Model and Artificial Neural Networks in the Fermentation Process for Ethanol Production

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The biological processes are each time more important in the productive processes due to its versatility, high production rate in specific products and high value of the generated products. These processes present a complex behavior where several mechanisms are involved to obtain specific products.

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 is computer technical 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 in this work is related with the fermentative ethanol production using the microorganism *Saccharomyces cerevisae*. The process is represented by a deterministic model, constituted by a system of differential ordinary equations, that is solved by 4th order Runge-Kutta method. This model was validated properly in industrial scale and it is used as data source for studies of the dynamics of the process, development of the models with ANN as well as of the hybrid models. Brazil is the detainer of the most advanced technologies for fermentative ethanol production using sugar cane. The ethanol is considered an alternative fuel with a promising future in this time of ecological preoccupation.

For the construction of the hybrid model ("Gray Box" model) it was accomplished the training of a ANN that predicts the specific speed of growth of the microorganism when supplied the values of the product, cells and substrate concentrations.

After adjustment of the ANN, it is coupled to the deterministic model supplying the dynamic profiles of the variables: S, X and P (for the four reactors), efficiency and productivity. It was also developed a "Black Box" model of the biological process. This model, based in ANN, supplies the dynamic profile of S(4), X(4) and P(4) during 20h of process. All the models were properly tested and their efficiency were verified.

Key Words: Artificial Neural Networks, Ethanol, Hybrid Models.

Poster Presentation 3-34

Optimization of an Immobilized Enzymatic Microbioreactor via Numerical Simulation

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At the University of Tennessee at Chattanooga (UTC), research continues on the development and optimization of continuous flow enzymatic microbioreactors. Because of physical phenomena particular to the reduced scale, these very small devices have the potential for dramatically increased effectiveness (e.g., higher purity of product) over their larger counterparts. Applications for such microreactors include the production of beneficial drugs and the cleanup of toxic waste streams.

Since microreactors are such a new technology, little is known about how to optimize their design. Experimental testing of various reactor geometries yields valuable information about which designs are the most promising. However, testing can be both time-consuming and expensive, and the myriad of possible configurations (e.g., channel lengths, widths, flow obstruction shape and packing density, *etc.*) demands that testing be performed efficiently. One way to achieve such efficiency is to combine an experimental test program with computer simulations. By developing computer models of flow and reaction within the reactor channels, investigators can evaluate how changes in geometric and flow configurations affect reactor performance. Experimental tests are used to validate the models, and computational results are used to steer future tests. In this way, the approaches complement each other, and the design and optimization process is significantly enhanced.

In a previous study, the authors established the feasibility of using computational modeling to simulate the complex physics that occurs within enzymatic microreactors. This paper builds upon and extends this prior work. The commercial code CFD-ACE+, developed by CFD Research Corporation (CFDRC), is used to perform the simulations. The beneficial effect of reduced scale is quantified, and optimized reactor configurations are identified for a hydrogen peroxide/catalase reaction. Results are compared with experimental data to demonstrate the accuracy of the numerical model.

Poster Presentation 3-35

β-Galactosidase Production Using *Kluyveromyces marxianus* and Enhanced Cheese Whey by Fermentation

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The enzymatic hydrolysis of lactose by β-galactosidase plays an important role in the milk processing industry due mainly to: (1) production of lactose-hydrolyzed milk for consumption by persons with intolerance to lactose; (2) prevention of crystallization in dairy products; and (3) alternative means to reduce whey waste and its pollution potential and produce high value-added bioingredients. This work presents kinetic studies of β-galactosidase production using *Kluyveromyces marxianus* and enhanced cheese whey by fermentation. An experimental design was performed varying initial concentration of lactose and yeast extract. Initial conditions were: pH = 5.5, cellular concentration = 1.107 cells/mL. During the fermentation the analyzed parameters were: lactose consumption, cellular concentration and enzymatic activity. The enzyme was extracted from cells by centrifugation and chloroform extraction. Enzymatic activity was determined using the initial rates method of lactose hydrolysis reaction. An enzymatic activity of 28,0 UGl/mL [(μmol glucose)/(minmL enzyme) was obtained, using as culture medium cheese whey with lactose (50 g/L), enhanced with salts K_2HPO_4, $(NH_4)_2SO_4$ and $MgSO_4$ (concentrations of 5.0; 6.0 and 0.6 g/L, respectively) and yeast extract (12 g/L). In these conditions the cellular concentration reached was 4.8 g/L of dry mass. The cheese whey enhanced with salts and yeast extract proved to be an appropriate medium for cellular growth and β-galactosidase enzyme synthesis.

Poster Presentation 3-36

Starch Hydrolysis with Thermopressurized Aqueous Phosphoric Acid and Free Maltosugars Bioconversion to Oxygenated Carotenoids

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Heavy suspensions (40g% w/v) of cassava roots native starch granules ("*fecula*") were adjusted to pH between 1.5 and 3.5 with aqueous phosphoric acid and submitted to thermopressurization in the range of 152 - 176°C (4 to 8 atm) up to 15 minutes. The ammonia-neutralized starch digest was then adjusted to a 3-5g% reducing sugars content and supplemented with minimum amounts of salts and / or N sources. This nutritional triad of C (maltosaccharides) / P / N (ammonium phosphate) sources were then inoculated with *Xanthophyllomyces dendrorhous* (formerly: *Phaffia rhodozyma*) for growth and pigmentation up to 5 days at 22°C and 180 rpm.

The best culture media formulation both for biomass (948 mg dry yeast /100 mL medium) and astaxanthin production (477 µg oxygenated carotenoid / g dry yeast cells) was a glucose + maltosaccharides mix arrising from starch acidified to pH 1.75, processed at 152°C (4 atm) for 15 min, partially neutralized with aqueous ammonia to pH 6, and supplemented with 0.25g% of dry solids obtained from "*manipueira*", the waste water resulting from grinding and washing of cassava root starch granules. Addition of 0.05 g% of KNO_3 also improved the red yeast. pigmentation pattern.

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Poster Presentation 3-37

Development of a Methodology for the Determination of Viable Cells in a Three Phase Fluidized Bed Reactor

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An experimental study was carried out in a Three Phase Fluidized Bed Reactor (TPFBR), to degrade biologically a synthetic effluent of milk industry. In this study three different concentration of milk wastewater substrate (462, 825 and 1473 mg O_2/L) were tested. Small PVC particles were fluidized in this reactor, which are used as support for microbial growing. The aiming of this work was develop a methodology to prepare the samples to obtain the cells in segregated form to the determination of the number of viable cells on the support particles and in liquid phase. For the definition of the method, in each process carried out, the number of ultrasounds treatments (Branson Sonic Power–Sonifier) and the amount of filtrations through the isopore polycarbonate filters with 0.5-µm diameter pores (Millipore) was varied, in order to obtain a optimum parameter, compared to the contents of proteins and Colony Forming Units (CFU). As a result of the methodology development to be used in preparation of the samples to be plating on nutrient agar (Biobrás) two sequential ultrasound treatments (0.2 A during 2 min) was used, with subsequent ultrasound treatment in the filter.

Poster Presentation 3-38 Student

Scale-up of Anaerobic Processes Involving Soluble Substrates: The Use of CFD Analysis for Experimental Design

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Biological conversion of cellulosic biomass to fuels and chemicals would employ fermentors of 1 million gallons and more, but financially accountable parties require convincing evidence that such operations cannot fail before they will fund construction of first-of-a-kind operations for such applications. Pilot and demonstration operations may be used to address this issue, but these operations delay project initiation, present their own financial challenges, and may not address investor concerns. However, computational fluid dynamics (CFD) models can be applied to relate the performance of large and laboratory scale systems but have received relatively little consideration in this context. Analysis of scale-up of anaerobic processes involving soluble substrates is an important stepping stone on the way to accurately predicting performance of anaerobic processes featuring cellulosic biomass. In this presentation, kinetic models to describe yeast fermentations are developed and integrated into CFD software to provide initial predictions of yeast fermentations with soluble sugars at a large scale. Strategies are then suggested to more accurately simulate expected commercial conditions.

Key words: computational fluid dynamics, CFD, yeast, fermentations, kinetics, scale-up

Poster Presentation 3-39

Biological Hydrogen Production from Synthesis Gas: Preliminary Techno-Economics and Reactor Design Issues

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The biologically mediated water-gas shift (WGS) reaction may be a cost-effective technology for the conditioning of synthesis gas. NREL researchers have isolated a number of photosynthetic bacteria that can perform the water-gas shift reaction, in which carbon monoxide is oxidized to carbon dioxide while simultaneously water is reduced to hydrogen. The overall stoichiometry of this reversible reaction is:

$$CO + H_2O \xleftrightarrow{hv} CO_2 + H_2$$

If this process is used to treat a biomass-derived synthesis gas stream, the resulting gas stream, now enriched in hydrogen, could be considered completely renewable.

One significant advantage to using bacteria to perform the water-gas shift reaction is their ability to operate at ambient temperature. Because the reaction occurs at ambient temperature, the reaction is not equilibrium-limited (at 25°C, K_{EQ}~5x10^4). The advantages of low operating temperature and lack of equilibrium limitation make the biological shift reaction a promising alternative the conventional two-stage, high temperature, high-pressure catalytic process. However, transfer of the sparingly soluble carbon monoxide from the gas phase to the liquid phase limits the overall reaction rate.

In previous work[1], we performed experiments with trickle-bed reactors (TBRs), which are known to be very effective for gas-liquid reactions where the mass transfer rate is limited by the resistance in the liquid phase. This work demonstrated that a simple reactor model could be used to scale performance data between two different reactor scales.

We will present a process flow diagram and preliminary techno-economic analysis of the WGS process, and discuss the potential applicability and the significant cost drivers of the biological WGS process. One of these drivers is the volumetric productivity of the bioreactor. We will show new laboratory data with increased reaction rates, and demonstrate how these increases in reactor productivity lead to decreased capital and operating costs in the overall process.

1.Bioreactor Design Studies for a Novel Hydrogen Producing Bacterium, Edward J. Wolfrum and Andrew S. Watt, *Applied Biochemistry and Biotechnology 98-100:611-626 (2002).*

Poster Presentation 3-40

A Green Process to Obtain Acethaldehyde: Oxidation of Ethyl Alcohol

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Acethaldehyde is an important intermediate product to obtain a large class of chemical products and the conventional way to be product is through petrochemical process. In this work an alternative process is proposed based on the oxidation of ethanol, which may be obtained by sugar cane fermentation process. A fixed bed catalytic reactor is used to have a high conversion and complete selectivity process to obtain acethaldehyde.

Nowadays, the control and safety of reactors are important features in the design as well as in operations of industrial processes that carry out complex reactions with constraints of thermal stability and/or selectivity as, for example, exothermic oxidation reactions. Taking this into account, a dynamic model of the reactor is very useful to study both the start-up period and the effects of a sudden (accidental) change in the operational conditions, particularly in the thermal reactor stability.

Inside of this context, heterogeneous bidimensional dynamic model for fixed bed catalytic reactor with an appropriate solution procedure was developed in this work; which considers variations in the physical properties of the fluid and in the heat and mass transfer coefficients, as well as the heat exchange through the jacket of the reactor. The model consist of mass and heat balance equations for the catalyst particles as well as for the gas phases, which include the momentun balance. The heterogeneous model includes the resistance to mass and heat transfer at the gas-solid interface and consider the resistance inside the catalyst particle. The catalytic oxidation of the ethanol to acetaldehyde over Fe-Mo catalyst was used, which is a strongly exothermic reaction.

The developed model has show to be able to predict the main characteristics of the dynamic behaviour of fixed bed catalytic reactor, including the inverse response phenomena. This knowledge is fundamental for the development of an efficient control strategy as well as for the simulation studies and reactor design, with the purpose of safe and efficient operating.

Poster Presentation 3-41

Evaluation of the Agitation Effective Power in Aerated and Non-Aerated Systems for Yeast and Filamentous Fungi Suspensions

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Many industrial processes involving microorganisms take place under aerobic conditions, usually with a combination of agitation and aeration of the medium. Besides supplying the metabolic needs of the fermentation agent, aeration also reduces the power required by the impellers. The aim of this work is to study the influence of aeration on effective power consumption for different types of impellers. A mechanically and aerated stirred system with baffles was used, containing different microbial suspensions in water. Data has been collected for suspensions of the yeast *Saccharomyces cerevisiae* and the fungus *Aspergillus niger*, and correlations were obtained between the ratio of agitation effective power, applied to aerated systems (P_g) and non-aerated systems ($P_{o\neq}$) and the dimensionless aeration number (Na). The curves obtained with the microorganisms tested have followed the same profile as in previous work, published in the literature for water.

Under anaerobic conditions the dimensionless variables involved are the power number (N_p) and the Reynolds number (Re), and correlations between them could be observed, showing how N_p tends to be constant in baffled systems, with turbulent flow, for the geometries and impeller diameters used in this study. This behaviour was observed both for water and for the aqueous suspensions of *Saccharomyces cerevisiae* and *Aspergillus niger*, and were similar to those reported in the literature.

Poster Presentation 3-42

Comparison of Strain Performance in Fed-batch Fermentation of Hydrolyzates

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An organism for production of fuel ethanol from lignocellulosic hydrolyzates should have a high inhibitor tolerance and be able to convert monomeric sugars in the hydrolysate. In the present work five different strains of Saccharomyces cerevisiae were studied: CBS 8066 – a widely used laboratory strain, commercial bakers yeast, TMB 3000 – a strain isolated from a spent sulfite liquor fermentation plant, TMB 3006 - a genetically modified strain based on TMB 3000, and TMB3400 – a genetically modified strain based on another industrial strain. The aim was to compare the strain specific fermentative capacity to further understand the inhibition caused by the hydrolysate. Furthermore, the ability of the genetically modified strains – expressing XR, XDH and XK – to ferment xylose in a hydrolyzate medium was also investigated.

The ability to ferment hydrolyzates was highly strain specific also in fed-batch cultures. With TMB 3000 and TMB 3006, respectively, the average ethanol productivity was increased by as much as 69-75% compared with CBS 8066. A likely explanation to this difference is that TMB 3000 and TMB 3006 have a higher ability to convert the inhibitors present in the hydrolysate. Therefore growth inhibition does not occur and a decrease in ethanol productivity is avoided. Enzyme activity measurements and fermentation with growth-arrested cells supported this hypothesis. The two strains expressing enzymes that enable xylose fermentation consumed part of xylose present in the hydrolyzate and fermented it to ethanol.

Poster Presentation 3-43

The Effect of Temperature on the Anaerobic Methane Fermentation Process for the Digestion of Organic Wastes

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The effects of temperature on the anaerobic methane fermentation process for the digestion of organic wastes were investigated. Temperatures were adjusted to 30, 35, 40, and 45°C, respectively, in the batch anaerobic digestion, while in case of continuous anaerobic digestion, they were adjusted to 40, 45, 50, and 55°C, respectively. The substrates (food wastes) were mixed with inocula (prepared methanogenic fluid) at a ratio of 1:1 in the batch anaerobic digestion, and several parameters such as pH , sCOD, and the amounts of gas production have been studied. During fermentation, pHs of all reactors tested showed more than 7. It was found that the removal rates of sCOD in the different temperature conditions correlated with the digestion time through an equation of the Grau kinetic model type. The value of k'_1 showed 0.14 (30°C), 0.35(35°C), 0.38(40°C) and 0.6(45°C), respectively. It means that substrate removal velocity increased as temperature elevated. The starting time of gas production was also reduced as the temperature increased. In the continuous anaerobic digestion, the highest removal rate of sCOD was found in the reactor operated at 50°C, and the rate of sCOD in the reactor of 55°C was similar to that of 40°C. As the temperature reached to 50°C, the amount of methane produced increased.

Poster Presentation 3-44

Biorefinery Optimization Tools – Development & Validation

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The concept of a biorefinery has been proposed as a means of improving the economics of bioethanol production. Bioethanol can be used as a neat fuel or as a gasoline or diesel additive. However, transportation fuels are relatively low-price commodities, and ethanol currently produced from corn in the U.S. relies on subsidies and by-product revenue to compete in this market. The next generation of bioethanol production technologies will produce ethanol from low-cost cellulose. While cellulosic processes can be optimized to produce high yields of ethanol, it may be preferable to co-produce high-value by-products to maximize profitability. Organic chemicals, solvents and biopolymers have all been proposed as possible cellulosic ethanol co-products from a biorefinery. *Mathematical optimization methods can be used to evaluate the economics of various biorefinery product slates and configurations.* There exists precedent for such an approach. The petroleum refining industry has long used Linear Programming techniques to select feed and product slates and optimize petroleum refinery economic performance.

A spreadsheet-based model has been developed to optimize the economic performance of future biorefineries. The refinery is modeled by a system of equations and inequality constraints that describe the performance of the real processes, pretreatment, fermentation, etc. Data from previous NREL studies were used to build this system and include yield, capital and operating cost information. An economic objective function has been formulated and mathematical techniques employed to find the optimum (maximum profitability) set of design/operating conditions satisfying all the constraints placed on the model. The model has been validated using previous design studies and can be used to screen and evaluate potential R&D alternatives.

Poster Presentation 3-45

Anaerobic Fermentations of Paper Sludge Hydrolysate to Hydrogen by Extreme Thermophilic Bacteria

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Hydrogen is regarded as the key energy carrier in the future energy economy. However, sustainable hydrogen production is still very expensive. For sustainable hydrogen production from biomass, the cost of biomass is one of the major cost drivers. This study addresses the application of an agro-industrial waste stream as feedstock for the production of hydrogen by extreme thermophilic microorganisms.

Paper sludge is a waste product from the paper industry and world-wide available in large quantities. A substantial part of the paper sludge is composed of carbohydrates, mostly cellulose and some hemicellulose. Prior to fermentation the paper sludge is enzymatically hydrolyzed to make a fermentable feedstock consisting of hexose and pentose monosaccharides. Small scale fermentation experiments using *Thermotoga elfii* and *Caldicellulosiruptor saccharolyticus* have previously shown hydrogen production from paper sludge hydrolysate with distinct strain differences in nutrient requirements.

In this study hydrogen production from the hexose and pentose monosaccharides in paper sludge hydrolysate using *Thermotoga neapolitana* is presented. Nutrient requirements as well as inhibitory effects are addressed. Additionally, results of fermentations on a larger scale under controlled conditions are presented. These fermentations allowed accurate determinations of hydrogen yields and hydrogen production rates for extreme thermophilic microorganisms. Finally, the efficiency of a hydrogen production process based on paper sludge hydrolysate will be discussed.

Poster Presentation 3-46

Biobased Products via Syngas Fermentation

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The long-term goal of this research effort is to develop the biological and engineering process technologies to convert biomass to useful products through syngas fermentation. The syngas approach employs carbon monoxide (CO) and hydrogen (H_2), rather than sugars, as the building blocks for synthesis of chemicals from biomass. Our desired result is to convert biobased syngas to value-added products that can supplement or replace nonrenewable fossil fuel-based chemicals, lubricants, and plastics. Although much is known about the metabolism of CO and H_2 by microorganisms, very little has been done toward exploiting this information toward development of a system for bioprocessing of syngas because several technological issues must be overcome.

We have assembled a multidisciplinary team to address this complex problem. We propose to identify, characterize and adapt a microbial fermentation system for capturing carbon and hydrogen from syngas and convert these chemically simple precursors to value added, chemically complex molecules, which will have utility as biorenewable products. By taking a systems perspective in this research, we hope to more rapidly advance the development of this technology. The success of our research program will lead to a new industry of gasifying biomass and using the resultant syngas to produce plastics, fuels, chemicals, lubricants, and other biobased products.

This poster will summarize the current status of our research efforts and provide a roadmap for future research.

Poster Presentation 3-47

Biodegradation Studies on Chemical Laboratory Effluent

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The wastewater from academically controlled pollution laboratories is composed by organic matter and a wide range of chemicals and heavy metals and is one of the most hardly-treated wastewaters. The biodegradability of laboratory effluent, measured as microbial survival rates, were determined as part of an investigation of the viability of biological treatment of chemical wastes. A series of eight experiments was developed to study the survival rates of microbial population of aerobic sludge from a conventional activated sludge plant of a local gelatin industry under various concentrations of laboratory effluent and domestic wastewater (1:1-1:64). Prior to the experiments, hydroxide precipitation using sodium hydroxide (~30% w/v) at pH=8.0 was performed with the laboratory wastewater. The experiments consisted in a series of equal volumes of aerobic sludge and a mixture of laboratory and domestic wastewaters placed on a rotary platform shaker at 37ºC and 100 rpm for 17 days to test the inhibitory effect of the laboratory wastewater on the bioactivity of the microorganisms. The rate of microbial survival varied from 5×10^5 to 1.8×10^8 cells/ml. Microorganism isolated from sludge samples at the end of the experiments contained low proportion of gram-positive relative to gram-negative bacteria. These communities are mainly composed of gram-negative straight, curved, and coccoid bacteria. Further studies will be conducted to test if the use of biological process for the treatment of laboratory effluents may be possible.

Poster Presentation 3-48

Use of Anion Exchange Resin to Recover Succinic Acid from a Continuous Fermentation

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Succinic acid is an important biobased product that can be used as a starting material in the production of numerous industrially important chemicals such as; 1,4-butanediol, tetrahydrofuran, 2-pyrrolidinone, n-methyl 2-pyrrolidinone, and gamma-butyrolactone. In many cases succinic acid can be directly substituted for maleic acid/anhydride in current industrial production processes. Numerous organisms are available for production of succinic acid through a fermentative route that provide acceptable conversion and productivity. However, a key step in making the biobased production of succinic acid cost competitive is the recovery of the product out of the fermentation broth. One approach is the use of ion exchange resin to remove the succinic acid/succinate from the fermentation as it is produced. Several types of anion exchange resins were evaluated for this application and then a continuous fermentation was demonstrated using a proprietary strain of *Actinobacillus succinogenes*. The pH of the fermentation was initially controlled by addition of ammonium hydroxide. As the fermentation progressed a group of anion exchange columns were used to remove succinate from the fermentation broth and recycle liberated ammonium hydroxide back to the fermentor to maintain pH control.

Poster Presentation 3-49

Ethanol Production from Hydrolyzates of Steam Exploded Biomass by Immobilized-cell Bioreactors

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The production of ethanol from enzymatic hydrolyzates coming from the cellulose and the hemicellulose of steam exploded biomass was investigated using bench scale bioreactors containing beads of *Pichia s*. and *Saccharomyces c* immobilized.

Two process geometries were explored: the continuous fermentation on fluidised bed (FBR) and the batch fermentation in a stirred bioreactor with suspended beads of alginate.

Various amounts of *Pichia s*. and *Saccharomyces c*. were tested in order to ascertain the effect on both the process productivity and the sugars utilization efficiency. Pure and mixed cultures of the two considered yeasts simultaneously metabolize both glucose and xylose already in the early 24 hours of the process, the consumption rate depending on the feed composition.

The viability of the yeasts under the settled conditions was explored over a multiple fermentation cycles. The process yields were higher with immobilized cells in both the geometries explored and the systems show good operational stability.

Poster Presentation 3-50 Student

Optimisation of Steam Pre-Treatment of H_2SO_4-Impregnated Corn Stover for Enhance Enzymatic Digestibility

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Among the available agricultural by-products, corn stover with its yearly 10 million tons, is the most abundant promising raw material for fuel ethanol production in Hungary. Due to the structural features, such as lignin and the highly ordered, crystalline structure of cellulose, lignocellulosic biomass is resistant to hydrolysis and only limited and slow conversion occurs without pre-treatment.

Pre-treatment of corn stover with H_2SO_4 impregnation and steam for enhancing the enzymatic digestibility has been investigated. Twelve different combinations of reaction temperature, time and pH were applied. After pre-treatment, the material was separated into a solid residue and a filtrate. The best pre-treatment condition (10% dry matter (DM) (w/w) 200°C, 5 min, 2% H_2SO_4) solubilized 50% of the hemicellulose and 20% of the lignin, while 80% of the cellulose remained in the solid fraction.

The solid residue was enzymatically hydrolysed with 5% DM (w/w) at 50°C using 25 FPU/g DM biomass. The best condition of pre-treatment increased the enzymatic conversion of cellulose to glucose four times in corn stover, compared to untreated material. The highest achieved conversion after 24 hours enzymatically hydrolysis was about 85%.

The fermentability of the pre-treated liquor after steam explosion was also investigated using Saccharomyces cerevisiae and no inhibitory effect was observed. Detailed results of the hydrolysis and the fermentability tests, and the changes in the lignocellulosic structure will be presented.

Poster Presentation 3-51

Controlled Immobilization of Biocatalytic Enzymes in Separative Bioreactors

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This work addresses the barriers to enzyme use in bioreactors, such as, enzyme inactivation during immobilization, product inhibition of enzymatic activity, and destruction of attachment site upon removal of inactive enzyme. The ANL *separative bioreactor* simultaneously produces and separates ionized bioproducts and is based on modified electrodeionization technology comprised of a unique porous ion-exchange resin wafer. The enzymes of the separative bioreactor are genetically engineered with a specific "tag" that facilitates chemically reversible immobilization to a specific "capture" resin. Selective enzyme capture-resins are incorporated in the resin wafer, thus the tagged enzymes are immobilized within the bioreactor to create the ANL two-in-one separative bioreactor. The tagged-enzyme/capture-resin technology overcomes the barriers to enzyme-based separative bioreactors with: a chosen site on the enzyme for immobilization (non-destructive enzyme immobilization), simultaneous product formation and separation (removal of product inhibition), and non-destructive removal of enzymes from the bioreactor (in situ enzyme regeneration). The tagged-enzyme capture-resin immobilization provides a technology platform for enzyme-based bioreactors of future biorefineries.

Poster Presentation 3-52

Potential of Ethanol Production by Immobilized Thermophilic Bacteria *Thermoanaerobacter* HY10 at Extreme Loading Rates

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Currently the area of using thermophilic bacteria for ethanol fermentation of hemicellulose sugars from lignocellulosic biomass has increased. The interest in thermophiles arises from a broad array of advantages in commercial scale bioethanol production obtained at elevated temperatures.

In this presentation work done on the extreme thermophile *Thermoanaerobacter* HY10 will be presented. Performance of HY10 converting xylose in a immobilized fermentation system at 70°C is compared to data obtained in a continuous stirred tank reactor. The advantages and disadvantages of an immobilized fermentation system is discussed related to organic loading rate, reactor size, substrate utilization and issues of contamination.

Poster Presentation 3-53

Modeling of Biomass Conversion to Mixed Alcohol Fuels (MixAlco Process) Using ASPEN-Plus

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The MixAlco Process converts biomass to mixed alcohol fuels, such as propanol, butanol, and pentanol. The processing steps are: (1) Pretreatment of raw biomass with lime, (2) Fermentation of lime-treated biomass to carboxylate salts using a mixed culture of acid-forming microorganisms, (3) Drying of Carboxylate salts, (4) Thermal conversion of carboxylate salts to ketones, and (5) Hydrogenation of ketones to alcohols. Ability to use wide variety of biomass feedstocks, absence of aseptic process conditions, and ability to use inexpensive equipment are some of the advantages of this process.

All the processing steps of this MixAlco process are modeled using Aspen Tech's Aspen Plus process simulation software. ASPEN PLUS is used because of the thorough treatment of thermodynamic interactions and its status as a widely accepted process simulator. The physical property data for many of the key components used in the simulation for the pretreatment process are derived from the In-house database (INHSPCD) developed by National Renewable Energy Laboratory (NREL). The standard NRTL (Non-Random Two Liquid or Renon) route is used as the main property method. The Aspen-Plus model developed is used to calculate energy costs of MixAlco process with energy recovery options for different processing steps.

Poster Presentation 3-54

Minimizing β-Glucosidase Activity Degeneration in Foam Fractionation

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Foam fractionation is a promising technique for concentrating proteins (which may be enzymes) because of its simplicity and low operating cost. Cellulase is a key enzyme for producing ethanol from biomass. The use of foam fractionation to recover fermentation-produced cellulase may lower the overall separation cost and, hence, the total cost of cellulase. In this foam fractionation study, one of the components of cellulase, β-glucosidase, is investigated. The effect of pH and superficial velocity in foam fractionation is determined in order to minimize β-glucosidase activity degeneration due to foaming.

Poster Presentation 3-55

On-Line Fermentation Monitoring by FTIR

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We are using an FTIR with an ATR fiber optic probe to monitor saccharification and fermentation of corn mash in benchtop reactors. The complex corn mash matrix is more difficult to monitor than a defined media. The purpose of this study is to determine if a chemometric model can be used to predict reactant and product concentrations during the reactions. Many of the constituents we want to monitor are nutrients for the yeast or products of the yeast's metabolism. The stoichiometry of the metabolic reactions causes the constituents to exhibit collinearity. The PLS1 chemometric model cannot distinguish between constituents that are collinear.

To eliminate the problem of collinearity, we are using prepared standards that do not contain fermentation matrix. Experimental design is used to generate sample sets that are uncorrelated and evenly represent the range of component concentrations. By adding matrix containing samples to the prepared standard training set, the predictive ability of the model is greatly improved.

Poster Presentation 3-56

Xylitol Production by Immobilized Yeasts from Sugarcane Bagasse Using a Fluidized Bed Reactor: Influence of Aeration and Mass of Carrier

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Xylitol is a polyalcohol with sweetening and anticariogenic properties. This work deals with a new approach to biotechnological production of this polyalcohol from sugarcane bagasse: the use of immobilized cells and fluidized bed reactor (FBR). The influence of aeration and mass of carrier on xylitol production from sugarcane bagasse hemicellulosic hydrolysate was evaluated using a FBR containing cells of *Candida guilliermondii* immobilized on porous glass. Seven batch fermentation runs, each lasting 72 hours, were conducted according to a 2^2 factorial design with 3 centerpoints. Increasing the mass of carrier resulted in a decrease in xylitol volumetric productivity, which, however, improved when the aeration rate was increased (95% confidence level). When the aeration rate and the mass of carrier were 150 ml/min and 100g respectively, the xylitol yield was low (0.25 $g.g^{-1}$), but the productivity was the highest (0.44 $g.l^{-1}.h^{-1}$), probably because the cell metabolism is faster when more oxygen available to the yeasts. When the mass of carrier was increased, the oxygen availability was even higher, due to a break in the air bubbles, resulting in lower productivity caused by more production of biomass instead of xylitol.

Poster Presentation 3-57

A Hollow-Fiber Membrane Extraction Process for Recovery and Purification of Lactic Acid from Fermentation Broth

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An energy-efficient hollow-fiber membrane extraction process is successfully developed to separate and recover lactic acid produced in fermentation. Lactic acid is an important specialty chemical that can be used to synthesize bio-based industrial products, including polylactic acid (PLA) and lactate esters. PLA and lactate esters are biodegradable and considered safe and green. Lactate esters in many situations can replace toxic solvents. While many fermentation processes have been developed for lactic acid production, an economical method for lactic acid recovery from the fermentation broth is still in need. Continuous extraction of lactic acid was achieved by using Alamine 336 in 2-octanol contained in a hollow fiber membrane extractor. In this process, the extractant was simultaneously regenerated by stripping with NaOH or ammonium hydroxide in a second membrane extractor, and the final product is a lactate salt, which can be acidified to lactic acid or reacted directly with alcohol to form lactate ester. The effects of the Alamine content, flow rate, solution pH, and lactic acid concentration on the extraction rate were studied. A mass-transfer model considering both diffusion and chemical reaction was developed and can be used for process scale up.

Poster Presentation 3-58

Immobilization of Whole Yeast Cells on Alternative Matrices for the Production of Sugar Inverted Syrup

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Yeast cells can be immobilized on a variety of media, such as calcium alginate, porous cellulose carriers, polyurethane foam and fibrous matrices. The high void volume, permeability, and low cost of fibrous matrices make them particularly attractive. For this study, loofa sponge and sugar cane bagasse, two types of fibrous, were chosen for immobilizing whole yeast cells, because in addition to having all advantages mentioned before, these kinds of supports are ready available.

Both matrices were previously coated with polyethyleneimine (0.2%) to acquire polycationic characteristics and three yeast strains of *Saccharomyces sp.* with different properties used: *Saccharomyces cerevisae* CB-IX (osmotolerant strain), *Saccharomyces cerevisae* IR2 (flocculent strain) and *Saccharomyces*, Fleischmann (commercial baker's yeast). Cells adhered strongly to the matrices and about 0.5-0.85 g of cells were bound per gram of dry support. The invertase activity values from 2.5 to 12.5 U/mg of dry support were in agreement with the immobilization yields, showing that there is a total biocompatibility of both supports with the yeast cells. However, under the conditions tested, loofa sponge was found to have a better performance than sugar cane bagasse.

The yeast strains with either osmotolerant or flocculent properties displayed a higher cell efficiency (> 80%) than the commercial strain (55%), suggesting that such special properties also help to achieve a high cell loading on loofa sponge.

The present study shows that the cell retention property of the fibrous supports can be enhanced by treatment with PEI. Cell adhesion was strong and such conditions as high ionic concentration and extremely high pH, which normally disrupt the ionic interactions, failed to desorb the cells from the matrix surface. The simplicity of the immobilization technique, the strong binding and the low cost of the loofa sponge can help to find future applications for yeast cells immobilized for the hydrolysis of concentrated sucrose syrups.

Acknowledgements: This work was supported by research grants from the FAPESP-Brazil.

Poster Presentation 3-59

Organosolv-Based Biorefining – Process Considerations and Product Profiles

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Biorefining technologies that convert biomass into multiple value-added co-products are needed for woody biomass to economically compete with fossil carbon resources. Many currently proposed technologies for the production of ethanol and other chemicals from woody biomass are not economic because they rely on a single revenue stream.

Ethanol-based organosolv pulping technology, when applied to woody biomass fractionation, provides a major improvement in the long-term economic viability of producing xylose, resins, adhesives and lubricants, as well as sugar platform chemicals, including ethanol. Lignol Innovations Corp. has acquired all of the rights to the ethanol-based organosolv pulping technology previously known as the Alcell Process, together with the pilot plant and intellectual property.

Lignol is upgrading this technology to operate as a biorefining technology for the production of chemicals and fuels from woody biomass. Canada's effort to ratify the Kyoto Accord is representative of governments emphasizing limiting the use of petrochemicals while encouraging the development of economically viable industries based on renewable resources.

The results of Lignol's pilot plant scale studies on softwood residues will be presented and discussed within the context of presenting design considerations for the initial commercial-scale plant to be commissioned in 2005. Additionally, the unique applications of the Lignol Process-produced products will be presented.

Poster Presentation 3-60

The Effect of β-Glucosidase on the Foamability of Cellulase

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In an effort to determine whether cellulase can be removed continuously using foam fractionation from a fermentation process, the foamability of *Trichoderma reesei* cellulase under different conditions was investigated. β-glucosidase was added to aqueous cellulase solutions to determine its effect on the resulting protein mixture foamability and total protein concentration in the recovered collapsed foam. The concentrations of the β-glucosidase and total cellulase were varied to determine their relative additive effect on the surface tension and the foam volume. Since, cellulase is relatively hydrophobic with respect to it's component, β-glucosidase, it was expected to be more likely to produce foam than the less hydrophobic β-glucosidase. In fact, cellulase addition to β-glucosidase tended to enhance foam formation. Interestingly, the addition of β-glucosidase to cellulase caused the surface tension to initially drop, followed by an increase at intermediate concentrations, with a drop again for even larger β-glucosidase concentrations.

Poster Presentation 3-61

Evaluation of Recombinant Green Fluorescent Protein *(GFPuv)* Purification Through Different "HiTrap" HIC Resins

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Cultures (37°C/24h/100rpm) of transformed *Escherichia coli* cells expressing recombinant green fluorescent protein (GFPuv) were centrifuged (1000 g/ 30 min/4°C). The pelleted cells were resuspended in extraction buffer (25 mM Tris-HCl, pH 8.0; 1.0 mM β-mercaptoethanol; 0.1 mM PMSF), and each 450 μL aliquot was subjected to one of two methods of extraction: either exposure to freezing/thawing/sonication (FTS) cycles prior to three-phase partitioning (TPP) or directly to TPP extraction. The proteins extracted from both methods were divided into five samples. Each sample was eluted through one "HiTrap" column pre-packed with a different hydrophobic interaction chromatography resin: methyl; butyl; octyl; phenyl (low substitution) or phenyl (high substitution) sepharose. The fluorescence intensity of the eluted samples was related to μg *GFPuv*/mL (excitation/emission maxima at 394/509nm). The eluted samples were also run on a 12% SDS-PAGE with protein bands visualized with Coomassie staining. A single band between 27 kDa (standard GFPuv) and 29 kDa was visualized for every type of HIC column used. The effectiveness of TPP extraction for GFPuv purification was observed to be similar in every lane for each column type.

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Poster Presentation 3-62

Succinic Acid Adsorption from Fermentation Broth and Regeneration

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Over twenty four adsorbents have been tested for uptake of succinic acid (SA) from aqueous solutions. The best resins were then tested for successive loading and regeneration using hot water. The key desired properties for an ideal adsorbent are high capacity, complete stable regenerability, and specificity for the product. The best resins have a stable capacity of _0.06 g succinic acid/g resin at moderate concentrations of SA. Several sorbents were tested more exhaustively for uptake of succinic acid and for successive loading and regeneration using hot water. One resin, XUS 40285, has a good stable isotherm capacity, prefers succinate over glucose, and has good capacities at both acidic and neutral pH.

Succinic acid was removed from simulated media containing salts, succinic acid, acetic acid, and sugar using a packed column of adsorbent resin, XUS 40285. The fermentation byproduct, acetate, was completely separated from succinate. A simple hot water regeneration successfully concentrated succinate from 10 g/L (inlet) to 40-110 g/L in the effluent. If successful, this would lower separation costs by reducing the need for chemicals for the initial purification step.

Despite promising initial results of good capacity (0.06 g succinic/g sorbent), 70% recovery using hot water, and a recovered concentration of >100 g/L, this regeneration was not stable over ten cycles in the column. Alternative regeneration schemes using acid and base were examined and more sorbent screened. Tests were performed with both simulated broth containing succinic acid at various concentrations and with actual broth provided by MBI. Seven of the most promising resins were tested for regenerability and stability using a modified extraction procedure combining acid and hot water washes. Two (XUS 40285 and XFS) showed both good stable capacities for succinic acid over ten cycles and more than 95% recovery in a batch operation. These results indicate that sorption for succinic acid continues to be promising. However, it will need additional column and process tests focusing on the regeneration of the resins and the concentration of the succinic acid in the effluent streams.

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Poster Presentation 3-63

A Technique to Quantify the Population of Viable Cells in a Three Phase Fluidized Bed Reactor

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An experimental study was carried out in a Three Phase Fluidized Bed Reactor (TPFBR), to degrade biologically a synthetic effluent of milk industry. In this study three different COD concentration of milk wastewater substrate (462, 825 and 1473 mg O_2/L) were tested. Small PVC particles were fluidized in this reactor, which are used as support for microbial growing. The aim of this work was to develop a technique to prepare samples and obtain cells in segregated form to determine the number of viable cells on both support particles and liquid phase. In defining the technique, for every COD concentration used, the number of ultrasound treatments and the number of filtration steps through the isopore polycarbonate filters with 5.0 μm diameter pores were varied. The lethality and the efficiency of disintegration of the sonication and filtration conditions were evaluated by colony counts on solid media and compared to the protein content. The technique will allow quantification of the dynamics of the population sizes of solitary and biofilm bacteria in particles used as support for microbial growing.

Poster Presentation 3-64

Ethanol/Water Pulping of Sugarcane Bagasse: Kinetic Studies and Enzymatic Bleachability of the Pulps Obtained

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The ethanol/water pulping process of sugarcane bagasse was estudied at 170°C, 185°C and 200°C. The obtained pulps were analyzed by kappa number (residual lignin content) and viscosity (preservation of the polymerization degree of the cellulose). The best temperature for the pulping achieved was 185°C: the constituents of pulps were maintained in desirable levels and the values of viscosity and kappa number were intermediates. Viscosity of the pulps ranged from 4.12 to 9.32 cP and the kappa number ranged from 23.59 to 31.18. In the sequence, pulping was carried out under argon pressure (0.1 to 2.0 MPa) and varying the reaction time from 1 to 2 h at constant temperature (185ºC). Pulps obtained from the organosolv cooking (ethanol/water) of sugarcane bagasse at 185°C for 2.5 h were bleached with the xylanase enzyme obtained from the fungus Thermomyces lanuginosus IOC-4145 and submitted to alkaline extraction.

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Poster Presentation 3-65

An Adaptation of GOD-PAP Method for the Quantification of Glucose in the Presence of Sucrose

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GOD-PAP method was developed for glucose determination in whole blood, serum and plasma. In this work we propose an adaptation of this kit to be used to quantify glucose in the presence of sucrose. Glucose concentration (C_{glu}) is given by the equation:

$$C_{glu} = 1.18\ C_{meas} - 0.26\ t - 7.52$$

where C_{meas} is the measured absorbance in a given time (t in min). This equation has a maximum deviation of 5% for C_{glu} from 20 to 180 μg glucose mL^{-1}.

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Poster Presentation 3-67

Comparison of Different Methods Used for Detoxification of Rice Straw Hydrolysate and Their Influence on Xylitol Production

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During acid hydrolysis of hemicellulosic materials, the monosacharides liberation, mainly xylose, is followed by formation or release of compounds such as acetic acid, furfural, 5-hydroxymethylfurfural and lignin derivatives. When hemicellulosic hydrolysate is used in fermentative processes, these compounds limit the utilization of sugars by the microorganism and consequently interfere with the products formation. The identification of these compounds and the choice of the best hydrolysate detoxification method are important to improve the efficiency of the fermentative processes.

This study evaluates different methods of treatment, based in pH alteration and adsorption on activated charcoal, applied to rice straw hemicellulosic hydrolysate before it was used as a fermentation medium for xylitol production by the yeast *Candida guilliermondii*. The best results for the fermentative process ($Y_{P/S}$ = 0.72 g/g; Q_P = 0.55 g/L.h) were attained with the use of the treatment: Adjustment of the initial pH (0.4) to 2.0 (with solid NaOH); Addition of activated charcoal (1 gram of charcoal per 40 grams of hydrolysate) under agitation at 150 rpm, 45°C, for 60 min; Increase in the pH to 6.5 (with solid NaOH). The results demonstrated that the lignin derivatives are the most potent inhibitors present in this kind of hydrolysate, and their removal occurred in a selective form, depending on the pH employed in the treatment.

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Poster Presentation 3-68

Increase of Tannase Production in Solid State Fermentation by Aspergillus Niger 3T5B8

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Tannase or tannin-acyl-hydrolase (E.C. 3.1.1.20) catalyzes the hydrolysis of ester and depside bonds in hydrolysable tannins, as tannic acid, releasing glucose and gallic acid. Several types of microorganisms are known as tannase producers. Species belonging to *Aspergillus* and *Penicillium* genus were reported as the best tannase producers. The applications and perspectives of uses of this enzyme are in food and pharmaceutical industries. The aim of this work was to optimize the production of tannase by *Aspergillus niger* 3T5B8 in solid state fermentation system. The media were based in wheat bran with addition of tannic acid. For optimization were tested the tannic acid/wheat bran ratio, different moisture levels, addition of supplementary nitrogen sources, addition of supplementary phosphate and the concentrations of supplementary nitrogen and phosphate added to the medium. The results showed that best medium was with 15% of tannic acid, 37.5% of initial moisture, 1.7 % of ammonium sulfate and 2.0 % of sodium phosphate. The presence of phosphate showed a great importance for optimization, because promoted increase in the synthesis level and a very expressive decrease in the maximum production time, from 72 to 24 hours of fermentation. The optimization process promoted a increase 861% in yield (from 2.77 to 26.62 $U.g^{-1}$) and 2783% in productivity (from 0.038 to 1.109 $U.g^{-1}.h^{-1}$).

Poster Presentation 3-69

Cellulase Retention and Sugar Removal by Membrane Ultrafiltration During Lignocellulosic Biomass Hydrolysis

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Biomass conversion processes are not economically competitive with petroleum conversion to fuels and chemicals at present. Membrane technology could potentially reduce production expenses in a variety of biomass applications. Because enzymatic hydrolysis of cellulose represents a significant fraction of production costs, it would be economically beneficial to recover and reuse the expensive cellulase enzyme by employing ultrafiltration membrane separations. We are studying ultrafiltration as a means to recover cellulase after simultaneous saccharification and fermentation and as a means to isolate the saccharification and fermentation reactions. Our work focuses primarily on the latter, as separate saccharification and fermentation reactions can be optimized with respect to temperature and other variables. In addition, the use of ultrafiltration membranes to remove inhibitory glucose and cellobiose from the saccharification reactor may result in a more efficient saccharification process.

In several laboratory-scale saccharification experiments, the utility of ultrafiltration membranes to extract glucose from a mixture of cellulase enzyme (CE) and lignocellulose (LC) from pretreated corn stover was examined using polyethersulfone membranes with 50,000 Dalton molecular weight cutoff. After solids are removed by vacuum filtration, typical permeate fluxes through the ultrafiltration membranes are 90±10 L/m2-h, while cellulase retention is approximately 80-90% and glucose transmission is nearly 90%. Saccharification experiments were conducted using LC concentrations of 15% (w/w) and enzyme loadings of 5-20 Filter Paper Units (FPU)/g cellulose. Saccharification efficiency in the different experiments is gauged by measuring the cellulose conversion and specific glucose yield from cellulase (mass of glucose generated/FPU) over seven days. Saccharification experiments processed with ultrafiltration are compared to control experiments. To date, cellulose conversions both with and without ultrafiltration were measured at 65-75% using an enzyme loading of 20 FPU/g cellulose. Future experiments, in which ultrafiltration is applied several times over the course of saccharification, may show an enhanced cellulose conversion along with an increased specific glucose yield, provided the cellulase enzymes are recovered and reused.

Poster Presentation 3-70

Selection of Anion Exchangers for Detoxification of Dilute Acid Hydrolysates

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It has previously been shown that treatment with an anion exchanger efficiently increases the fermentability of a spruce dilute-acid hydrolysate by *Saccharomyces cerevisiae*[1]. However, the mechanisms for detoxification are dependent on both the functional groups and the properties of the polymer matrix[2].

Six different anion exchanger resins were therefore selected for detoxification experiments with a hydrolysate that originally had very low fermentability. The resins were based on different polymers and represented both strong and weak anion exchangers.

Fractions of the hydrolysate from each of the resins were analyzed for monosaccharides, furfural, HMF, phenols, sulfate, acetic, formic and levulinic acid. The strong anion exchangers initially trapped monosaccharides, which were released when the sorbed sugars were replaced by compounds with a higher affinity towards the resin. As expected the aliphatic acids were strongly retarded and sulfate had the strongest affinity. pH and UV absorbance were also determined and the samples were fermented with different results. All resins had the ability to increase productivity and ethanol yield, however the results varied considerable between the anion exchangers tested.

[1] Larsson *et al.* (1999) Appl. Biochem. Biotechnol. 77-79, 91-103

[2] Nilvebrant *et al.* (2001) Appl. Biochem. Biotechnol. 91-93, 35-49

Poster Presentation 3-71

Engineered Fluid Transporting Fractals for Improving Bioprocessing Efficiencies

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Amalgamated Research Inc. has recently developed the concept of using engineered fluid transporting fractals for controlling the scaling and distribution of fluids. Fluid scaling and distribution are common requirements in many disparate processes. Fractals allow fluid properties, such as eddy size or concentration distributions, to be adjusted in a highly controlled manner. This control is obtained by introducing symmetries into the fractal structures. Benefits can include reductions in process size, increased efficiencies and energy savings.

An ongoing DOE project, DE-FC07-01ID14016, has demonstrated that ion exchange and chromatography processes can be significantly reduced in size by designing process equipment around fractals. The project has also demonstrated benefits in distillation applications.

Poster Presentation 3-72

Removal of VOCs Using Biotricking Filters

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This work reports the result of studies using biotricking filter with fibriform and poly-urethane form packing, respectively, for treatment of VOCs in an air stream. Effects of packing material, specific surface area of the packings, volumetric loading of VOCs, the pressure drop and the empty bed residence time (EBRT), were tested. Results show that the elimination capacity of VOCs in the biofilter system can be evaluated on the basis of unit surface area of packing materials. Surface properties may be a major concern in choosing a packing, The removal efficiency of VOCs decreased as the gas velocity and VOCs concentration in the inlet gas increased. The pressure drop in the biofilter packed fibriform was 5mmH_2O at a representative space velocity of 360/hr. The removal efficiency exhibited a decreasing trend at lower EBRTs, with the efficiency dropping to as low as 92% at an EBCT of 8 sec.

Poster Abstracts for Session 4

Biotechnology for Fuels and Chemicals - Past, Present, and Future

Poster Presentation 4-09

Biodiesel: The Fuel of the Future

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Biodiesel has transformed from an experimental fuel into the fastest growing alternative fuel on the market. This renewable fuel is domestically produced from any fat or vegetable oil, such as soybean oil, through the chemical process transesterification. Biodiesel works in any diesel engine with few or no modifications necessary. After nearly a decade of research and in-field demonstrations, biodiesel has emerged as a leading choice among fleet managers because of its ease of use, high performance and emissions reduction. Biodiesel has played a significant role in federal energy legislation, and nearly half of the states have passed legislation favorable to the growth of the fuel.

Although there has been some consumer backlash against genetically modified crops for consumption as food, the biodiesel industry is well positioned to provide a market for genetically modified soybeans and other crops. Soybeans can be modified to contain a higher oil content, thus providing more oil for biodiesel production. They can also be modified to improve certain characteristics of biodiesel. Increasing the oleic acid content may improve fuel stability. Removing saturates may improve cold flow properties for biodiesel. In addition to improving performance characteristics, genetically modifying soybeans may offer reduced emissions. Preliminary research suggests beans can be modified to decrease emissions of nitrous oxides when the fuel is burned in a diesel engine.

Poster Presentation 4-10

Optimization of Biodiesel Enzymatic Production from Castor Oil in Organic Solvent Medium

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Biotransformation of vegetable oils through the use of enzymes as catalysts has been a matter of intense investigation nowadays. Among several raw materials available, castor oil, obtained from the growing native castor plant, is one of the most versatile products with applications in food, pharmaceutical and cosmetic industries. Furthermore, the possibility of using biodiesel as an additive to mineral diesel, to result in a sulfur-free, with a higher-cetane number fuel from a renewable resource has motivated the biomodification of vegetable oils towards the reduction of environmental investments and import needs. In this context, this work is aimed at investigating the production of fatty-acid ethyl esters (FAEE) from castor oil using n-hexane as solvent and two commercial lipases, Novozyme 435 and Lipozyme IM, as catalysts. For this purpose, a Taguchi experimental design was adopted considering the variables temperature (35-65^{o}C), water (0-10%) and enzyme (5-20%) concentrations and the oil to ethanol molar ratio (1:3 to 1:10). Afterwards, an empirical model was built so as to assess the main and cross variable effects on the reaction conversion and also to maximize the biodiesel production for each enzyme. It is shown that complete conversion in FAEE is achieved for some experimental conditions tested.

Poster Presentation 4-11

Exhaust Emissions and Performance of Diesel Engine Operating on Biomass-Based Oil Blends and their Water-Fuel Emulsions

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We experimentally evaluate a performance and exhaust emissions of a single cylinder, four stroke direct injection diesel engine operating on diesel fuel containing 50% volume waste soybean oil (Blend 50), waste soybean oil containing 50% waste polystyrene pyrolysis oil (Blend PS), soybean oil methyl ester (VDF) and their water-fuel emulsions. The combustion characteristics and emissions such as NOx, CO, HC, HCHO, O_2, CO_2 , smoke degree and particle size distribution of exhaust particulate are compared with the case of JIS #2 Diesel fuel. Fuel consumption and indicator diagram in cylinder were measured, and carbon deposit on cylinder head were observed after 400 hours operating on Blend 50. Based upon evaluation of engine performance and emissions, biomass blends, and water emulsified fuels appear to be renewable, ecologically acceptable fuels of diesel engine. Diesel engine/Generator can be operated on biomass fuels such as Blend PS and Blend 50 to produce electric power as the distributed power system. Also discussed are the usability of biomass-based fuels as alternative fuel, as well as availability for treatment processes of the waste plant oil and waste plastics.

Key words: Diesel Engine; Waste Soybean Oil; Polystyrene Pyrolysis Oil; Biomass Based Fuels; Refuse Derived Fuel; Engine Performance; Exhaust Emission; Water Emulsified Fuel

Poster Presentation 4-12

Pre-Esterification of Free Fatty Acids by Solid Acid Catalysts

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Free fatty acids contained in waste fats or oil may deactivate the alkaline catalyst which is widely used for biodiesel production. The free fatty acids have been esterified by sulfuric acid before the transesterification to get higher yield. But sulfuric acid used for the esterification process may not be recovered and cause a secondary environmental pollution problem. Strong acidic resins have been used to overcome the problems with sulfuric acid. The resins have a major disadvantage that may not be used at a high temperature. A new solid acid catalyst, which has a higher thermal stability, has been made at our laboratory. The catalyst has been tested for the esterification not only of several free fatty acids but of waste oils from food industry. The performance data of the new catalyst will be presented under the various operating parameters like temperature, methanol, and catalyst concentrations. The performance of the catalyst will also be compared with that of Amberlyst 15.

Poster Presentation 4-13

Efficient Ethanol Production by a Novel Bioprocess

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Due to the expected depletion of fossil-fuel-based energy sources, research on alternative, renewable sources has recently gained prominence. From this point of view, fuel ethanol production from sugars has been investigated using *Saccharomyces cervisiae* and *Zymomonas mobilis*. Ethanol production from various sugars has thus been attempted using a recombinant *E. coli* strain. However, higher productivity and cost efficiency of such a bioprocess is desirable. We previously found that coryneform bacteria could retain main metabolic capabilities under growth-suppressed conditions. Using this property, high conversion yield and much less by-products in high cell density coryneform bacterial continuous reaction was possible.

Coryneform bacteria are widely used in the industrial production of amino acids, but these bacteria do not possess a natural ability for ethanol production. In order to use the bacteria for ethanol production, a recombinant strain containing pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase (*adh*) genes from *Z. mobilis* was constructed. Using the recombinant strain, we attempted to establish a novel bioprocess for continuous ethanol production.

Poster Presentation 4-14

Production of Lactic Acid by Novel Bioprocess Using Coryneform Bacteria

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Biomass represents the highest potential as a sustainable source of renewable energy and industrial chemicals of the future. Products of its primary degradation can be used as raw materials in a variety of industrial processes including production of ethanol, lactic acid and various kinds of chemicals. Lactic acid has a wide range of industrial applications in fields such as preservatives, pH regulators and biodegradable plastics. We previously found that coryneform bacteria could maintain main metabolic capabilities under growth-suppressed conditions. This property is advantageous to establish a novel bioprocess with high productivity due to high cell density in reactor, less by-products, high conversion yield of reactants and complete continuous reaction. Using these features, a novel bioconversion process for lactic acid production from glucose was developed.

Poster Presentation 4-15

Bioethanol Co-location with a Coal-Fired Power Plant

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Bringing the first bioethanol plant on line in the U.S. will be a major step toward commercializing the cellulosic biomass to ethanol industry; however, presently there are large economic hurdles that must be overcome in order for this goal to be attained. Major economic savings can be realized by co-locating a cellulosic biomass to ethanol plant with a coal-fired power plant. In addition, coal fired power plants benefit by burning the lignin residue produced by the bioethanol plant, thereby reducing their SO_x, NO_x, green house gases (GHG), and CO_2 emissions. To this end, NREL has recently completed two subcontracts investigating the possible synergies of co-locating a bioethanol plant with existing coal fired plants in New York, Indiana, and a green-field site in Nebraska. Results from these subcontracts show promising rates of return for potential investors.

By co-locating, the bioethanol facility saves the largest single capital investment cost by not having to purchase a boiler/turbogenerator unit (~ 30-40 MM$). Other cost savings are realized via labor, warehousing, site development, and wastewater treatment facilities. A coal-fired power plant can benefit by being able to sell excess steam and electricity, instead of letting it go unused, or operating below capacity. However, its greatest benefit will be displacing coal by burning the lignin residue produced by the bioethanol plant. Lignin residue is a clean (renewable) fuel that would drastically reduce GHG and SO_x emissions, as well as reducing NO_x and CO_2 emissions. With environmental regulations becoming more stringent in the fossil fuel based power industry, the benefit of burning a clean fuel can potentially save power plants from having to purchas NO_x and SO_x reduction systems and may add life to older plants that do not comply with current and future regulations.

Poster Presentation 4-16

Two-Step Preparation for A Catalyst-Free Biodiesel Production; Hydrolysis and Methyl Esterification

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Two-step preparation for biodiesel fuel production was developed. The first step consists of triglycerides hydrolysis for fatty acids formation in subcritical water, and the second is methyl esterification of the hydrolyzed products by supercritical treatment of methanol. By the two-step preparation, we can lower the reaction temperature and pressure without the use of catalyst. Reaction parameters to affect the conversion on both hydrolysis and methyl esterification will be presented, followed by a proposed optimum reaction condition.

The obtained products showed comparable methyl esters formation with those prepared by the conventional alkaline-catalyzed method and our previous supercritical methanol method. Biodiesel produced by our two-step preparation method was proved to fulfill the quality specification on free glycerol and glycerides content as described in some biodiesel standard in the USA and European countries. This new production method can cover any type of raw material, from virgin vegetable oils to wasted vegetable oils. This two-step method will be more useful for biodiesel from waste vegetable oils.

Poster Presentation 4-17

Biodiesel Fuel from Vegetable Oil by Various Supercritical Alcohols

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Our research group has developed a catalyst-free biodiesel fuel production method by the application of supercritical state of methanol. To study the effect of alcohol species on the transesterification of triglycerides and esterification of fatty acids, ethanol, 1-propanol, 1-butanol and 1-octanol were selected. The difference of reactivity in transesterification and esterification was studied at temperature 350 and 300°C. As we expected, results showed that the reaction time for transesterification was found to become longer as the alkyl chain of alcohol is longer. Complete conversion of vegetable oil to alkyl esters was observed for all alcohols studied.

Five fatty acids mostly present in vegetable oil were studied for esterification reaction. We found that esterification of fatty acids depended also on the alcohol species. Interestingly, the rate of esterification reaction was faster than that of transesterification. It means that, compared with transesterification, esterification of fatty acids could be carried out at lower temperature at relatively shorter reaction time. These lines of evidence would be important in developing biodiesel fuel since biodiesel produced from longer alkyl chain of alcohol may improve the cold flow properties.

Poster Presentation 4-18

Stabilization of Photochemical Energy Conversion Potentiality of Cyanobacterial Thylakoids by Osmolytes and Immobilization

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Thylakoids isolated from Nostoc sp. and *Lyngbya arboricola* and *Scytonema geitleri* were immobilized with crosslinked albumin and gelatin polymers, alginate and polyurethane in absence and presence of osmolytes (1 M) sucrose, trehalose, sorbitol and glycine betaine, and exudates obtained from the liquid culture of *S. geitleri*. The photochemical reduction of 2,6 dichlorophenol indophenol was undertaken as a measure of thylakoids' energy conversion potentiality. The storage of native and immobilized thylakoids at -16°C for 600 h in dark showed a considerable photoreduction of the dye, which lowered on further increase in the storage period. The lowering in the reduction of dye was minimum in the thylakoids immobilized with albumin and thereafter gelatin and polyurethane. Though, compared to *Nostoc* sp., thylakoids isolated from terrestrial forms possessed low capacity to reduce dye, they were active even after storage for longer duration. Application of osmolytes enhanced stability of the thylakoids on storage. The effect of osmolytes on stability was in the order of trehalose > sucrose > exudate > sorbitol > glycine betaine. The functional stability recorded by continuous illumination of the native and immobilized thylakoids also exhibited similar pattern as storage stability, but the span of stability was considerable only up to 120 h.

Poster Presentation 4-19

Conversion of Municipal Solid Waste to Carboxylic Acids Using A Mixed Culture of Mesophilic Microorganisms

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Waste biomass was anaerobically converted to carboxylate salts by using a mixed culture of acid forming microorganisms. Municipal solid waste (MSW) and sewage sludge (SS) were used as fermentation substrates. MSW serve as the energy source (carbohydrates) while sewage sludge as the nutrient source (minerals, metals, and vitamins). Four fermentors were arranged in series and solids and liquids were transfer in opposite direction. Fresh biomass was added to the first fermentor (which contains the highest carboxylic acid concentration) and fresh media was added to the fermentor four (which had the most digested biomass). All fermentations were performed at 40C. Calcium carbonate was added to the fermentors to neutralize the acids to their corresponding carboxylate salts. Iodoform was used to inhibit methane production. Urea was added as a nitrogen source.

Countercurrent fermentation allows both high conversions and high product concentrations. Excellent reproducibility was demonstrated for batch and countercurrent fermentations. 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. Product concentrations up to 25 g/L were found, with productivities of 1.4 g total acid/(L liquid.day). Mass balances with closure between 93 and 105% were obtained for all systems. Continuous Particle Distribution Modeling (CPDM) was applied to correlate batch fermentation data to countercurrent fermentation data and predict product concentration over a wide range of solids loading rates and residence times. CPDM for lime-treated MSW/SS fermentation system predicted the total acid concentration and conversion within 20 % of the experimental results.

Poster Presentation 4-20

Complete Recycling of Effluent from the Bottom of Ethanol Distillation Column in Ethanol Continuous Fermentation Using Self-Flocculating Yeast

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An integrated green ethanol continuous fermentation process with complete recycling of ethanol distilled effluent was established. Continuous fermentation of ethanol was carried out in air-lift suspended bed bioreactors operated in series using self-flocculating yeast SPSC (a fusant of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*) as the fermentation strain.

The feasibility of the complete recycling of ethanol distilled effluent was discussed. A mathematic model was developed to predict the accumulation of inhibitive by-products in fermentation broth. The concentration of inhibitive by-products could reach a steady state after a certain time operation based on the mathematic model. Additionally, a two-month continuous fermentation experiment proved that complete recycling of ethanol distilled effluent is feasible in our ethanol continuous fermentation process. An industrial scale demonstration plant of this technology is under construction now in China.

Poster Abstracts for Session 5

Biobased Industrial Chemicals

Poster Presentation: 5-07

Using Landfill Gas Energy as a Source of Renewable Power, Localized Biodiesel Production and Hydrogen Vehicle Refueling Stations

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According to the United States Environmental Protection Agency, landfills are the largest source of methane emissions from human activity in the United States, accounting for 37% of America's emissions. The Agency also estimates that landfill methane is 21 times more effective at trapping heat in the atmosphere than is carbon dioxide, so lessening its impact is vital to assisting worldwide efforts to reduce "greenhouse gas" emissions. Substantially reducing vehicle emissions is another widely supported goal for addressing the world's greenhouse gas phenomena.

This paper describes how developing landfills gas recovery projects can substantially mitigate landfill and vehicle emissions while providing localized vehicle refueling infrastructure. In addition to renewable electric power, such project developments can support value added biodiesel production for diesel vehicles and hydrogen production for fuel cell vehicles. All three endeavors, run concurrently, can realize greenhouse gas emissions reductions on a much broader scale than is currently being attempted.

DTE Biomass Energy, Inc. an Ann Arbor, Michigan based company and a national leader in developing landfill gas-to-energy projects throughout the United States has teamed with Biodiesel Industries, Inc. a Nevada based biodiesel fuel producer on several hybrid project developments. The projects contemplate employing landfill gas-to-energy facilities and 3,000,000 gallon per year modular biodiesel production units. Hydrogen production facility sub-projects are also planned. Such projects would use the waste heat and portions of the electricity generated from landfill gas. Essentially, the developers plan to use a greenhouse gas to concurrently make electricity, biodiesel fuel and hydrogen for fuel cell vehicles.

Poster Presentation 5-08

Conversion of Rice Straw for Production of Bioethanol and Other Valuable Products using The Danish Bioethanol Concept

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Rice Straw has been pursued as a suitable lignocellulosic substrate for production of bioethanol and other valuable products – like biogas. This is due to the vast surplus of straw in areas growing rice – especially in the US and in major parts of Asia. Rice Straw holds a great potential for production of ethanol as it has a lignocellulosic composition similar to corn stover and wheat straw. The utilization of rice straw as a substrate in production of bioethanol and biogas using the Danish Bioethanol Concept has recently been studied. The results from these studies will be the focus of the presentation.

The process to which the rice straw was subjected, includes pretreatment by wet oxidation, enzymatic hydrolysis, sugar fermentation by *S. cerevisiae* and a mutant of *T. mathranii*, plus a subsequent production of biogas. Recoveries following the pretreatment process as well the ethanol yields obtained from the two fermentations will be included in the presentation. Production of biogas in combination with bioethanol further improved the overall biomass utilization.

Poster Presentation 5-09

Application of the Danish Bioethanol Concept to Different Types of Biomass Waste

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Danish Bioethanol Concept (DBC) is based on the philosophy of using a combination of processes to utilize the full potential of the raw material by producing multiple valuable products. The main components of the concept, consists of an alkali wet oxidation pretreatment unit, a mesophilic SSF reactor for glucose conversion, a thermophilic fermentation converting xylose to ethanol and a process water purification step producing biogas. The presentation will focus on the possibility of adapting the DBC to different types of lignocellulosic biomasses such as bagasse, rice straw, corn stover, wheat straw, and sisal waste. Variations caused by the difference in the lignocellulosic structure of the different biomass types influences the process performance In the presentation we discuss the adaptations needed to obtain the highest possible biomass conversion.

Poster Presentation 5-10

Extraction of Low Grade Energy in the Form of Hot Water at 65 to 70 Degrees Celsius from Compostable Materials Such as Municipal Solid Waste (MSW) pre Human Consumption Plant and Vegetable Waste and Animal Manures

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Field spreading of MSW and animal manures is outlawed in many jurisdictions due to the potential for pathogenic bacterial cultures to migrate into ground water. There is also a body of evidence to suggest that these pathogens may migrate directly in to the cell structure of plants presented with untreated MSW and manures. pre human consumption food waste is costly to process and is generally barred from landfills.

One very efficient method of rendering these waste feed stocks harmless is to kill the pathogenic bacteria through the process of composting during which colonies of thermophilic bacteria act on the waste materials in the presence of oxygen to achieve and maintain elevated temperatures in excess of 50 degrees Celsius for a continuous period spanning a minimum of three days. Having undergone this elevated temperature cycle, the materials are then considered safe for field spreading.

The above process is far from innovative or new. In fact it is used in many municipalities throughout North America.

This paper will address an innovative method for extraction of low level energy in the form of hot water at a temperature of 65 to 70 Celsius from the composting process utilizing a matrix of super thermal conductors in the form of evacuated two phase heat exchangers generically called “heatpipes”. This matrix is self powered by the energy it contacts and rapidly transfers that energy into an insulated water tank. The hot water is produced in quantities such that a typical municipal composting facility can produce excess energy sufficient to heat a 5 to 10 acre greenhouse operation contiguous to the composting plant without causing any change in the “pasteurization” process within the composting cycle.

A 10 acre commercial greenhouse as described here will generate approximately 12 tons of waste plant material weekly during its typical growing cycle. When plant vines are removed prior to planting new seedlings, the plant waste tonnage increases ten-fold minimum. Due to the inclusion of plastic support clips and twine in these vines, disposal is costly and complex but is quite simple if composting is used; even consuming the plastic twine and clips in the composting process. Essentially the energy extraction process and the greenhouse heating are accomplished at no cost after the capital costs are amortized. Further, the waste feedstocks from the greenhouse are consumed by the composting process freeing the greenhouse owner from the cost of tipping fees for disposal of his waste. By way of comparison, a typical 10-acre commercial green house producing hydroponically grown tomatoes operating in the County of Essex, the Province of Ontario, Canada, spent $500,000.00 in natural gas to maintain heat to a required level during the winter of 2000/2001.

The above technology is “patent applied for” in Canada and the United States with world wide patent protection pending. For additional information please view www.agrilab.org

Poster Presentation 5-11

Utilization of Lignin Biomass Component in Composites with Polyolefins

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Lignin is an amorphous natural polymeric material. About $50x10^6$ t/year of lignin is produced from woody plants in paper making worldwide. Rising oil prises and the high energy intensity in production of synthetic polymers favors the future use of lignin biomass component in materials application rather then for energy production. In our research, the new type of photo- and biodegradable polymers was developed by blending of polypropylene and polyethylene with lignin polymers isolated from waste products of pulp and paper industry. A series of polyolefin blends containing 10, 20 and 30 wt% of lignin was examined from the view point of the influence of lignin blending on processing stability, mechanical properties, UV-light and long-term heat degradation as well as biodegradability.

The results obtained indicate that lignin acts as effective processing stabilizer during production of polyolefin blends. Moreover, it initiates photodegradation of the composites at long-term UV exposure, particularly of those containing 20 and 30 wt% lignin. Biodegradation of composite blends shows that lignin causes modification of surface of synthetic matrix. The characterization of biodegraded polymers by GPC and FTIR indicates that ligninolytic enzymes produced during the cultivation process with *Phanerochaete chrysosporium* initiate partial biodegradation of synthetic polymer matrix. It can be supposed that the lignin component of composite materials will affect the behaviour of polyolefins in outdoor weathering as initiator of their oxidative degradation by UV light and by lignin-degrading microorganisms.

Poster Presentation 5-12

Vegetable Oil as Substitute for Mineral Oils

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Investigations on food quality vegetable oils show favorable dielectric characteristics as insulating material for electrical devices. In order to comply with possible applications, characterizations of different seed oils (and some chemical derivatives) have been carried out. Results are compared to specified values for unused mineral oils (IEC 60296): the electrical properties of vegetable products are closed to the properties of conventional mineral oils. Particularly, breakdown strength under AC stress is at least as high as for mineral oil. We have prepared blended oils based on semi-refined rape-seed oil and derivatives that satisfy operation requirements in transformers. Viscosity and pour point can be fitted to appropriate values. Flash and fire points of oil exceed normative requirements by far. Thermal properties (specific heat, thermal conductivity and coefficient of expansion) are superior to mineral oil ones, and comparable to those of high temperature hydrocarbons or silicones. Nevertheless, regarding typical transformer life cycles of 30-50 years, ageing causes problems that could reserve biodegradable oils to sealed equipments. First evidence is provided that rape-seed based oil possesses suitable properties for use in transformer with superior environmental, safety and health properties compared to those of conventional mineral oils.

Poster Presentation 5-13

Production of Succinic Acid by *Anaerobiospirillum succiniciproducens* from Wood Hydrolysate

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Anaerobiospirillum succiniciproducens grew on a minimal salts medium containing wood hydrolysate. When supplemented with 10 g corn steep liquor l-1 (CSL) as a complex nitrogen source, succinic acid concentration of 24 g l-1 was obtained by batch culture of *A. succiniciproducens* in wood hydrolysate-based medium (equivalent to 27 g glucose l-1), resulting in the succinic acid yield of 88%. These results suggest that succinic acid can be produced economically from inexpensive wood hydrolysate and CSL. Detailed results will be presented along with potential strategies for the improvement of the process.

[This work was supported by the Korea Energy Management Corporation and by the BK21 project from the Ministry of Education.]

Poster Presentation 5-14

Batch and Continuous Cultures of *Mannheimia succiniciproducens* MBEL55E for the Production of Succinic Acid from Whey and Corn Steep Liquor

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Mannheimia succiniciproducens MBEL55E isolated from bovine rumen is able to produce a large amount of succinic acid in a medium containing glucose, peptone and yeast extract. In order to reduce the medium cost, whey and corn steep liquor (CSL) were used as substrates for the production of succinic acid by *M. succiniciproducens*. Anaerobic batch cultures of *M. succiniciproducens* in a whey-based medium containing CSL resulted in the production of succinic acid with the yield of 71% and productivity of 1.18 g/L/h. Anaerobic continuous culture of *M. succiniciproducens* in a whey-based medium containing CSL resulted in a succinic acid yield of 69% and a succinic acid productivity of as high as 3.90 g/L/h.

[This work was supported by the Korea Energy Management Corporation and Bioinfomatix Co.]

Poster Presentation 5-15

High Level Production of Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by Fed-batch Culture of Recombinant *Escherichia coli* in a Pilot Scale Fermentor

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Production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB/V)] by fed-batch culture of recombinant *Escherichia coli* harboring the plasmid pJC4 containing the *Alcaligenes latus* polyhydroxyalkanoate (PHA) biosynthesis genes was examined in pilot-scale fermentors varying K_La value with air supply only. In 30 L fermentor having the K_La value of 0.11 s^{-1}, the final P(3HB/V) concentration obtained was 29.6 g/L, resulting in the productivity of 1.37 g P(3HB/V)/L-h. In 300 L fermentor having the K_La of 0.03 s^{-1}, the P(3HB/V) concentration was 20.4 g/L, resulting in the productivity of 1.06 g P(3HB/V)/L-h.

[This work was supported by the National Research Lab program and G7 project of the Ministry of Science and Technology.]

Poster Presentation 5-16

High Level Production of gamma-polyglutamic Acid by Fed-Batch Culture of Bacillus licheniformis

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Production of gamma-polyglutamic acid (PGA) with high productivity is essential for the development of cost-effective process for gamma-PGA production. While the biosynthetic mechanisms and characteristics of -PGA have been studied extensively, little work has been done on gamma-PGA mass production. In this work, efficient production of gamma-PGA by fed batch culture of *Bacillus licheniformis* was studied. Novel nutrient feeding strategies were developed, which led to the production of as high as 50 g gamma-PGA per liter. Detailed strategies employed and the results obtained will be reported.

[This work was supported by the National Research Lab program and G7 project of the Ministry of Science and Technology.]

Poster Presentation 5-17

Characterization of Surfactin from *Bacillus subtilis* for Application as an Agent for Enhanced Oil Recovery

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Surfactin produced by *Bacillus subtilis* (ATCC 21332) was used to examine the effect of altering salt concentration, pH, and temperature on surfactin activity (as measured by reductions of surface tension). These parameters are some of the conditions which define oil reservoir characteristics and can affect the application of surfactants. The Biotechnology for Oilfield Operations research program at the Idaho National Engineering and Environmental Laboratory (INEEL) has successfully produced surfactin from potato process effluents for possible use as an economical alternative to chemical surfactants for improved oil recovery (IOR). Surfactants enhance the recovery of oil through a reduction of the IFT between the oil and water interfaces, or by varying the wettability index of the rock matrix. The current study investigated changes of surfactin activity under a range conditions by measuring surface tension.

Surface tension was determined using video image analysis of inverted pendent drops. Experimental variables included sodium chloride (0 to 10%), pH (3 to 10), and temperature (up to 70°C for 30 days). Each of these parameters, as well as selected combinations, resulted in discreet changes of surfactin activity, useful consideration for exploitation of surfactin as an enhanced oil recovery agent.

Poster Presentation 5-18

The Manufacture of Synthesis Gas Ffrom Biomass Aand Production Off Alcohols Aand Electric Power Using the Pearson Thermo-Chemical Steam Reforming and Catalytic Conversion Processes

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Pearson Technologies, Inc. ("PTI") has constructed a 30-ton per day wood waste to alcohol facility in North Mississippi. The process consists of a syngas producing front-end with a Fischer-Tropsch back-end. Syngas is produced using a multi-stage steam reformer (gasifier) with a "cold gas" efficiency of 81%. The number of stages and operating conditions of the reformer are dependant upon the end-use of the gas (i.e. if the end use of the gas is to fuel boilers, air exclusion in fuel bunkers, feeders, etc. need not be so stringent). Also, the reformer design is dependant on the reactivity of the feed and the analysis of the feed. Should the feed be high rank coal, more than "one" stage may be needed. Temperatures and pressures will or may be adjusted. The Pearson reformer is the only, or one of a small number of, syngas generators whose mol ratio of products (H_2 / CO) can be significantly adjusted with operating conditions. The back-end is a fairly straightforward Fischer-Tropsch synthesis loop, using a proprietary catalyst developed by Pearson Technologies, Inc ("PTI"). Single-pass conversion to ethanol is from 15% to 60% with a total conversion of 99+ %, with recycle.

Poster Presentation 5-19

The Effect of Germ and Fiber Removal on the Production of Ethanol from Corn

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Removal of non-fermentables, such as the germ and fiber, from corn is expected to increase ethanol production capacity in dry milling operations. The increase in capacity and the production of germ and fiber by-products, such as corn oil and corn fiber oil, could potentially increase profit margins and reduce reliance on a single product. Ethanol fermentations were conducted using both whole corn and corn with germ and a portion (~50%) of the fiber removed. Ethanol production increased 11.7% in the germ and fiber removed corn vs. the whole corn. Distillers Dried Grains and Solubles (DDGs) protein content increased from 30% to 36% and phosphate levels were 60% lower in corn with germ and fiber removed vs. whole corn. The protein increase and reduction in phosphate should offer a higher value swine and poultry feed as opposed to the current use of DDGs as a cattle feed.

Poster Presentation 5-20

Kinetics of *Monascus ruber* Secondary Metabolites Production on Rice, Under Variable Initial Cell Concentration

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Solid state cultivation of *Monascus* sp. on rice is an important process for the production of red pigments used in food industry. This work analyses the influence of initial cell concentration on red pigments, citrinin (nefrotoxin) and mevinolin (with hypocholesterolemic properties) kinetics production on solid state fermentation.

Each 30 g of substrate (rice) was inoculated with the following vegetative cell mass of *Monascus ruber* UFPE 3196: $0.325x10^{-3}$ g, $1.625x10^{-3}$ g, $2.438x10^{-3}$ g, and $3.25x10^{-3}$ g.

The samples were analysed for: humidity of substrate, secondary metabolites (red pigments, citrinin and mevinolin) on HPLC in C18 column, and red pigments on a spectrophotometer at 500 nm.

The humidity reached 65% in all assays and the maximum absorbance was 2.6 U for the culture with $0.325x10^{-3}$ g of initial cell mass and the other three cultures reached 1.8 ± 0.17 U. The analysis for citrinin and mevinolin didn't showed differences between the inoculum conditions (0.02 g/g of dry product and 0.08 g/g of dry product, respectively, at the end of the cultivation).

Mevinolin was produced at just the 3rd day of cultivation and its value (0.2 mg/g of dry product) was almost constant until the end of the assay. Citrinin have been made by the 15th day and had a maximum value at 21st day (0.8 mg/g of dry product) but it decreased to almost 0 (zero) at the end of the culture (27th day). That maximum value of citrinin correlated with the higher production of the red pigments (0.66 and 0.47 mg/g of dry product) and absorbance of 2.55 U. The red pigments concentation were constant after the 21st day while citrinin decreased, that could be an interesting process strategy.

The assays showed that it could be possible to reduce the initial cell mass used for the cultivation (actually, $3.25x10^{-3}$ g is used) and it could be smaller than $0.325x10^{-3}$ g, used in this work.

Poster Presentation 5-21

Relationship Between Secondary Metabolites Production and Batch Growth Kinetics of *Monascus purpureus* sp.

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Monascus purpureus sp., synthesize useful secondary metabolites like pigments, a hypocholesterolemic agent (lovatastin) and antibiotics. Among the pigments, the red ones has been used for coloring foods, specially meat and sausage products, being an important substitute for the highly toxic nitrite.

Kinetics of growth and red pigment production of M. purpureus CCT 3802 were achieved in liquid medium cultures (Bio Flo III, New Brunswick Scientific, SL), having as variable agitation frequencies (N = 500 and 700rpm) and dissolved oxygen level (DO controlled at 10% and higher than 70%). In each culture, the level of N and DO, results different values of maximum specific oxygen uptake rate, $q_{02máx}$, observed during the exponential phase of the cultures. There is a direct correlation between q_{02} and DO, as usually observed in other processes.

The lowest value of $q_{02máx}$ (about 2.45 mmol O_2/g cell/h) and DO (10%) is associated with low value of red pigment production and specific growth rate (μ_x = 0.11 h^{-1}). The low red pigment production, in this situation, is likely to be due to the dissolved oxygen limitation. Under values of $q_{02máx}$ higher than presented above, the red pigments production was increased, although the citrinin (toxic metabolite) and yellow pigments (synthesized whit the same pathway of the red pigments), were increased in a higher proportion.

In this work, appropriate values of DO and q_{02}, were determined in order to enhance (more than three times) the ratio between red pigment production and citrinin production.

Poster Presentation 5-22

Optimizing the Three Precursor Concentration for Aureomicin Biosynthesis Through Solid Fermentation

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Solid state fermentation (SSF) is an aerobic harvesting method that employs supports or substrates than can be industrial waste such as rice husk, wheat bran and potato skins aside from presenting operational advantages over submerged fermentation. On such basis, our task force has dedicated itself to producing different antibiotics through this technique or technology.

In this report we present the results obtained with the ***Streptomyces aureofaciens ATCC 10762*** for the production of aureomicin testing different precursors that participate in its biosynthesis. The fermentation systems were set up in test tubes using wheat bran as a support and harvesting media which contained sacarose, corn syrup liquor, amonium dibasic sulfate, calcium carbonate, peanut oil and mineral salts with a initial humidity of 60%.
The concentrations of the precursors tested were: glutamic acid 0.5%, 1.0 %, 1.5%, 2.0%, metionin 0.05%, 0.075%, 0.100%, 0.150%, 0.200%, asparagin 0.5%, 0.75%, 1.0%, 1.5%. Fermentation system follow up is being done measuring the pH variables, sugar concentration, humidity and the antibiotic production (USP XXII) obtaining the following results:

PRECURSOR	OPTIMAL CONCENTRATION (%)	AUREOMICIN PRODUCTION (u/G)
Glutamic acid	1.0	59.20
Metionin	0.05	70.65
Asparagin	0.75	1.0

Poster Presentation 5-23 Student

Effects of Xylose Reductase Sources on Xylitol Production in Recombinant *Saccharomyces cerevisiae*

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Two different xylose reductase genes were introduced into *Saccharomyces cerevisiae* to explore the xylitol production patterns in batch and fed-batch cultures: xylose reductase gene (XYL1) of *Pichia stipitis* and aldose reductase gene (GRE3) of *Saccharomyces cerevisiae*. Although glucose utilization and ethanol formation of the two recombinant strains were not different in batch cultures, the xylitol productivity of the strain containing the *S. cerevisiae GRE3* gene was 50% of that of the strain harboring the XYL1 gene of *P. stipitis*. Such a difference in xylitol productivity was confirmed in fed-batch cultures using ethanol as a cosubstrate for regeneration of NAD(P)H. *S. cerevisiae GRE3* gene product showed a strong preference to NADPH, while the degrees of cofactor specificity of *P. stipitis* gene for both NADPH and NADH were almost identical. Similar amounts of xylose reductase were expressed in both recombinant strains, but a strict preference to NADPH in the *S. cerevisiae* with the *GRE3* gene limited cofactor availability for xylose conversion and concomitantly resulted in lower xylitol productivity compared with the recombinant strain containing the *P. stipitis XYL1* gene whose product exhibited almost the same cofactor specificity to NADPH and NADH.

Poster Presentation 5-24

Development of Itaconic Acid Fermentation by *Aspergillus terreus*

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Itaconic acid is an unsaturated organic compound produced in a branch of the Kerbs cycle. Industrial application covers copolymer production for textiles and latexes, usage as emollients and adhesives. The economically feasible biological itaconic acid production has two key parameters: the proper and reliable inoculum preparation and the high productivity to decrease fermentation time. Here we report the systematic analyses of optimal inoculation and fermentation strategy.

The sporulation of *Aspergillus terreus* was examined on a series of agar nutrients in the function of time, as well as the efficiency of washing solutions was evaluated. Rice based inoculum was designed for larger scale application: beside the amount of moisturizing solution, the effect of supplementary materials were investigated in Plackett-Burman experimental design. The most effective compounds were further analyzed in Box-Behnken design. To increase productivity, the role of starting substrate concentration, the timing of extra substrate addition and the possibility of semi-continuous fermentation was analyzed in a series of bench-top and pilot-plant fermentations. Based on the results of this systematic study, optimal inoculation and fermentation methodology can be developed.

Poster Presentation 5-25

Development of a Brewery's Spent Grain Dilute-Acid Hydrolyzate Media for Polyols Production by *Debaryomyces hansenii* CCMI 941

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Non-detoxified brewery's spent grain dilute-acid hydrolyzate, a pentose-rich media, was tested for xylitol production by *Debaryomyces hansenii* CCMI 941 under semi-aerobic conditions. The xylitol production was evaluated in two-fold concentrated media, non-supplemented or supplemented with inorganic salts and vitamins, or different complex nutrients, namely, yeast extract, casamino acids, wheat bran extract, corn steep liquor and combinations of these.

For all media tested, short lag phases were observed and polyols (xylitol and arabitol) were the main metabolic products found. Xylitol was preferably produced, typically in a xylitol to arabitol concentrations ratio above 3. Supplementation enabled simultaneous utilization of sugars (glucose and xylose, and xylose and arabinose) and led to higher xylitol productivity. The best results were found for yeast extract- and wheat bran extract-supplemented media, 24 g xylitol L^{-1} and 25 g xylitol L^{-1}, respectively in 48 hours. The results reported showed that this hydrolyzate can have a potential use for the production of xylitol by D. *hansenii* once it was not needed any previous detoxification stage or yeast adaptation.

Poster Presentation 5-26

Genetic Engineering of Glucose/Xylose Co-fermenting *Saccharomyces* Yeast for Co-production of Ethanol and Various Industrial Enzymes

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We should strive to make the cost for the production of cellulosic ethanol as low as possible. One way to reduce the overall cost for the production of cellulosic ethanol is to produce high valued co-products or by-products during the production of ethanol. One class of co-products could be various industrial enzymes that are high priced products.

One important industrial enzyme is phytase, which is used as a supplement in animal feed to improve phosphorus nutrition and to reduce phosphorus pollution of animal excreta.

Saccharomyces yeast has the GRAS status and has been used for the preparation of food and drinks for human consumption for thousands of years. Thus, it can be used for the production of any enzyme or special protein including those for human and animal consumption. In this presentation we focus on the expression and secretion of a bacterial phytase in our glucose/xylose co-fermenting *Saccharomyces* yeast.

Poster Presentation 5-27

Extraction of Oryzanols and Corn Oil from Distillers Dried Grains and Solubles

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For the ethanol industry that employs the dry mill process, a large proportion of the profit is tied up in the processing and sale of distillers dried grains (DDGs). The value of DDGs has decreased over time from $0.09 per pound to $0.04 per pound. Recently there has been interest in the extraction of steryl ferulate esters “oryzanols” from corn fiber. The extraction of oryzanols from DDGs could potentially provide a high value product to improve the profitability of dry mill facilities. DDGs, from a commercial dry mill facility were extracted by a variety of processes and the yield of oil and oryzanols were compared with values in the literature and with the yield of oil and oryzanols extracted from fiber obtained from a wet mill facility. Hexane extraction of DDGs yielded 0.036 % oryzanols and 9.9 % crude oil compared to the recovery of 0.15 % oryzanols and 3.8 % crude oil from wet mill corn fiber. Ethanol extraction of DDGs yielded 0.034 % oryzanols and 10.7 % crude oil. In addition the ethanol extraction also provides a method to recover glycerol from DDGs.

Poster Presentation 5-28

Applications of a Specialty Polymer Derived from a Biobased Monomer

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MBI International is currently developing a group of novel polymers with unique characteristics. The polymers form films where one side of the polymer film is non-polar and the other side is highly polar. These polymers have been referred to as two-dimensional (2D) polymers. A key component in the formation of these polymers is a α, β-unsaturated carboxylic acid or ester. Many such compounds are biobased, such as fumaric acid and itaconic acid that are produced by fermentations or are downstream products from biobased sources, such as 2(5)-furanone, a by-product from the alkaline oxidation of cornstarch to form (S)-3-hydroxy-γ-butyrolactone. MBI international is developing a wide range of applications for these polymers: To create a novel non-thrombogenetic coating for use in coating medical implants. To form conductive films that may be used in the formation of OLEDs for display technologies. To develop an anti–infective coating that kills bacteria on contact. To develop heavy metal absorption systems for removal of pollutant from water supplies. This presentation will concentrate on the applications being developed for these 2D polymers.

Poster Presentation 5-29 Student

Expression of Yeast Alcohol Acetyltransferase Genes in *Escherichia coli* and *Clostridium acetobutylicum* for the Production of CoA Esters

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Recent research has shown that *C. acetobutylicum* can be grown on a variety of feedstocks. Concurrent with this investigation is an exploration of important and useful compounds that can be produced via microbial fermentation using biomass. This report focuses on the production of commercially useful esters by *C. acetobutylicum*. Isoamyl acetate and ethyl acetate are esters used for food and beverage flavorings. Alcohol acetyltransferases (AATase I and AATase II) from the yeast Saccharomyces cerevisiae are responsible for the production of these esters from acetyl-CoA and alcohols. The *ATF1* and *ATF2* genes have been cloned and expressed in *E. coli* and A*TF2* has been expressed in *C. acetobutylicum* to study the effectiveness of expressing AATases in bacteria. Isoamyl acetate production from the substrate isoamyl alcohol was determined by head-space gas analysis on a gas chromatograph. Data indicates that AATase I produces almost twice as much isoamyl acetate as AATase II when expressed from a high-copy expression vector. Mutant strains of *C. acetobutylicum* that have altered levels of acetyl-CoA are being studied for their effect on ester production. The wide range of substrates available in *C. acetobutylicum* makes it an ideal organism for investigating ester production in bacteria.

Poster Presentation 5-30

Continuous Production of Butanol by *Clostridium acetobutylicum* Using a Fibrous Bed Bioreactor

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An investigation was undertaken to explore the influence of pH and dilution rate in continuous cultures of the mutant strain from *Clostridium acetobutylicum* ATCC 55025. The fermentation were initially started as batch runs and transferred to continuous operation while entering solventogenic phase. A 150-ml fibrous bed bioreactor (FBB), batch incubation of the first fermentor culture at pH 5.4 and 35 °C, could ferment 20 g l^{-1} dextrose producing high biomass and butyrate concentration. Varying the dilution rate in the range of 0.25 h^{-1} to 3 h^{-1} and the pH from 3.5 to 5.5, the butanol yield and productivity were examined in the second stage. In this work, producing butanol by the uptake of butyric acid converted from carbohydrates has proven to be very efficient. Compared to the conventional ABE fermentation, the FBB greatly improved butanol yield and increased the fermentation rate and butanol tolerance by the bacteria, making butanol production from corn an attractive alternative to ethanol fermentation.

Poster Presentation 5-31

Peroxidase as the Biocatalyst Agent in the Biotransformation of Isosafrole into Piperonal Effected by *Paecilomyces variotii*

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Paecilomyces variotii was used as a biocaalist agent capable of oxidizing isosafrole with 100% of conversion. The biotransformation process was carried out by using a broth rich peroxidase. Peroxidase activity (0.12 U mL^{-1}) was followed by guaiacol and hydrogen peroxidase. The transformation of the xenobiotic substrate was performed in 100 mL glass flask containing 30 mL of *Paecilomyces variotii* culture broth, 2mL of 10mM substrate solution, 3mL of citrate buffer solution (pH 3.0) and 1 mL of 23 mM hydrogen peroxide solution, on magnetic stirring with controlled temperature (28 °C). After 24h reaction, a sample of 2 mL was withdrawn, worked up and analysed by GC and GC/MS. Results indicated the production of piperonal as the sole reaction product. Piperonal is an aldehyde of great commercial importance for the flavor and fragrance industries.

Poster Presentation 5-32

Peptidelipid Surfactant Production By *Bacillus subtilis* Grown On Low Cost Raw Materials

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The production of peptidelipide by *Bacillus subtilis* was investigated using renewable, low cost and easily available substrates (crystal sugar, sugar cane juice, molasses, glycerol, mannitol, soybean oil). Among these, crystal sugar has shown the best results in terms of surface tension reduction. However, culture broth from sugar cane fermentation, presented the greatest emulsifying properties. Different concentrations of crystal sugar, added to the production medium, improved both cell growth and biosurfactant synthesis, the best yield being achieved for 10 g/L of substrate, in a 48 h-period. A remarkable effect on biosurfactant production was evidenced by changes on the initial pH and additions of yeast extract, EDTA and microssalts on the production medium. Experiments performed in biorreactor indicated that biosurfactant production and growth is an associated process, the culture broth presenting surface tension, micelar critical concentration and emulsification index values of 29,6 dyne/cm, 82 and 57%, respectively. These results showed that the investigated strain is very promising for the production of biosurfactant using low cost raw material.

Poster Presentation 5-33

A Computational Framework for the Discovery of Novel Biobased Industrial Chemicals

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The large number of chemical transformations involved in biochemical pathways and the even larger number of enzymes that catalyze these transformations contribute to the complexity of biological systems. Analysis of the available genome sequences, as well as results from prior and current biochemistry research, suggest that while many of these transformations are common in most of the organisms, there are many organism-specific pathways, and many new enzymes and transformations, that remain to be discovered. We have focused on the development of a computational framework that identifies novel biotransformation pathways based on the rules of enzyme function and evaluates the alternative pathways with respect to their thermodynamic feasibility.

The framework is based on graph theoretic methodologies. Graph theory provides the foundation for applying mathematics and associated algorithms to represent biochemistry. The output of the pathway generation algorithm is a set of molecules and paths connecting them based on likely enzyme-catalyzed transformations. The application of this framework to aromatic amino acid biosynthesis will be discussed.

Poster Presentation 5-34

The Procter & Gamble Chemicals and Biomaterials Program

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P&G Chemicals' current business is primarily oleochemical based, constructed on the processing of renewable coconut and palm kernel oils, and partially petrochemicals based. Products include fatty acids, alcohols, methyl esters, glycerine and dimethyl tertiary amines. These materials are used in production of a variety of personal care products as surfactants, humectants, stabilizers, etc. This has served the company well for over 100 years, but future expansion will be designed to take advantage of new, economical feedstocks that are becoming available. This includes understanding the sourcing and processing of materials that are recovered from the domestic agricultural sector to economically produce materials of use to P&G consumer brands. To facilitate achieving this objective, P&G Chemicals has entered into a strategic alliance with Archer Daniels Midland to explore novel uses for biotechnologically and traditionally processed agriculture materials. We are also investigating other types of "waste" streams for applications. The poster will summarize some of the efforts we are making in this area.

Poster Presentation 5-35

Heavy-Duty Diesel Emissions Characteristics of Glycerol Ethers

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In the process of refining a fat or oil to produce methyl esters, glycerine is generated as a co-product in an amount equal to 10 % of the esters by weight. The global glycerine market is estimated to be 750,000 to 800,000 metric tonnes per year. If, as anticipated, biodiesel grows to 1 billion gallons worldwide, an extra 400,000 tonnes of glycerine would reach the market. Maintaining high value uses for the biodiesel co-product will be critical for biodiesel and oleochemical economics. Thus in the interest of maintaining glycerine value, isobutyl ethers of glycerol have been suggested as components in diesel or biodiesel fuel.

In the present study, the glycerine derivative will be examined at Ricardo, Inc. for potential use as a blend component in biodiesel and diesel fuel. The glycerol ethers will be tested in an Iveco Cursor 8 engine with Euro III emissions standards. The base fuels will be a U.K. low sulfur diesel fuel and a biodiesel fuel derived from rapeseed oil. European heavy-duty cycles, both transient and steady state (ETC and ESC) will be examined through engine performance data. Engine-out regulated and non-regulated emissions will be reported. Due to health concerns about diesel exhaust, a particle size distribution will be measured during the ESC cycle and compared to the reference diesel fuel. Several hypotheses regarding the effect of the glycerol ethers on diesel emissions will be discussed.

Poster Presentation 5-36

Lactic Acid Production from Cheap Raw Material

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Lactic acid is feed stock monomer of biodegradable plastic that has both hydroxyl and carboxyl functional groups. It has also been used as preservatives and acidulant since prehistoric era. When lactic acid is produced by fermentation, the raw material cost occupies the main portion of the total production cost. Therefore, cheap nutrient sources, such as starchy material and corn steep liquor, are more desirable than refined sugars and yeast extract.

From the optimization study for batch culture of *Enterococcus faecalis* RKY1 using some cereal sources as main nutrient source, the following general principles were derived; 1) heat treatment of liquefied (or saccharified) cereal broth was undesirable for producing lactic acid, 2) by adding small amount of yeast extract, lactic acid fermentation can be stimulated. The lactic acid productivity in batch culture of strain RKY1 was reached more than 5 g/L/hr using the saccharified whole wheat flour. This work was supported by Ministry of Commerce, Industry, and Energy through the Korea Institute of Industrial Technology Evaluation and Planning

Poster Presentation 5-37

Succinic Acid Production from Glucose by Two-Step Bioconversion Process

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Succinic acid is a dicarboxylic acid produced as an intermediate of tricarboxylic acid cycle and also as one of the fermentation products of anaerobic metabolism. It has been considered as an important chemical because it can be used for the precursor of 1,4-butanediol, tetrahydrofuran, and gamma-butyrolactone and for the application in polymers, foods, pharmaceuticals, and cosmetics. Currently most succinic acid is produced commercially through chemical synthesis. However, much attention has recently been focused on the biological production of succinic acid by microorganisms as an alternative to chemical synthesis. We have designed a two-step bioconversion process for succinic acid production from glucose via fumaric acid using fumaric acid producing fungus and E. faecalis RKY1. The fumaric acid producing fungus was able to produce fumaric acid from glucose with a yield of 0.46 g/g-glucose, and E. faecalis RKY1 could convert the fumaric acid from this culture broth to succinic acid with a yield of more than 0.9 g/g-fumaric acid. Furthermore, we have optimized the culture conditions of two-step bioconversion process for succinic acid production from glucose.

This work was supported by grant No. R05-2000-00175-0 from the Korea Science & Engineering Foundation.

Poster Presentation 5-38

The Effects of Trace Contaminants on Catalytic Processing of Biomass-Derived Feedstocks

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Trace components in biomass feedstocks are potential catalyst poisons when catalytically processing these materials to value-added chemical products. Trace components include inorganic elements such as alkali metals and alkaline earths, phosphorus or sulfur, aluminum or silicon, chloride, or transition metals. Protein components in biomass feedstocks can lead to formation of peptide fractions (from hydrolysis) or ammonium ions (from more severe breakdown) both of which might interfere with catalysis. The effects of these components on catalytic hydrogenation processing has been studied in batch reactor processing tests and compared with results from processing actual biomass-derived products, including wheat millfeed hydrolysates and dairy manure hydrolysates. Results using cleanup technologies, such as ultrafiltration and carbon adsorption, with the biomass-derived feedstocks will also be presented.

Poster Presentation 5-39

Anaerobic Fermentation of Biomass Generated Producer Gas to Ethanol and Other Useful Products

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Fermentation of producer gas to fuel ethanol and other useful products at economically competitive prices is the prime research focus of the multi-discliplinary, multi-institutional research team in Oklahoma. The producer gas is obtained from gasification of low-cost biomass. The research is comprised of the holistic process starting from biomass growth to production of ethanol and is driven by a need for a low-cost sustainable, renewable energy source that would be beneficial both economically as well as environmentally.

This work focuses on one component of the holistic approach, namely utilization of producer gas in a bioreactor to produce ethanol. Switchgrass is gasified in a fluidized bed gasifier in a controlled amount of air to minimize oxygen. The producer gas is cleaned and stored in tanks and later utilized in a bubble-column bioreactor with a four-liter working volume. A novel anaerobic clostridium species (P7) is used for the fermentation of producer gas to ethanol and butanol and their corresponding acids. The bioreactor is initially operated using bottled gasses of hydrogen, carbon monoxide, and carbon dioxide at similar compositions as the producer gas (with a balance of nitrogen). Once a steady cell concentration is achieved, the gas feed source is switched from bottled gases to producer gas. Cell growth has been achieved and maintained with accurate pH control in the presence of both gas sources, demonstrating the successful integration of producer gas with the bioreactor. Product, cell, and pH profiles will be compared between the producer gas and bottled gas studies.

Poster Presentation 5-40

Algal Hydrogen Production—Physiology and Process Development

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Algal H2 production (r) and collection (l)

Sulfur deprivation of *Chlamydomonas reinhardtii* cultures gradually inactivates photosynthetic O_2-evolution capacity in the algae to levels below that of O_2 consumption by respiration. With the subsequent establishment of anaerobiosis, the culture redox potential starts to fall, but the in situ photochemical activity of photosystem II (PSII, the light reaction associated with O_2 evolution) abruptly drops to ca. zero. Shortly thereafter, PSII activity reappears, and then H_2 photoproduction commences as shown at the right. The abrupt loss of photochemical activity results from the over-reduction of the plastoquinone (PQ) pool (electron transport carrier pool located between PSII and PSI, the light reaction associated with H_2 production), caused by the lack of both O_2 and other pathways available to re-oxidize the PQ-pool. Once hydrogenase (the O_2-sensitive enzyme that releases H_2) activity is induced, over-reduction of the PQ-pool is relieved, partial recovery of PSII photochemical activity is observed, and H_2 can be collected. Since most of the electrons used directly by the algae for H_2 photoproduction originate from water oxidation by residual PSII activity, the redox state of the PQ-pool serves a regulatory function in the system. Given that PSII activity, even at reduced levels, results in the co-production of O_2, sulfur-deprived cells must have a mechanism to dispose of the gas, since hydrogenase activity requires the continued presence of an anaerobic environment. Both chlororespiration and mitochondrial respiration seem to be involved in this process, and storage products such as starch and protein provide the substrate. Recent improvements to the original algal batch process, first reported three years ago, now allow continuous production of pure H_2-gas, and this process will be described. This work was supported by the USDOE Hydrogen program.

Poster Presentation 5-41

The Higher Alcohols Biorefinery: Improvement of the Catalyst for Ethanol Conversion

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The concept of a biorefinery for higher alcohol production is to integrate ethanol and methanol formation via fermentation and biomass gasification, respectively, with conversion of these simple alcohol intermediates to higher alcohols via the Guerbet reaction. 1-Butanol results from the self-condensation of ethanol in this multistep reaction occurring on a single catalytic bed. Combining methanol with ethanol gives a mixture of propanol, isobutanol, and 2-methyl-1-butanol. All of these higher alcohols are useful as solvents, chemical intermediates, and fuel additives and, consequently, have higher market values than the simple alcohol intermediates.

Several new catalysts for the condensation of ethanol and alcohol mixtures to higher alcohols were designed and tested under a variety of conditions. Reactions of methanol–ethanol mixtures gave as high as 100% conversion of the ethanol to form high yields of isobutanol with smaller amounts of 1-propanol, the amounts in the mixture depending on the starting mixture. The most successful catalysts are multifunctional, with basic and hydrogen transfer components.

Poster Presentation 5-42

Renewable Carbon-Feedstock to Industrial Chemicals

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In this presentation, we will illustrate that renewable carbon feed stock such as granular starch or cellulose derived from biomass can be converted to industrial chemicals using in-vitro and or in-vivo biocatalytic systems. This process converts the raw-starch hydrolysates or cellulolytic end- products concomitantly to the desired final product. This process will thus provide improved productivity, reduction in undesirable impurities and offer higher product yield. Several examples will be presented to illustrate the versatility of this approach and its implications on the biobased industrial chemicals.

Poster Abstracts for Session 6A

Biomass Pretreatment and Hydrolysis

Poster Presentation 6A-07

Effect of Hemicellulose and Lignin Removal for Batch and Flowthrough Pretreatment on the Enzymatic Digestion of Corn Stover Cellulose

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Dilute acid hemicellulose hydrolysis in co-current reactors (e.g., catalyzed steam explosion) recovers hemicellulose sugars with relatively high yields and can provide good digestibility of cellulose by enzymes. However, flowthrough pretreatment can give even greater hemicellulose recovery, remove much more lignin, and enhance enzymatic digestibility. Furthermore, while digestibility is often only related to hemicellulose removal for co-current systems, the improved digestibility for flowthrough reactors appears to be related to lignin removal as well. Comparison of co-current and flowthrough performance suggests that adding dilute acid to the batch reactor solubilizes lignin, but the lignin may precipitate in forms that interfere less with enzyme action, improving digestion relative to native biomass or uncatalyzed hydrolysis. Flowthrough reactors appear to remove lignin before it condenses, particularly at higher flow rates, improving performance even more. An evaluation index is being developed to relate digestibility to reaction conditions and key features of pretreated samples with the goal of clarifying the relationship between the digestibility of pretreated corn stover cellulose and its structural features. The new insight gained can lead to novel processes that obtain even higher yields of hemicellulose sugars at acceptable concentrations and enhance cellulose digestibility.

Poster Presentation 6A-08

Ammonia Fiber Explosion (AFEX) for Pretreatment of Corn Stover: Recent Research Results

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The ammonia fiber explosion (AFEX) process treats lignocellulosic biomass with liquid ammonia under pressure followed by explosive pressure release to enhance conversion of structural carbohydrates (cellulose and hemicellulose) to fermentable sugars. AFEX has some unique properties compared with other pretreatments. We are involved in an in-depth comparative study involving five universities and the National Renewable Energy Laboratory on the pretreatment and hydrolysis of corn stover. This paper will present our latest research results on AFEX pretreatment of corn stover.

By adjusting the parameters of AFEX (temperature, moisture content, treatment time, ammonia to biomass ratio) it is possible to convert over 90% of corn stover glucan to glucose and xylan to xylose at moderate cellulase loadings. Sugars produced by AFEX are not degraded and appear to be well-fermented. Mass balance closure for AFEX-treated corn stover currently approaches 95%. A variety of physical and spectroscopic tools have been applied to better understand the fundamental reasons for the effectiveness of the AFEX process and some preliminary conclusions will be provided along these lines.

Poster Presentation 6A-09

The Development of a Pilot-Scale Hydrogen Energy System From Livestock Wastes and Raw Garbage

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The establishment of a carbon circulation system is important for prevention of global warming. Raw garbage and livestock wastes are resource of greenhouse gas emissions that produce a large amount of methane (CH_4) and carbon dioxide (CO_2). Biogas fermentation for livestock wastes and raw garbage is useful to reduce these emissions. In this research, in order to raise the methane concentration, a high efficiency reactor for converting CO_2 and H_2 to CH_4 was developed by using the acclimated-methanogen. The fixed bed biogas reactor (10L) was used for methane fermentation from livestock wastes and raw garbage. High-concentration of vitamin B_{12} as a by-product was obtained, and more than 90% of CH_4 was achieved. CH_4 is convertible to hydrogen and benzene with the hydrogen conversion equipment which uses zeolite as catalyst. Benzene is applicable as chemical materials. Since this hydrogen is high purity, it is usable to a fuel cell. This study can be considered for establishing a hydrogen supply system, and this hydrogen energy can contribute to creation of new local industry.

Poster Presentation 6A-10

Influence of Oxygen Content in Purge Gas on Biomass Pyrolysis Process

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The importance of pyrolysis is still growing up together with developing of the pyrolysis systems. From the other side we have increasing amount of biomass, starch and cellulose containing wastes which come from different areas of life (e.g. agriculture, forest industry, municipal solid waste etc.). The pyrolysis can either join a treatment method for such type material or a method for energy and/or material recovery.

Biomass pyrolysis can be described as the direct thermal decomposition of the organic substances in the absence of oxygen to yield an array of useful products – gas, liquid, and solid – a wide range of fuels, solvents, chemicals etc. In this studies, the term of "the absence of oxygen" has been changed to "with different amount of oxygen in purge gas". For this purpose four different purge gases have been prepared, composed and used in pyrolysis process, namely: pure nitrogen, simulated flue gas (with 6% of oxygen), simulated air (with 21% of oxygen) and pure oxygen. Such activity – of course – significantly changes the reaction resulting changes in pyrolysis products and kinetics.

The aim of this work is to gather data and knowledge, which should be useful when modeling, design and operation of thermal conversion processes are considered. The experiment has been performed on Thermogravimetric Analyser: SDT 2960 Simultaneous TGA-DTA - TA Instruments connected to, and controlled by a PC where all data from pyrolysis process has been stored. The samples were prepared from typical waste paper – as a source of biomass – (but not a glossy paper or highly printed newspaper) by cutting into small pieces exactly fitted to the platinum cup.

In this paper, basic kinetic parameters such as activation energy, pre-exponential factor, standard entropy of activation and standard enthalpy of activation have been calculated, discussed and compared. Relation between those parameters and oxygen concentration in purge gas is indicated.

Poster Presentation 6A-11

Predicting Performance of Batch, Flowthrough, and Mixed Batch Hemicellulose Hydrolysis by Coupled Mass Transfer and Reaction Models

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The uncatalyzed hydrolysis and removal of xylan from corn stover is markedly improved when operating conditions are changed from batch to continuous flowthrough, and the increase in hemicellulose removal with flow rate is inconsistent with predictions by typical first order kinetic models. Thus, mass transfer and other effects could be important in controlling the hydrolysis rate, and several models were applied to investigate whether incorporation of fundamental kinetic and mass transfer forces could explain xylan removal in both batch and continuous flowthrough reactors. One of these is based on just simple diffusion, and a second expands the model to include the effects of solution concentration in a biphasic leaching approach. In addition, a branched pore model is evaluated that includes reaction within a particle, desorption of the products from this reaction, diffusion into internal pores, and leaching from the pores to the solution. Experiments were run with a mixed batch system to evaluate model parameters, and data from batch and flowthrough reactions were then compared to model predictions.

Poster Presentation 6A-12

Fed-Batch of SO_2-Impregnated and Steam-Pretreated Spruce in Simultaneous Saccharification and Fermentation for Production of Ethanol

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Simultaneous saccharification and fermentation (SSF) of steam-pretreated wood is a strategy, which has been frequently mentioned in the literature as a means to increase yield and production rate. It has also been suggested, right or wrong, that the method is less sensitive to infection than is separate hydrolysis and fermentation (SHF). However, the method also suffers from a weakness: If the concentrations of inhibitors present in the slurry after steam pretreatment are too high, the yeast may become severely inhibited or even stop working.

One alternative to increase the tolerance towards high inhibitor concentrations is adaptation of the fermenting organism to the liquid prior to SSF. Another alternative, which was used in this study, is fed-batch of the steam-pretreated wood to the fermenter vessel. This also helps overcoming the problems of using heavy initial solids loadings to reach high ethanol concentrations. If the initial viscosity is too high mixing becomes difficult, but by using fed-batch techniques the problem becomes less important. In this study, the experiments were performed in 30-litre fermenter equipment to ensure good mixing.

In comparison with SSF or with SHF – performed batch wise at the same solids content – fed-batch resulted in better utilisation of the wood and also in a higher ethanol concentration. A higher ethanol concentration is of extreme importance to decrease the energy requirements in distillation to reach reasonable ethanol production costs. Results from this study will be presented.

Poster Presentation 6A-13

Hydrothermal Pretreatment for Barley Straw Conversion to Ethanol

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Pretreatment is an essential and important step in the biomass to ethanol process. Several physical, chemical and biological processes have been developed to pretreat lignocellulosic biomass prior to biologic conversion. Hydrothermal pretreatments are low cost and high yields technologies to prepare the cellulose fraction to enzymatic attack and to recover sugars from hemicellulose fraction.

In this study operational conditions for hydrothermal pretreatment of barley straw were investigated. Utilization of hemicellulolytic enzymes to enhance the hydrolysis of hemicellulose fraction and obtain high recovery of soluble sugars was assayed.

The work reports the compositional analysis of hydrolysate liquors and water insoluble fiber, enzymatic digestibility and ethanol production by Simultaneous Saccharification and Fermentation Process of barley straw pretreated at different experimental conditions.

Poster Presentation 6A-14

Kinetics of Xylooligosaccharides Hydrolysis

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Xylooligomers are important intermediates in hemicellulose hydrolysis for pretreatment and sugar recovery from cellulosic biomass and also have potential applications in such areas as pharmaceuticals, feed formulations, agricultural applications, and functional foods. They are generally not considered in describing the kinetics of hemicellulose hydrolysis, and lumped as one or perhaps two species when they are included. However, xyloolgiomers have a range of chain lengths, and it would be useful to better understand how these species react during hydrolysis. Thus, pure xylooligosaccharides with degrees of polymerization (DP) from 1 to 5 were treated with just water in small batch reaction tubes at 200°C for 6 to 30 minutes, and concentrations of the starting species and reaction products were measured by an HPLC system. Decomposition of each of the starting species could be described by simple first order kinetic models under these conditions, and kinetic constants were developed to describe these reactions. However, use of these constants did not accurately predict formation of lower DP species from the longer chains according to simple models for reactions in series unless oligomer degradation reactions were integrated into the kinetic expressions.

Poster Presentation 6A-15

Impact of Fluid Velocity and Contact Time on Corn Stover Pretreatment in a Flowthrough Reactor

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Flowthrough pretreatment has been shown to have important advantages including reduced chemical use, less corrosion, high hemicellulose sugar yields, and high cellulose digestibility but suffers from high energy requirements for pretreatment and ethanol recovery because of high water consumption. Significant benefits could result if we can understand the mechanisms for flowthrough systems and apply that knowledge to develop a way that can keep their key attributes while overcoming their limitations. Our initial experimental results showed increasing flow rate could significantly enhance hemicellulose and lignin removal and improve cellulose digestibility. Moreover, flow seemed to have a similar affect on hemicellulose hydrolysis as temperature and acid concentration. In this study, a set of flowthrough reactors with the same internal volume (16.8mL) but different diameters (½, ¾, and 1 inch, O.D.) were applied to investigate the effect of fluid velocity in promoting hemicellulose hydrolysis and lignin removal during dilute acid pretreatment of corn stover. The impact of liquid–solid contact time on these features was also evaluated by applying a second set of flowthrough reactors with the same diameter (½ inch O.D.) but different lengths (60, 120, and 180mm). The effect of fluid velocity and liquid–solid contact time on the enzymatic digestibility of cellulose was also observed. The results from these studies can give us new insights into the factors that influence hemicellulose hydrolysis and cellulose digestion.

Keywords: fluid velocity, liquid-solid contact time, flowthrough pretreatment, enzymatic hydrolysis, corn stover

Poster Presentation 6A-16 Student

Optimization of the Steam Pretreatment Step in the Production of Bioethanol from Salix

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In Sweden fast-growing salix is considered as a promising raw material for bioenergy, e.g. for production of heat and power. An alternative is to use the hydrocarbon fraction in salix to produce a liquid fuel, ethanol, and to use the solid residue, mainly lignin, for heat and power. This study is focused on the ethanol production.

To obtain high overall yields of sugar and ethanol, pretreatment of the material is necessary prior to enzymatic hydrolysis. In this study the steam pretreatment step has been investigated and optimized with respect to the yields of glucose and xylose as well as the overall ethanol yield (after fermentation). The production of degrading byproducts, such as furfural and HMF, which act inhibitory on the yeast, has also been taken into consideration.

Both 1-step and 2-step configurations have been investigated. The material was impregnated prior to pretreatment either with SO_2 or dilute sulfuric acid. The temperature was varied within the range from 180°C to 220°C while the residence times were set to 4, 8 or 12 minutes.
The obtained results from this on-going study will be presented.

Poster Presentation 6A-17

Dilute-Acid Hydrolysis of Hemicellulose from Agricultural Renewable Biomass

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Renewable biomass such as rice hull, rice straw and coconut shell was subjected to dilute acid hydrolysis designed to achieve high pentosan recovery. Sulfuric acid was used for converting these materials and investigated parameters were reaction temperature, acid concentration and dry matter substance.

Experiments were carried out 0.2%~1.0% range of a sulfuric acid and a temperature was adjusted at 100~140~. The higher concentration of sulfuric acid and temperature was induced the higher contents of monosaccharides. However, a relative value of yield and selectivity to xylose compare with other monosaccharides was not increased. The optimal conditions of hydrolysis to be aimed at xylose yield and selectivity was 0.8% and 140~. Whereas, the increased acid concentration and temperature was presented formation of furfural which was toxic compound. High xylose yields can be achieved when the acid-insoluble content (i.e. 1% dry matter substance loading) is low. In an attempt to improve the yield further, control of reaction time and the size of substance is presently performed to increase the productivity and purity of xylose. The results from this study will be presented.

Poster Presentation 6A-18 Student

Extraction of Sugar from the Fiber Fraction in Wheat Grain to Enhance Fuel Ethanol Production

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Today there are many plants that utilize wheat to produce fuel ethanol. However, most of these plants only make use of the starch fraction in the wheat grain to produce ethanol. Besides starch, the wheat grain also contains proteins, hemicellulose and to some extent also cellulose. Presently, the latter carbohydrates are not used in the ethanol production. Instead, only the starch fraction is hydrolyzed while the residue is used to produce animal fodder.

In this study, the possibility to utilize the fiber fraction in starch free wheat grain in the ethanol production was investigated. The streams in a full-scale ethanol process were analyzed with respect to their content of hemicellulose and cellulose. The purpose was to make a first decision whether it is of interest to continue with a more fundamental research on ethanol production from the fiber fraction in wheat.

The material best suited for ethanol production was chosen for a more thorough investigation to optimize the pretreatment of the material. Three pretreatment methods will be evaluated – acid hydrolysis, enzymatic hydrolysis and combined pretreatment and enzymatic hydrolysis. Preliminary results on the potential to liberate sugar from starch free wheat grain residues will be presented.

Poster Presentation 6A-19

Ammonia Fiber Explosion Process (AFEX): A Rapid and Flexible Laboratory Scale Unit

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Effective, economical pretreatments enhance bioconversion of recalcitrant lignocellulose to fermentable sugars, making biofuels such as ethanol more economically viable. Various lignocellulose pretreatments, each with some advantages and shortcomings, have been developed. A unique process referred to as Ammonia Freeze Explosion (AFEX) is one pretreatment alternative. In the AFEX process, the combined chemical and physical effects of cellulose decrystallization, limited hemicellulose hydrolysis, deacetylation of acetyl linkages, reduced lignin content and increased surface area, enable nearly complete enzymatic conversion of lignocellulose to fermentable sugars.

We have developed a small scale (bench-top) AFEX unit to study and optimize important process variables. The unit consists of an ammonia tank, a reactor, heater, temperature and pressure monitoring devices and connecting elements. A measured quantity of biomass with a specific moisture content is placed in the reactor. The desired amount of liquid ammonia is delivered to the reactor. The ammonia-biomass mixture is gradually heated to a desired temperature/pressure, held for a given time, and the pressure rapidly released ("exploded"). The flexible experimental system allows us to rapidly test various temperatures, moisture contents and ammonia loadings to optimize their effects on the overall fermentable sugar yields.

Poster Presentation 6A-20

Production of Succinic Acid from Ammonia Fiber Explosion (AFEX) Pretreated Biomass Hydrolysates

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The Ammonia Fiber Explosion (AFEX) process was used to pretreat corn stover, bagasse, rice straw, alfalfa, and soybean straw. A laboratory scale (300 ml) stainless steel reactor was used. The pretreated materials then were hydrolyzed with an industrial cellulase to produce a mixture of fermentable intermediates, which consisted of mostly glucose and xylose. The rates of hydrolysis of the pretreated materials were significantly higher than those obtained with the untreated materials. The hydrolysates obtained with the pretreated biomass were used for production of succinic acid without further treatment. Succinic acid-producing microbial strains capable of utilizing both glucose and xylose were used in these fermentations. The results of biomass hydrolysis and succinic acid fermentation will be discussed.

Poster Presentation 6A-21

Study of Important Variables on Acid Hydrolysis of Wheat Straw Hemicellulose for the Bioconversion of Xylose into Xylitol

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Lignocellulosic materials represent a renewable and widely available resource with potential application in diverse bioconversion processes. Their major components (cellulose, hemicellulose and lignin) vary in amount according to the species. Acid hydrolysis of the hemicellulosic fraction results in a hydrolysate rich in fermentable sugars, mainly xylose, which can be used to produce xylitol. Xylitol is a sugar of high commercial interest, not only because it has sweetening and anticariogenic properties, but also because it can be used as a sugar substitute by diabetics. This study presents the results of hydrolysis of wheat straw in different conditions with the aim to reduce the formation of inhibitory compounds normally present in the hydrolysate, such as acetic acid, furfural and hydroxymethylfurfural. Hydrolysis was conducted in a 40L reactor, according to the conditions established on a factorial design. The factors and levels tested were: temperature (120, 130 and 140°C), acid concentration (0.5, 0.75 and 1.0%), reaction time (20, 30 and 40 minutes) and solid to liquid ratio (1:15, 1:17.5 and 1:20). In all the hydrolysates, xylose was the predominant sugar, followed by glucose and arabinose, both found in small quantities. The concentrations of acetic acid, furfural and hydroxymethylfurfural were low. At 140°C and 1.0% acid concentration, the xylose concentration was maximal (15.37 g/L), while at 120°C and 0.5% acid concentration it was minimal (7.17 g/L). In both cases, the time of hydrolysis was 20 minutes and the solid-liquid ratio was 1:15. The best result was obtained with the higher temperature and the higher acid concentration. A complete hydrolysis of xylose was not possible when the lower temperature and lower acid concentration were used.

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Poster Presentation 6A-22

Dilute Acid Hydrolysis of SEDAP Treated Oak Wood

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SEDAP (Steam Explosion Dilute Acid Process) is combined steam explosion with dilute acid process for the treatment of lignocellulosic biomass. SEDAP involves a mechanical step for comminuting of the biomass and reducing the hemicellulose fraction from the biomass. Dilute acid process has been applied only for hemicellulose hydrolysis as a method of pretreatment for enzymatic hydrolysis. It is understood that the denaturants from sugar decomposition, especially xylose, inhibit the microbial fermentation to ethanol or other chemicals production. That is why the hemicellulose fraction of the treated biomass should be minimized in order that the dilute acid treatment may be used as a means of cellulose hydrolysis. About half of hemicellulose were removed and 95~98% of cellulose were remained in the treated solid after SEDAP with Oak wood chips at the same time. The obtained solid from SEDAP was hydrolyzed under various conditions. The temperature was varied over the range of 180 ~ 220 and sulfuric acid concentration was that from 0.1 up to 2.0%(w/w). Glucose yields and xylose decomposition were detected through each experimental run. Each hydrolyzate used for the ethanol fermentation with *Brettanomyces custersii* H_1-39, which is hyperfermentable yeast mutant endurable to toxic components from xylose decomposition. The experiments were conducted statistically by response surface methodology and fractional factorial design to optimize the process conditions of dilute acid hydrolysis and to maximize the ethanol production.

Poster Presentation 6A-23

Softwood Hydrolysis in a Shrinking Bed Flow-through Reactor

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The advantages of countercurrent operation in dilute-acid hydrolysis of biomass have been shown in both mathematical simulations and in proof-of-concept experiments. This led to the installation of a pilot-scale countercurrent reactor at the National Renewable Energy Laboratory (NREL) in 2000. During the 6 months it was operated, not all technical problems were overcome. Especially, the mechanical transport of the solid material upwards proved difficult to achieve at high temperature and/or high conversion, as the material became too mud-like. The material used in all these experiments was yellow poplar, a hardwood species that loses much of its lignin already in the prehydrolysis stage. In the following countercurrent stage, even more lignin is solubilized, and the material becomes mud-like. Softwood on the other hand, only loses about 10% of its lignin during hydrolysis, and seems to retain its structure. The result of this difference is that softwood is less muddy, and therefore probably easier to operate in a countercurrent reactor.

To investigate this difference further, and to predict the mechanical behaviour of softwood in a countercurrent reactor, we have used the shrinking bed flow-through reactor developed at NREL.

Poster Presentation 6A-24 Student

Optimisation of Pretreatment of SO_2 Impregnated Corn Stover for Ethanol Production

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One way to reduce the negative consequences of the greenhouse effect is to produce fuel from biomass. Corn stover, which is the corn stalk and the leaves without root and corncob, has very low economic value but it contains 38% cellulose and 32% hemicellulose, which make it a promising material for ethanol production.

In this study, corn stover is steam pretreated using SO_2 (with a concentration of 1-3% of the water content in the corn stover) as a catalyst to make the cellulose more accessible to the enzymes in the enzymatic saccharification and to hydrolyse the hemi≠cellulose. The steam pretreatment experiments are performed at temperatures from 160 to 210ºC for 1-10 minutes.

The solid material after pretreatment is hydrolysed enzymatically to determine the sugar yield and then fermented to determine the ethanol yield. The pretreatment is optimised with respect to sugar yield in the hydrolysate and ethanol yield after the fermentation. Results from this study will be presented.

Poster Presentation 6A-25 Student

Surfactants in Enzymatic Hydrolysis of Lignocellulose

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Lignocellulose is a potential substrate for ethanol production. However, high cellulose conversion requires high enzyme loading, which makes the process less economically feasible. Addition of surfactants to enzymatic hydrolysis of lignocellulose increases the conversion of cellulose into soluble sugars. 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. Non-ionic surfactants were found to be the most effective. Studies of adsorption of the dominating cellulase of *Trichoderma reesei*, Cel7A (CBHI), during hydrolysis showed that non-ionic surfactants reduced enzyme adsorption to the lignocellulose substrate.

Our conclusions from studies of lignocellulose and delignified substrates are that improved conversion of lignocellulose with surfactant can be explained by reduction of the unproductive enzyme adsorption to the lignin part of the substrate. This is due to hydrophobic interaction of surfactant with lignin on the lignocellulose surface, which releases unspecific bound enzyme. A new approach with mixed charged and non-ionic surfactants has been introduced to further improve the positive effect of surfactant addition. A study with non-ionic surfactants (alkyl poly(ethylene oxide)) with various amounts of ethylene oxide in the hydrophilic head was performed. The results showed that the increase in hydrolysis of lignocellulose increased with the length of the ethylene oxide chain.

Poster Presentation 6A-26

Pretreatment of Barley Husks for Ethanol Production

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Barley husks, produced worldwide in large quantities as a by-product of the barley milling industry, were identified as a potentially promising feedstock for ethanol production. This biomass consists mainly of glucan (29 %), xylan (24 %), arabinan (4 %) and lignin (14 %). Enzymatic hydrolysis is not enough to hydrolyze these polysaccharides to simple sugars and, therefore, chemical and physical methods are also required.

Several promising pretreatment methods, with and without addition of acid, and enzymatic processes for conversion of cellulose and hemicellulose to fermentable sugars were evaluated. Pretreatment was performed either in a steam pretreatment unit or in a microwave oven. The influence of acid concentration, temperature and residence time on the sugar and ethanol yields was investigated.

Results from this study will be presented.

Poster Presentation 6A-27

Application of Xylanase from *Thermomyces lanuginosus* OC-4145 in Enzymatic Hydrolysis of Corncob and Sugar Cane Bagasse

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Xylanases present great potential in several biotechnological applications as they catalyze the hydrolysis of internal -1,4-D-xylose. Recently, the interest for the bioconversion of xylan-containing lignocellulosic materials to D-xylose, has increased, due to the production of several bioproducts with high aggregated value. A thermophilic strain of the filamentous fungus Thermomyces lanuginosus has been investigated for xylanase production, on semi-solid fermentation in a medium containing corncob. In relation to xylanase activity, the highest yield (ca. 850 U/mL) has been reached at 45°C, after 96 hours of semi-solid cultivation. The enzyme was used for treatment of corncob and sugar cane bagasse. The cellulosic materials were pretreated using NaOH 1N solution, during 20 hours at 30°C and 150 rpm. Some samples had also been treated thermally in autoclave, for 90 minutes. Additionally, the waste materials were characterized in relation to xylose content, using total acid hydrolysis. The enzymatic hydrolysis was carried out using either distilled water, universal or phosphate buffers at 40°C and 150 rpm, for different time intervals. The highest degrees of enzymatic hydrolysis were 22 and 55%, achieved by using 3000U/g (dry material) of xylanase for bagasse and corncob, respectively, which have been treated both chemical and thermally.

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Poster Presentation 6A-28

A Modified Polymer Depolymerization Kinetics Model to Describe the Thermochemical Hydrolysis of Hemicellulose

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Dilute acid and water-only pretreatment methods have been studied extensively, and several predictive models have been developed based on homogeneous first order kinetics. Because oligomeric saccharide intermediates can be present in significant amounts, a few models have been refined to include the formation and subsequent degradation of oligomers, but these treat oligomers as a few lumped species. Thus, little insight is provided on the fate of the range of individual solubilized oligomers. Recently, a simple depolymerization model was developed to account for the distribution of oligomer chain lengths expected during hemicellulose hydrolysis by assuming that hemicellulose linkages are broken and subsequently hydrolyzed at random creating oligomers with a distribution of chain lengths. However, this model overestimates sugar release when its predictions are compared to experimental data. Therefore, a modified depolymerization model was developed. This modified model includes a term describing declining hemicellulose reactivity coupled to a hydrolysis rate constant that varies as a function of the instantaneous conversion of hemicellulose. The predictions from the modified model are compared to pretreatment data from various researchers and other models, and an analysis is offered of the oligomers that may be solubilized during hydrolysis.

Poster Presentation 6A-29

Enhancement of Enzymatic Digestibility of Used Newspaper by Surfactant Addition in Ammonia-Hydrogen Peroxide Pretreatment

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Waste paper is approximately half of municipal solid waste making it a potential source of bioenergy. Newspaper is mostly derived from softwood and exhibits low enzymatic digestibility because of its high lignin content and dense structure. Also, chemicals such as fillers, ink, and other additives make it difficult to enzymatically hydrolyze. Our previous study showed that ink had a significant effect on enzymatic digestibility when pretreating with an ammonia-hydrogen peroxide mixture in a shaking bath. In this process, ammonia was used as deinking purpose and hydrogen peroxide as swelling purpose for cellulose fiber. Ink particles released from the fiber surface were removed by physical shock provided by shaking bath. Ink components must be separated from the slurry to increase enzymatic saccharification. Otherwise ink is left in the space between fibers and interferes with enzymatic digestibility. To enhance ink removal from the fibers, surfactant can be added in the pretreatment process. The surfactant added, also, can improve the enzymatic digestibility by preventing the loss of enzyme activity due to the high adsorption of enzyme on the cellulose surface. In this study enzymatic digestibility was investigated under various conditions such as surfactant type, enzyme dosage, residual surfactant amount after pretreatment, temperature, etc.

Poster Presentation 6A-31

Study on Methane Fermentation and Production of Vitamin B12 from Alcohol Waste Fluid

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Biogas fermentation from alcohol waste fluid was studied in the laboratory to evaluate the anaerobic digestion process and the production of vitamin B12 as a by-product. The anaerobic digestion using the acclimated-methanogens was performed at two hydraulic retention times (HRTs) for 20 and 10 days. The performance of the fermentation system depends on the HRT and the addition of trace metals ion into the reactor. The fermentor at the HRT of 10 days and at the optimum trace metals concentration (10ml/l), the methane production and the vitamin B12 yield in culture broth were two times and five times than that using the conventional prescription of trace metals, respectively. Furthermore, an effective method for extraction and purification of vitamin B12 from digested fluid was developed.

Poster Presentation 6A-32

Recirculation of Condensate Streams in Fuel Ethanol Production from Softwood Based on SSF

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The effect of process condensate recycling on ethanol production from steam- pretreated softwood, based on simultaneous saccharification and fermentation (SSF) was investigated. The process configuration consists of four evaporators connected in a serious, and each evaporator produce a condensate corresponding to 14% of the process stream after SSF. The fractionation of volatile acids was distributed evenly in each condensate fraction.

The condensates contain only volatile substances and showed no negative effects on either fermentation of the steam pretreatment hydrolysate or SSF of the steam pretreatment slurry. It was possible to replace 100% of fresh water by recycling the total condensate fractions without affecting the ethanol yield and the residence time. The only influence noticed was the absence of lactic acid production, which is otherwise obtained when the pretreated material is diluted with fresh water.

The possibility to use the condensates will make it possible to eliminate the use of fresh water in the process, except for the live steam used in the pretreatment, which will have a very positive effect on the wastewater management and thereby improve the economical feasibility of the process.

Poster Presentation 6A-33

Enzyme Activity in Fed Batch Simultaneous Saccharification and Fermentation

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It has been investigated the efficiency of using a cellulolytic enzyme mixture in the fed batch mode to carry out SSF. The lignocellulosic substrate has been poplar wood, which has been steam exploded at severity logRo 4.13 (214°C, 6 min.) and then water washed or delignified with NaOH. The cellulolitic mixture has been obtained by mixing Celluclast 1.5L and Novozym 188 at ratio 5:1.

The SSF has been carried out by adding the biomass in two steps: at the beginning of the experiment and at 72 hours, half time of the whole test. The amount of the added biomass has been such to restore the initial ratio enzyme to cellulose.

For both stages it has been determined the SSF efficiency and the content of free protein in the fermentation broth.

By carrying out SSFs with samples having different lignin content it has been pointed out that the enzyme activity and the residual activity in the second stage increase by decreasing the lignin content.

In all cases, a glucose accumulations at high reaction time reveals an inhibition of the *Saccharomyces c.* as a consequence of rising concentration of metabolic products and lignin derivatives.

Poster Presentation 6A-34

Evaluation of Post-Hydrolysis Processes of Brewery's Spent Grain Autohydrolysis Liquor to Produce a Pentose-Containing Culture Media

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Brewery's spent grain was used as a lignocellulosic source to produce a readily fermentable pentose-containing hydrolyzate, by a two-step process consisting of an autohydrolysis producing oligossacharides followed by an enzymatic or chemical hydrolysis with sulphuric acid to produce monomeric sugars.

Enzymatic hydrolysis was tested with several commercial enzymes with endo-1,4-β–xylanase, β-xylosidase, acetylesterase and cellulase activities. The chemical hydrolysis was studied at several operational conditions (time, temperature and sulphuric acid concentration) by using the combined severity concept (CS) in the range 0.83-2.01, where the highest CS value corresponds to the standard quantitative acid hydrolysis. Under the tested conditions chemical hydrolysis proved to be more efficient, once pentose recoveries by enzymatic hydrolysis were always lower. Furthermore, a total recovery of monosaccharides together with a low content of inhibitors was possible to obtain at the optimized condition of CS = 1.16.

The post-hydrolysis liquor obtained was easily fermented by *Debaryomyces hansenii* CCMI 941, in semi-aerobic shake flasks experiments. In the tested conditions it was possible to obtain xylitol and arabitol as the main products. Further assays demonstrate that hydrolyzate concentration and casamino acids supplementation have a positive effect on polyols production.

Poster Presentation 6A-35

Optimization of Brewery's Spent Grain Dilute-Acid Hydrolysis for the Production of Pentose-Rich Culture Media

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The dilute-acid hydrolysis of brewery's spent grain to obtain a pentose-rich fermentable hydrolyzate was investigated. The influence of time, temperature, and sulphuric acid concentration on carbohydrate hydrolysis was assessed by the combined severity parameter (CS) in the range 1.39-3.06. When the CS increased, the pentose sugars concentration increased to a maximum at CS of 1.94, whereas the maximum glucose concentration was obtained for a CS value of 2.65. The concentrations of furfural, HMF as well as formic and levulinic acids, and total phenolic compounds increased with the increase of severity.

The optimum conditions were found at a CS value of 1.94 where more than 90% of feedstock pentose sugars were recovered in the monomeric form, together with a low content of furfural, HMF, acetic, formic and levulinic acids, and total phenolic compounds. This hydrolyzate containing glucose, xylose, arabinose (ratio 1:6:3) was supplemented with inorganic salts and vitamins and was readily fermented by the yeast *Debaryomyces hansenii* CCMI 941 without any previous detoxification stage. The yeast was able to assimilate all sugars, furfural, HMF and acetic acid and high biomass yields and productivities (0.90 g L^{-1} h^{-1}) were observed. Detoxification with activated charcoal resulted in a similar biomass yield and a small increase in volumetric productivities (11%).

Poster Presentation 6A-36

Comparison of the Microbial Inhibition and Enzymatic Hydrolysis Rates of Liquid and Solid Hydrolysates Produced from Pretreatment of Biomass with Carbonic Acid and Liquid Hot Water

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Achieving production of renewable ethanol from lignocellulosic biomass would be promoted if hydrolysates could be produced that allow rapid and high yield conversion of cellulose to fermentable sugars and have a low inhibition to fermenting organisms such as *Saccharomyces cerevisiae*. This research quantified the enzymatic digestibility of the solid component and the microbial inhibition of the liquid component of pretreated aspenwood hydrolysates. Products of liquid hot water and carbonic acid pretreatment were compared. Pretreatment temperatures tested ranged from 180^{0}C to 220^{0}C, reaction times were varied between 4 and 64 minutes.

The research was conducted in 2 parts. Part one was In-vitro inhibition of yeast in liquid hydrolysate, done by measuring the rate of glucose uptake by an anaerobic culture of *Saccharomyces cerevisiae* growing in hydrolysates produced at different reaction severities. The degree of inhibition was correlated to the severity of pretreatment and to the presence or absence of carbonic acid. Part 2 focused on enzymatic hydrolysis of pretreated solids by measuring rates and yield of glucose accumulation through enzyme digestion of pretreated solids. Hydrolysis rates were correlated to severity of pretreatment and to the presence or absence of carbonic acid.

Both microbial inhibition rates and enzymatic hydrolysis rates showed no difference between pretreatments containing carbonic acid and pretreatments containing no carbonic acid. When microbial inhibition rates were examined with increasing reaction severity, the inhibition increased as the reaction severity increased, but only above a midpoint severity parameter of 200^{0}C for 16 minutes. Below this midpoint severity parameter there was little to no inhibition of the yeast. When enzymatic hydrolysis rates and yields were examined with increasing reaction severity, both the rates and yields displayed an increase from the lowest tested reaction severity to the highest tested reaction.

Poster Presentation 6A-37

Modeling of Carbonic Acid Pretreatment Process Using ASPEN-Plus

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Use of carbonic acid instead of sulfuric acid for the pretreatment of biomass would offer environmental benefits for the production of renewable fuels and chemicals. The viability of this substitution depends on the economics of the process. Laboratory work in the Department of Environmental Studies at Baylor University has assessed the process parameters of the carbonic acid system. Economic costs and benefits of the process is determined using Aspen Tech's ASPEN PLUS process modeling software.

Aspen Tech's ASPEN PLUS process modeling software is being used to model carbonic acid pretreatment of biomass process. ASPEN PLUS is used because of the thorough treatment of thermodynamic interactions and its status as a widely accepted process simulator. The physical property data for many of the key components used in the simulation for the pretreatment process are derived from the In-house database (INHSPCD) developed by National Renewable Energy Laboratory (NREL). Because of the need to distil ethanol and to handle dissolved gases the standard NRTL (Non-Random Two Liquid or Renon) route is used as the main property method. The pretreatment reactor is modeled as a "black box" reactor due to unavailability of reaction kinetics. Stoichiometric data are used to define reactions. The Aspen-Plus model developed is used to calculate energy costs of carbon dioxide compression with energy recovery options for pretreatment process. Laboratory data is used to calculate ethanol revenue from carbonic acid pretreatment for different reaction severities. Model results indicating economic advantages and disadvantages of the carbonic acid system, compared to water-only and sulfuric acid based systems are discussed.

Poster Presentation 6A-38

Generation of Coproducts Derived from a Modified Hot Water Pretreatment of Corn Stover

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Ethanol production utilizing five and six carbon sugars recovered from corn stover hydrolysate has been documented. Hot water pretreatment of corn stover has been shown to assist in the enzymatic hydrolysis of the biomass to fermentable sugars. Corn stover contains carbon sources other than carbohydrates including lignin (17-18% dry mass) and crude fat (1-2% dry mass).

The first objective of this study was to investigate the coproducts generated by modification of the hot water pretreatment method by the addition of varying concentrations of ethanol. Sample from this study were analyzed by GC/MS and contained free fatty-acids (Palmitic and Linoleic acids) and lignin derivatives (coniferyl alcohol, vanillin, etc.) that are soluble in ethanol-water mixtures.

Phase two of this study involved passing the pretreatment liquid stream through a tubular reactor containing Amberlyst 35 catalyst. This catalyst is sulfonic acid-based and has an ion exchange capacity of 5.48 meq/gram.

Analysis of this liquid stream by GC/MS found ethyl esters of Palmitic acid, Linoleic acid, Oleic acid and Steric acid which are components of bio-diesel. Phenolic compounds identified included 2 ethyl phenol and ethyl 3-(4-hydroxyphenol)-propenate.

Solids remaining following pretreatment were hydrolyzed by enzyme with minimal difference in results as compared to water only pretreatment at up to 50% ethanol.

Poster Presentation 6A-39

Enhanced Enzymatic Hydrolysis of Steam-Exploded Douglas Fir by Alkaline Oxygen Post-treatment

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Steam explosion is an effective way to pretreat biomass for subsequent bioconversion to ethanol. Although steam explosion has been successfully applied to hardwood and agricultural residues, it does not work very well with softwood. For example, lignin is typically concentrated to 40-50% in the substrate during the steam-explosion of softwoods. Condensation of lignin also occurred during the explosion. The high lignin content plus the condensed nature of the lignin are two of the biggest barriers to the effective enzymatic hydrolysis of steam-exploded softwood. For example, only 30% of the cellulose present in a steam-exploded Douglas fir substrate with a 42% of lignin content was hydrolyzed after the addition of 20 FPU of cellulase per gram glucose, after 48 hours.

In the present study, alkaline oxygen was used as a post-treatment to remove and/or modify the lignin in order to improve the hydrolysis of the steam-exploded Douglas fir. The results showed that the removal of lignin by oxygen significantly enhanced the hydrolysis. As the substrate was delignified from 42% to 12% with oxygen, a complete hydrolysis was achieved with 20 FPU of cellulase within 48 hours, and the time for complete hydrolysis was shortened to 24 hours with the 40 FPU of enzyme. The result also showed that enhanced hydrolysis is not only due to the removal of the lignin, but also influenced by the modification of the lignin. The oxygen-treated substrate containing more lignin (49%) was much faster to hydrolyze than untreated substrate with less lignin (42%). The changes in lignin and cellulose during oxygen treatment and their effects on subsequent enzymatic hydrolysis will be described.

Poster Presentation 6A-40

The Effect of Modified Pretreatment and Delignification Parameters on the Bioconversion Process

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Modified pretreatment parameters were investigated in attempts to improve the efficacy of the delignification steps in the bioconversion process, which consequently is one of the most costly process variables. Three different parameters were manipulated, including: chip size, moisture content, and post explosion particle size. All variables are believed to significantly affect the severity of steam pretreatment of softwood wood chips. It became apparent that a substantial improvement in delignification efficiency with a hot alkaline peroxide step was attainable by increasing the chip size and moisture content. Subsequent refining further improved alkaline peroxide delignification by 5 %, and led to increased yield from hydrolysis.

Therefore, it is apparent that cost saving in delignification can be achieved by the use of the optimum pretreatment chip conditions, which appear to be chips with a higher moisture content, chip size and post steam explosion particle size reduction. Further, improvements in peroxide delignification were also achieved with the addition of a chelation stage prior to peroxide delignification and stabilizing the peroxide delignification. With the addition of EDTA chelation and DTMPA stabilization the peroxide loadings could be reduced 40 % while maintaining rapid hydrolysis rates.

Poster Presentation 6A-41

SO_2-catalysed Steam Explosion of Corn Fibre for Ethanol Production

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It has been shown that a two-stage processing of corn fibre (SO_2-catalysed steam explosion and enzymatic hydrolysis) can be a very effective method for converting the available polysaccharides in the residue to monomeric sugars, as evidenced by the 81% conversion at optimum pretreatment conditions (190°C, 5min and 3%SO_2).

In the current study, we tested the hydrolysability of high concentration solids obtained from the optimum pretreatment conditions (190°C, 5min and 3% SO_2) by employing regular shaker-flasks. One of the disadvantages of two-stage generic bioprocessing (SO_2-catalysed steam explosion and enzymatic) is the generation of dilute process streams, particularly with regard to hemicellulose-rich prehydrolysate. To overcome this problem, steam-pretreated, water-washed corn fibre solids were enzymatically hydrolysed at 5% consistency with prehydrolysate to assess the effect of high concentration of sugars and inhibitory effects of prehydrolysate on the enzymatic conversion of polysaccharides.

The results indicated that sequential SO_2-catalysed steam explosion and enzymatic hydrolysis of high consistency solids (5%, 7.5%, 10% and 12%) resulted in very high conversion (95%) of cellulose in the corn fibre to glucose within 72 hours. In addition, because of the relatively low toxicity of the hemicellulose stream in corn fibre, this water-soluble stream has the potential to be used as the aqueous media for carrying out enzymatic hydrolysis.

Poster Presentation 6A-42 Student

Enzymatic Hydrolysis of Cellulose to Improve Pre-hydrolysate Sugar Concentration

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Bioconversion processes employing a two-stage hydrolysis can maximize recovery of carbohydrate from biomass by allowing independent optimization of hemicellulose recovery and cellulose hydrolysis. However, a drawback of this strategy is the generation of dilute process streams, which greatly impact the overall economics of the process. In an effort to improve the starting sugar concentration in the water-soluble pre-hydrolysate derived from steam-exploded Douglas-fir, we have explored supplementation with sugars derived from the enzymatic hydrolysis of the water-insoluble cellulose component. We have demonstrated that a 1:1 combination the cellulose hydrolysate with the pre-hydrolysate resulted in increased sugar concentration, improved rates of fermentation and superior ethanol concentrations. However, the effective dilution of the pre-hydrolysate by the cellulose hydrolysate resulted in inefficient galactose metabolism during fermentation. To prevent this dilution and achieve a potentially greater increase in sugar concentration, the enzymatic hydrolysis was conducted directly in the water-soluble pre-hydrolysate. However, the efficiency of hydrolysis in the pre-hydrolysate was reduced, owing to sugars present in the pre-hydrolysate, but also non-carbohydrate inhibitors (e.g., furans and lignin-derived phenolics). The use of a pre-hydrolysate detoxification step (e.g., solvent extraction, overliming, treatment with laccase or anion exchange resin) prior to hydrolysis resulted in a partial improvement in the rate and yield of hydrolysis.

Poster Presentation 6A-43

A Quantitative approach to Studying the Effects of Sugar Inhibition on Cellulase and β-glucosidase During Enzymatic Hydrolysis of Softwood Substrates

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End-product-inhibition has been shown to play an important role in determining the cellulose hydrolysis rate, particularly in the later stages of hydrolysis. End product inhibition leads to incomplete hydrolysis and inefficient utilization of the substrate. A quantitative approach was taken to determine the inhibition effects of glucose and other sugar monomers during cellulase and β-glucosidase hydrolysis of various cellulosic materials. Glucose and cellobiose have both demonstrated considerable inhibition of cellulase hydrolysis. However, this adverse effect became less significant when high substrate consistency conditions were used for enzyme hydrolysis. The inhibition effects of mannose, xylose and galactose on both cellulase and β-glucosidase activities were also determined at different sugar concentrations. It was interesting to note that, while little inhibition was detected towards the β-glucosidase activity, these sugars showed considerable inhibition of the cellulase activity during cellulose hydrolysis. This paper will also discuss the potential of combining cellulose hydrolysis with the water soluble hemicellulose stream to increase sugar concentration in the final hydrolysate.

Poster Presentation 6A-44

High Consistency Hydrolysis of Softwood Substrates

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Hydrolysis of lignocellulosic material is often performed at dilute substrate concentrations, when compared to starch hydrolysis, which is normally carried out at consistency of around a 20%. As a consequence, a dilute sugar stream and low ethanol content are often obtained after the hydrolysis and fermentation process steps. At the same time, a high energy input is required to extract the ethanol from the final liquor and a large volume of water is often "carried" throughout the process. It is generally recognized that a practical ethanol production process will require a concentrated sugar stream. However, a major obstacle encountered in previous efforts to use high consistency hydrolysis has been the difficulty in "liquefying" the solid lignocellulosic substrate. In this study, a combined ball-milling and high speed shaking method was used to initialize the liquefaction of the lignocellulosic substrate at high substrate consistencies (10-20% w/v). Different types of softwood substrates, including steam exploded wood, organosolv pretreated wood and kraft pulp, were used in this study. We have found that this method can hydrolyze softwood substrates of up to 20% consistency and provide good recovery of sugars. Glucose concentrations ranging from 8 to 15% were obtained from the different hydrolysates. Subsequent fermentation resulted in a good rate of glucose conversion to ethanol. The effect of end product inhibition during both enzyme hydrolysis and fermentation will be reported.

Poster Presentation 6A-45

Saccharification of Sugar Cane Bagasse Pith by a Heterogenerous Cellulase

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Previously we reported the cellulase production by mixed culture fermentation between *Penicillium sp.CH-TE-001* and *A. terreus CH-TE-013*, actually we are studying the effect of the enzymatic system obtained by them at the saccharification of native sugar bagasse pith.

This presentation describes the saccharification of native sugar cane bagasse pith with the heterogenerous cellulase system derived from mixture culture among these two filamentous fungi and the comparision with respect to each individually.

The 3% solutions of sugar cane bagasse pith at pH 4.8, was saccharified using 50 mL volume of culture filtrate in a glass cup at 50º C and 120 strokes/min. The saccharification was carried out for 24 h and liberated reducing sugars determined by DNS method.

The experimental results showed a major capacity of reducing sugar production with the culture filtrate obtained by mixture culture and it was possible to increase the native sugar cane pith saccharification in a quantity of 47% compared with the highest filtrate culture saccharing activity acting individually and under the same conditions.

Poster Presentation 6A-46

Evaluation of the Fermentation Conditions for Beta-Glucosidase Production with *A. niger* Using Different Lignocellulosic Materials

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The hydrolytic activity of fungal originated β-glucosidase is exploited in several biotechnological processes for increasing the rate and extent of saccharification of several cellulosic materials by hydrolyzing the cellobiose which is inhibitor of cellulases. *Aspergillus* species are known to be good producers of β-glucosidase. Previously we reported the screening of three different strains of *Aspergillus niger*, one of *Aspergillus oryzae* and one of *A. terreus* for β-glucosidase production using a simple plate assay utilizing p-nitrophenyl-β-D-glucoside as the sole carbon source. In this work we are reporting the liquid fermentation for β-glucosidase production with the *A. niger* C-6 strain in a basal culture medium with mineral salts, corn syrup liquor and different waste lignocellulosic materials as the sole carbon source.

Shake-flask cultures were carried out in 500 mL flask containing 100 ml medium. The inoculated flask were incubated in a rotary shaking incubator at 29ºC for 10 days. When *A . niger* C-6 strain was grown on basal medium containing sugar cane bagasse, the maximum enzyme titer was reached within 5-6 days and the activity of β-glucosidase was 8.5 IU/mL The results suggest that *A. niger* C-6 is a potential microorganism for optimizing the β-glucosidase production.

Poster Presentation 6A-47

Rapid Biomass Analysis: New Analytical Methods Supporting Biomass Pretreatment Research

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The ability to obtain an accurate chemical composition of biomass and biomass-derived samples using rapid and inexpensive methods is a key element supporting commercialization of processes that convert biomass to fuels and chemicals. New techniques are being developed at NREL that combine Near InfraRed (NIR) spectroscopy and Projection to Latent Structures (PLS) multivariate analysis for the rapid chemical characterization of corn stover and stover-derived solids. These rapid techniques can provide significant savings in time and money with precision and accuracy that matches traditional wet chemical methods. They also support and improve research by providing levels of information that would have been too costly to pursue using traditional methods

Aspects of NIR/PLS method development will be described including the collection of appropriate calibration samples, the development of robust spectroscopic methods, the importance of quality calibration data, the development and validation of multivariate analysis equations and the use of appropriate QA/QC techniques. Application of rapid analysis tools to the chemical characterization of dry solids, slurries and liquid samples produced during dilute acid pretreatment of corn stover will be presented.

Poster Presentation 6A-48

Methods for Quantitative Analysis of Uronic Acids in Biomass

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Hemicelluloses are very structurally diverse polysaccharides containing a variety of monosaccharides, uronic acids, acetyl and other functional groups. Xylans are the most common hemicellulose and can be considered the second most abundant polysaccharide in nature. Processes designed for converting biomass into ethanol need to utilize the sugars that can be obtained from xylan to be cost effective. The presence of uronic acids in xylans is believed to render the xylan more resistant to hydrolysis by dilute acid resulting in a potential loss of xylose that is not fermented into alcohol. Consequently, methods for analysis of uronic acid content in biomass have been assessed to determine the most efficient means of quantifying the uronic acid components that are present in feedstocks of interest for ethanol production. A literature study has identified hydrolysis and degradative methods of separating uronic acid components from the monosaccharides present in xylans. Various spectroscopic, HPLC, and GC methods for measuring uronic acid concentration have also been identified. This paper will examine the different methods of analysis comparing their precision and reproducibility, and the effort required to perform the analyses.

Poster Presentation 6A-49

Effects of Dilute Acid Hydrolyzate Components on Glucose Degradation

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Enzymatic cellulose hydrolysis is selective and promises high sugar yields at reasonable operating cost provided that low cost enzymes and enzyme reaction systems can be produced. However low cost enzymes have yet to be realized. Acid catalyzed cellulose hydrolysis has been known for many years and may be cost effective if low acid concentrations are used and high sugar yields can be obtained. To date only in rare circumstances have high sugar yields (>90%) been obtained from low acid concentration (<0.5% sulfuric acid) cellulose hydrolysis when taken to high conversion levels (>90%). Recent studies have indicated that components present in dilute acid prehydrolyzates may affect glucose degradation under the conditions used for dilute acid cellulose hydrolysis. This paper will examine the influence of various components on glucose stability at conditions used for dilute acid hydrolysis of cellulose.

Poster Presentation 6A-50

Hot-Washing of Pretreated Corn Stover Using Integrated Sunds Horizontal Screw and Jaygo Pretreatment Reactors with Pneumapress Automatic Pressure Filter

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A 200 kg (dry) per day Sunds (now Metso) horizontal screw pretreatment reactor integrated with a 130-L Jaygo high solids paddle type reactor configured as a surge tank, and a Pneumapress® model 3-C276 automatic pressure filter was used to wash pretreated corn stover and yellow poplar sawdust with hot water at greater than 130°C. The pretreated biomass slurries were not allowed to cool below 130°C before "hot-washing". Pretreatment was carried out at temperatures near 190°C, sulfuric acid concentrations between 0.5% and 2.5 wt-% and residence times of approximately 1 to 5 min. Pretreatment sugar yield, and hot-wash Pneumapress performance, lignin removal from solid residues, and enzymatic digestibilities of hot-washed filter cakes will be reported.

Poster Presentation 6A-51

Production and Hydrolysis of Cellulose from the PureVision Biomass Fractionation Process

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Biomass represents an important renewable energy source to provide the United States with a more environmentally friendly and sustainable energy source. Lignocellulosic biomass is composed primarily of three biopolymers: cellulose, hemicellulose and lignin. The cellulose fraction is of paramount importance because it is a polymer of glucose – the sugar most amenable to fermentation for fuel ethanol production.

It has long been recognized that enzymatic hydrolysis of cellulose is an environmentally benign means for producing glucose. The problem has been the high cost of the enzymes consumed in this process — a cost due at least in part to deactivation of the enzymes by binding to lignin in the biomass, thereby requiring enzyme usage in reagent quantities. To address this need, PureVision Technology, Inc. and Western Research Institute have been perfecting PureVision's patented fractionation process. This processes emphasizes production of a low lignin content product on which cellulase enzymes can act as true catalysts with a consequent reduction in enzyme consumption.

Details of the PureVision fractionation process will be presented along with data, which will define the purity of the cellulose product and its hydrolysis using cellulase enzymes.

Poster Presentation 6A-52

Computer Simulations of Water Structuring Adjacent to Microcrystalline Cellulose Iβ Surfaces

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Due to the abundance of cellulose and it's potential for becoming a major energy source through hydrolysis to ethanol, understanding the structure, function and degradation of this material is an active area of research. One way to study these intrinsic properties is through the use of molecular modeling techniques. In this work, we have examined the water/cellulose boundary layer through molecular dynamics simulations of a cellulose 1β crystal, with both the planar and step face exposed to the water, at 298K, 323K, 373K, and 408K. During the course of the simulations, structural movement of the top two cellulose chains was observed and characterized. The fluctuations were greater in the step face compared to the planar face, pointing to a less stable crystal. Differences between the step and planar faces of cellulose were also highlighted through the extent of water penetration into the crystal as a function of temperature. The average density of the water was plotted as a function of the distance from the crystal surface and displayed a maximum value of 1.4 g/cm3 directly above the first layer of the crystal, and a value of 1.1 g/cm3 at approximately 6.6Å from the surface. Hence, the water above the microcrystalline cellulose surface is highly structured, as can be confirmed from visualization of the simulations, creating possible barriers to rapid hydrolysis.

Poster Presentation 6A-53

Pretreatment of Corn Stover by Low-Liquid Ammonia Percolation Process

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A pretreatment method based on aqueous ammonia is being developed in our laboratory. This process uses a flowthrough packed column reactor (or percolation reactor), thus termed as ammonia recycle percolation (ARP) process. It has been proven to be effective for enzymatic hydrolysis of various biomass feedstocks including corn stover. In this work, the effects of various reaction and operational parameters of the ARP were investigated as it applies to pretreatment of corn stover. The amount of liquid throughput, reaction time, ammonia concentration, and reaction temperature are the primary factors influencing the reactions occurring in the ARP. Economic analysis of this process indicates that the amount of liquid throughput is one of the major cost items since it is directly related to the process energy cost. Further improvement of this process was therefore sought with emphasis on minimizing the liquid throughput. The effects of low-liquid throughput on the carbohydrate retention and delignification were investigated. The corn stover samples treated by this process were further evaluated by subjecting them to enzymatic hydrolysis and simultaneous saccharification and fermentation (SSF). This paper provides experimental details and overall assessment on the pretreatment of corn stover by ARP.

Poster Presentation 6A-54

Effects of Residual and Soluble Lignin on Enzymatic Hydrolysis of Cellulose

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Upon pretreatment, biomass undergoes considerable changes in physical properties and in chemical composition. There are a number of factors that are related with the digestibility of the cellulosic biomass. The lignin content is believed to be one of the major factors controlling the digestibility. Lignin in solid may shield the cellulosic component of biomass impeding the access of the enzyme to cellulose. The cellulase enzyme may also be adsorbed onto the lignin, the efficiency of the enzyme thus being negatively affected. In this investigation, attempts were made to determine the isolated effects of lignin on the enzymatic digestibility using pretreated corn stover and α-cellulose as the feedstocks. Alkaline or neutral pretreatment methods generate effluents containing sugar oligomers (primarily xylose oligomers) as well as soluble lignins. There is a strong interest of converting these oligomers into monomers using the cellulase enzymes. It is well known that most of the cellulase enzymes have xylanase activities. A plausible process scheme is that the pretreatment effluents containing sugar oligomers are fed into a hydrolysis reactor or a SSF reactor along with the pretreated biomass. For this reason, we have also studied the effects of soluble lignin on the enzymatic hydrolysis. Three different pretreatment methods including treatment with aqueous ammonia, hot water, and dilute-acid, were used in order to generate the various types of lignin. Digestibility studies were done with α-cellulose/pretreated corn stover adding various kinds of external lignins such as acid-soluble/insoluble lignin, water-soluble/insoluble lignin, and alkali-soluble/insoluble lignin of corn stover. Insoluble lignin (Klason lignin) was isolated by sulfuric acid in a two-stage hydrolysis process from pretreated corn stover. The data were analyzed to assess the isolated effects of the residual lignin and the soluble lignin on the enzymatic hydrolysis of the lignocellulosic biomass.

Poster Presentation 6A-55

Kinetics of Glucose Decomposition Under Extremely Low Acid and High Temperature Conditions

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Recent research work in our laboratory and at NREL has demonstrated that extremely low concentration of acid (pH near 2) and high temperature (200-230°C) is a reaction condition that can be effectively applied for hydrolysis of the cellulosic component of biomass. The conditions specified here being far apart from those of the conventional dilute-acid hydrolysis processes, the kinetic data are not currently available. In this study, the kinetics of glucose decomposition was experimentally investigated covering pH1.5-2.2 and 180°C – 230°C using batch reactors. The primary factors controlling glucose decomposition is the reaction medium, acid concentration, and temperature. Based on the experimental data, a kinetic model was developed and the best-fit kinetic parameters were determined. However, consistent discrepancy was found in the rate of glucose disappearance between the model based on pure glucose data and those observed in the actual hydrolysis of lignocellulosic biomass. This was taken as an indication that glucose recombines with acid solubilized lignin during the hydrolysis process, and it was incorporated accordingly into the overall model of glucose decomposition.

Poster Presentation 6A-56

Acid Hydrolysis of Corn Stover Hemicellulose by Low-Liquid Percolation Process

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Acid hydrolysis of biomass using a percolation reactor (fixed-bed flowthrough reactor) is a high-yield, yet energy-intensive process due to the large amount of liquid throughput applied in the process. We have investigated on acid hydrolysis of corn stover hemicellulose with a special interest of minimizing water throughput. In percolation reactor operation, the sugar yield has inverse relationship with sugar concentration. Large amount of liquid throughput increases the yield but lowers the sugar concentration. With repeated experiments over wide range of reaction and operating conditions, we have identified the process and operational conditions considered to be optimum from an economic standpoint. Such an optimum condition calls for low-liquid throughput and high sugar concentration. The process we devised therefore uses dry feedstock without presoaking, which is directly subjected to hot acid solution. Among the notable findings derived from our experiments are as follows. Liquid throughput less than one reactor void can be applied in order to maximize the sugar concentration. Use of high acid concentration improves the hydrolysis rate as well as the product concentration. There exists an optimum flow rate for a given reaction condition that maximizes xylose concentration and yield. The hydroysates of this process contain primarily xylose, its oligomers, and small amount of glucose. The xylose oligomers are easily hydrolyzed by dilute acid without causing decomposition. The oligomers are also hydrolyzed by the action of the xylanase normally embodied in "cellulase" enzymes. The enzymatic digestibility data for treated solid samples and additional details of the process and operation strategies including recycle of the reactor effluent are presented.

Poster Presentation 6A-57

Hydrolyzed Distiller's Grain Production, Fermentation and Animal Feeding Trials

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Over the past three decades ethanol production has increased from 200 million gallons per year to close to 2 billion gallons per year in the year 2002, with ethanol production expected to reach five billion gallons per year by 2012. The simultaneous co-production of 4.5 million tons per year of distillers grain (DG), an increase of 2.5 million tons per year, is expected to exceed the capacity of the current dried distiller's grain (DDG) and dried distiller's grain with solubles (DDGS) animal feed markets to absorb without erosion of prices. Dry mill ethanol producers are seeking ways to increase the protein content and quality of DG to help stabilize prices and increase the market share for a higher quality product. Dry mill ethanol producers are now evaluating new technologies to increase the protein content of DG, with an expected added benefit of increased ethanol production using the additional carbohydrates that become available from hydrolyzed DG (HDG). Bench-scale methods for steam explosion, SO_2, and dilute sulfuric acid pretreatment of distiller's grain (DG) were developed using a 2-L ZipperClave® and a 4-L steam explosion reactor. Fermentation protocols for pretreated DG (hydrolyzed DG or HDG) were developed at the bench-scale and those conditions used for an 800-L fermentation of a production of HDG using DG obtained from a mid-western dry mill ethanol plant. HDG fermentation residues were air-dried at low temperatures (45°C), screened, and provided to the Animal Science Laboratory at the University of Minnesota for animal feeding trials using National Forage Testing Association (NFTA) approved methods. Results of the pretreatment, fermentation and animal feeding trials will be presented.

Poster Presentation 6A-58 Student

Measuring and Modeling Oligomer Solubilities in Hemicellulose Hydrolysis

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We have hypothesized that oligomer dissolution and solubility could play important roles during hemicellulose hydrolysis for pretreatment of cellulosic biomass. In order to examine this hypothesis, oligomer solubility data or predictive models are needed. However, a thorough search of the literature reveals little information on oligomer solubilities, particularly for species relevant to hemicellulose hydrolysis. Therefore, a novel method using small quantities of sugar or oligomer was developed and used to determine solubilities. A known mass was added to a thermostated cell that was agitated and heated at a steady rate, and the refractive index was monitored until a sudden change in slope was encountered, signaling total dissolution of all the solids. A series of such experiments with various quantities and types of sugars and oligomers allowed us to develop solubility curves for different species over a range of temperatures. Then, several thermodynamic relationships, including the ideal solubility law, UNIQUAC, UNIFAC, the Flory-Higgins model, and the entropic free volume models, were evaluated for their ability to predict solubility data.

Poster Presentation 6A-60

The Role of Solids Concentration and Acetylation in Uncatalyzed Batch Hydrolysis of Corn Stover Hemicellulose

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Biomass provides the only known sustainable route for making organic fuels, chemicals, and materials, and biological conversion of cellulosics promises low costs vital to commercial success. High yields of glucose from cellulose can be realized by removing hemicellulose from biomass, but hemicellulose sugar recovery is too low unless dilute acid or flow systems are employed. Because costs could be reduced if hemicellulose could be removed efficiently with much less acid or flow, the objective of this paper is to better understand the role of solids concentration and acetylation in hemicellulose hydrolysis. Statistically significant differences in yields were demonstrated for solids concentrations of 5 and 20% in tubular batch reactors, and the greater solids loading increased solids hydrolysis, oligomer hydrolysis, and monomer degradation reaction rates. Furthermore, hemicellulose sugar recovery dropped significantly when acetyl groups were removed, and adding back acetic acid increased solid and oligomer hydrolysis rates and decreased monomer degradation. However, yields could not be restored close to levels for natural corn stover even when as much as five times the original acetic acid was applied. Kinetic models showed that first order rate constants typically applied to describe hemicellulose hydrolysis changed with both solids loading and acetic acid addition.

Key words: acetic acid, hemicellulose, kinetics, oligomers, pretreatment

Poster Presentation 6A-61

Softwood to Ethanol Process Design and Optimization

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Bioconversion of lignocellulosic residue to ethanol generates fuel that can be used as a substitute for, or an additive to liquid fossil fuels. In British Columbia, conversion of softwood residues from the forest industry to ethanol could sustainably support a 10% blend of ethanol in gasoline in Western Canada (British Columbia and Alberta).

The current UBC bioconversion process consists of six major stages: pretreatment, fractionation, enzymatic hydrolysis, fermentation, product recovery and waste treatment. A techno-economic model (STEAM) was previously developed to both assess research and economic progress. Through sensitivity analysis, the model can assist in focussing research efforts on the most costly operations.

The STEAM model identified fractionation and enzymatic hydrolysis as contributing 46% and 21% to final ethanol cost, respectively. As a result, a project was undertaken to evaluate the comparative impact of three different pre-treatment methods (SO_2 catalyzed steam explosion, acetic acid and organosolv) and on the overall economics of the bioconversion process. Our group has previously obtained experimental data for SO_2 catalyzed steam explosion and acetic acid pulping. To develop a database for the organosolv pretreatment technology a factorial design was created with three factors (time, temperature and liquor:wood ratio) at three levels. Preliminary experiments demonstrated that organosolv pretreatment reduced lignin content by 20-40% and the product was readily hydrolysed by cellulase enzymes.

Poster Abstracts for Session 6B

Plant Biotechnology and Feedstock Genomics

Poster Presentation 6B-07

Effects of Pretreatment on the Activity of Plant-Produced Cellulase and Xylanase Enzymes

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For bioconversion processes that convert lignocellulose to fermentation products, maximal utilization of the various polymeric sugars is essential. Complete and balanced cellulolytic and xylanolytic systems are required to achieve maximum hydrolysis of plant cell wall polymers. A critical parameter affecting the economic feasibility of industrial processes is the production of inexpensive and highly active cellulase and xylanase enzymes in bulk quantity. A promising approach to reduce enzyme costs is to genetically transform plants with the genes of these enzymes, thereby producing the desired enzymes in plants themselves. Extraction and recovery of active proteins or releasing active cellulase and xylanase from the plants during bioconversion could have a significant positive impact on overall lignocellulose conversion economics.

We measured the effects of various lignocellulose pretreatments employing a range of treatment pH values and temperatures on the activity of plant-produced cellulase and xylanase enzymes. The plant materials included transgenic tobacco plants expressing E1 (endoglucanase from *Acidothermus cellulolyticus*) and transgenic tobacco plants expressing xynZ (thermostable xylanase from *Clostridium thermocellum*). The E1 and xynZ activities were measured in untreated and pretreated tobacco leaves to characterize the effects of various pretreatments on the activity of these enzymes.

Poster Presentation 6B-08

Effective Digestion of Untreated Corn Stover by Microbial Enzymes

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Athenix is identifying high-value traits in microbes by screening for specific bioconversion activities, including breakdown of lignocellulosic substrates. Current methods for conversion of corn stover to its composite sugars require harsh physical and chemical pretreatment methods to break down hemicellulose and lignin polymers followed by enzymatic saccharification of cellulose. Though effective, such pretreatments are expensive to adapt to an industrial scale and partially destructive to the sugars. We are developing an improved method to release sugars from corn stover without such pretreatment. We have approached this by identifying microbes that facilitate enzymatic digestion of stover without the need for chemical pretreatment.

To this end, we have devised several screens to identify microbes that digest untreated corn stover, including a 96-well high throughput stover assay. We have used these methods to rapidly screen several thousand bacterial strains for ability to digest stover. We have also devised novel methods to screen fungi for lignocellulosic degradation activities. These efforts have led to the discovery of diverse fungal and bacterial species with robust stover digestion activities. Our current efforts focus on optimization of these strains for commercial applications, as well as gene cloning to identify relevant enzymatic activities.

Poster Presentation 6B-09

Biorefining Reproductive Organs of Plant Crops

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In its most general conception, a biorefinery consists of cascades of operations that segregate mass, separate molecules, and assemble molecules. The "bio" in biorefining suggests the molecules of interest are of biological origin. By this definition, the farm, the crop-processing plant, and even a petroleum refinery may be considered as biorefining operations or part of a biorefinery. One of the results of biorefining is the creation of concentrated or enriched fractions that serve as platforms from which additional cascades of biorefining operations can be applied. For grain, the biorefining may produce oil, protein, sugar, starch, fiber concentrates and other platforms that add both to the cost and profitability of the biorefining operation. In general the choice of the crop, the initial mass segregation, and the segregation/separation cascade determine the structure of all of the refining that follows. In US practice, mass segregation strategies are based on methodology established in past decades, centuries, or millennia. The methodology is often water-based. New paradigms for biorefining that emphasize benign conditions are being investigated by the USDA, Agricultural Research Service within the Quality and Utilization of Agricultural Products and Energy Alternatives National Programs. The paradigms emphasize disassembly of crops and utilization of intermediate platforms as well as the creation of the sugar platform. The new paradigms are enabled by capitalizing on the use of green solvents, tailored enzymes along with moderate temperatures that minimize energy use and undesired products. Two research efforts at WRRC reflect this vision. In one, the use of chilled ethanol as a replacement for water in the refining of wheat to starch and protein platforms creates possibilities for an increased number of very-high-value crop-component platforms. In another, solid phase or cold hydrolysis of starch by enzymes evolved to the task will reduce energy demand for conversion of starch to sugar platforms.

Poster Presentation 6B-10

Transgenic Maize-Produced Cellulases for Biomass Conversion

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The transgenic maize production system is ideal for producing recombinant industrial enzymes. Corn seed is an efficient system that allows protein to be stably stored for months to years without loss of enzyme activity. For industrial enzymes, high levels of expression are required for commercial viability and consequently drive the technology to achieve high expression. The technology encompasses utilizing promoters with high functionality, targeting the protein to tissues and subcellular locations that promote efficient accumulation, and breeding the transgenic line into elite germplasm for selection of higher-expressing lines. Preparation of parity yielding agronomic material is a key piece of cost-effective production. By-product credits can be obtained for materials remaining after enzyme removal. Regulatory paths are a critical piece of the overall plan, but for industrial enzymes these will differ dramatically from requirements for pharmaceutical products. Industrial enzymes are large-volume, large acreage products with medium-range margins for which field production is the major cost. Production of cellulases in transgenic maize is progressing rapidly. More than 200 independent transgenic events have been isolated for three genes, two cellulases and a β-D-glucosidase. Plants from these events are being cultivated in the greenhouse. Assays of first generation seed will begin in 2003. The seed-based expression of enzymes can be coupled with stover harvest and treatment from the same field to achieve cost-effective production.

Poster Presentation 6B-11

Plant Genomics at the Service of Energy Crops

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Plant biomass represents a promising source of renewable energy, which is likely to play an increasing role in the world's energy supply. In order to meet a growing demand, it is anticipated that large areas of land will be needed for energy crops. As a result, productivity will be a critical issue in ensuring the competitiveness of energy crops with traditional agricultural crops. It will also be essential to make them cost-competitive with other sources of renewable energy.

Quantitative traits such as biomass yield will determine which plant species are most suitable for energy production. Qualitative traits such as cellulose and lignin contents will also be important, since they impact energy content, conversion efficiency and recalcitrance to bio-transformation.

Since pressure for agricultural land is likely to restrict energy crops to marginal areas, tolerance to environmental stresses such as water or nutrient deficiency will be key features of successful energy crops. Using a functional genomics approach, Mendel Biotechnology has developed a portfolio of genes and technologies that can be used to address some of the critical needs of energy crops. In particular, we have discovered regulatory genes that have a dramatic impact on biomass production and drought tolerance. Some of our results and our strategy for energy crops will be presented.

Poster Presentation 6B-12

Modifying Lignin Composition to Enhance Ethanol Production from Maize Stover

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We are looking at changing lignin composition as a way to improve the efficiency of bio-fuel production from maize stover. In secondary cell walls carbohydrates are intimately associated with the hydrophobic polymer lignin. We hypothesize that the enzymatic or chemical hydrolysis of cell wall carbohydrates is impeded by the presence of lignin. Changing the content and subunit composition of lignin is expected to alter the interaction between lignin and carbohydrates and therefore affect the yield of fermentable sugars, ideally in a positive manner. Preliminary experiments with a set of near-isogenic maize mutants with altered lignin composition revealed that (1) changes in lignin composition could increase the yield of fermentable sugars by as much as 35%, and (2) lignin composition is a more important determinant of the yield of fermentable sugars than lignin content. We are currently using a deconvolution strategy to define a relationship between lignin subunit composition and the efficiency of hydrolysis of cell wall carbohydrates. This involves the analysis of a set of single and double cell wall mutants in terms of bio-fuel production, but, given the importance of lignin in the overall viability of the plant, also in terms of agronomic performance. This approach is expected to lead to the development of high-efficiency bio-fuel crops that still perform well agronomically.

Poster Presentation 6B-13

The Effect of Inoculum Conditions on the Growth of Hairy Root of *Panax ginseng* C.A. Meyer

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Plants have the potential to produce a large number of important metabolites such as pharmaceuticals, food additives, pigments, flavors, fragrances, and fine chemicals. The large-scale plant cell and tissue cultures for producing useful products have been considered as an attractive alternative to whole plant extraction for obtaining valuable chemicals. In plant cell and tissue cultures, cell growth and metabolite production are influenced by nutritional, environmental conditions and physical properties of culture system. In order to obtain a high growth rate of plant cell and tissue cultures, the culture conditions should be maintained at the optimum level.

This study investigates the effect of inoculum conditions such as the number of root tips, the length of root tips, the part of root tips, and the inoculum size and age of root on the growth and metabolite production of hairy root. Hairy root had the highest growth rate at 0.4 %(w/v) inoculum size than others in flask cultures. The final cell growth was not proportional to the increase of inoculum size. It was caused by limited substrate, dissolved oxygen, and limited space at the flask culture.

Poster Abstracts for Special Topics

Presentation SpT A-01

Development and Optimization of Ethanol- and Biodiesel-Blended Diesel

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Keeping ethanol and diesel fuel blended together in a homogeneous solution or suspension, especially under cold-weather conditions and/or in the presence of water contamination, requires the use of an ethanol cosolvent or emulsifier. An ethanol–diesel blending approach currently under development at the University of North Dakota Energy & Environmental Research Center (EERC) and Great Planes Fuel Development of Brookings, South Dakota, involves the use of biodiesel as an ethanol cosolvent.

EERC investigated the viability of ethanol- and biodiesel-blended diesel (EB diesel) on the basis of emissions, fuel economy, cetane, flash point, vapor pressure, cloud point, pour point, low-temperature fuel stability, and fuel stability in the presence of water contamination. Based on the findings and information resulting from this preliminary investigation, the EB-diesel concept represents a viable strategy for commercializing an ethanol-blended diesel fuel. Results supporting this contention will be presented.

Presentation SpT A-02

Pentose-Fermenting *Zymomonas mobilis*

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As one of the fastest and most efficient ethanol producers using glucose identified to date, *Zymomonas mobilis* was extensively characterized during the 1980s when research on its microbiology, biochemistry, genetics, and process optimization was conducted around the world. *Z. mobilis* has significant potential for large-scale production of ethanol, and several pilot scale and larger scale fermentation trials have been conducted using this microorganism. This natural "glucose-fermentor," however, is not capable of metabolizing the pentose sugars found in renewable lignocellulosic feedstocks. In 1993, scientists at NREL initiated an effort to metabolically engineer *Z. mobilis*, and since then have successfully introduced pathways for D-xylose and L-arabinose metabolism into this microorganism to enable pentose fermentation to ethanol. This presentation will describe the current state of development and application of recombinant *Z. mobilis* and outline future prospects.

Presentation SpT A-03

Construction of an L-arabinose Fermenting *Saccharomyces cerevisiae* Strain by Genetic Engineering and Evolutive Screening

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Bioethanol produced by microorganisms is an excellent alternative to fossil transportation fuels. Most industrial ethanol fermentations use the yeast *Saccharomyces cerevisiae.* However, although *S. cerevisiae* is able to ferment hexoses rapidly and efficiently it is unable to ferment pentose sugars.

Therefore, expanding the substrate fermentation range of *S. cerevisiae* to include pentose sugars is important for the utilization of this yeast in economically feasible biomass-to-ethanol fermentation processes. We have constructed an *S. cerevisiae* strain able to utilize the pentose sugar L-arabinose for growth and to ferment it efficiently to ethanol. After overexpression of a bacterial L-arabinose utilization pathway we were able to select an L-arabinose utilising yeast strain by sequential transfer in L-arabinose media. Molecular analysis of this strain revealed that the crucial prerequisite for efficient utilization of L-arabinose is a specifically reduced activity of ribulokinase. Moreover, high L-arabinose uptake rates and enhanced transaldolase activities favor utilization of L-arabinose. The strain exhibits a high ethanol production rate and high ethanol yields with L-arabinose as sole carbon source.

Presentation SpT A-04

Ethanol Production from Sugar Mixtures using *E. coli* FBR Strains

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Development of suitable biocatalysts for the biomass to fuels and chemicals industry remains a significant technical challenge. We have developed a series of stable *E. coli* strains that convert hexoses and pentoses to ethanol by transforming non-fermentative strains (Dr. David Clark, SIU) with pLOI297 plasmid (Dr. Lonnie Ingram, U of Fl) carrying the alcohol dehydrogenase and pyruvate decarboxylase genes from *Zymomonas mobilis*. Expression of ADH and PDC not only restored their ability to grow in anaerobic cultures, the plamid was retained in the absence of antibiotics. Furthermore, the modified strains produced ethanol from sugar mixtures and hydrolysates prepared from corn fiber at 90-93% of theoretical based upon beginning sugar concentrations.

When the strains fermented sugar mixtures, as expected, the glucose was used in preference to xylose. To allow for simultaneous sugar utilization, a catabolite repression mutant (ptsG) was constructed that simultaneously used glucose and xylose and produced ethanol at 94% of theoretical. This research demonstrated that metabolic engineering approaches can be used to modify *E. coli* to provide for more efficient fermentation of hemicellulose containing biomass hydrolysates.

Presentation SpT A-05

Optimizing Carbon Partitioning between Biosynthesis and Ethanol Production in Ethanologenic *Escherichia coli* KO11

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Previous studies have shown that high levels of complex nutrients (Luria broth or 5% corn steep liquor) were required for the rapid conversion of xylose (10%) to ethanol by the ethanologenic *E. coli* strain KO11. The basis for this nutrient requirement was determined to be an imbalance in the partitioning of pyruvate between biosynthesis (acetyl-CoA) and re-oxidation of NADH (ethanol production). Increasing acetyl-CoA availability by adding acetate, by adding pyruvate or by eliminating the wasteful consumption of acetyl-CoA (ackA deletion) increased cell yield and volumetric productivity by approximately 2-fold. These approaches all increased carbon flow through the oxidizing arm of the TCA pathway, and all could be replaced by the addition of glutamate. Glutamate is the most abundant free amino acid in *E. coli* and part of the osmotic stress response. The relatively high concentration of sugar (~0.6 M xylose) would require cells to increase intracellular glutamate, a compatible solute and osmoprotectant. Further studies revealed that glutamate could be replaced by the addition of small amounts of other osmoprotectants (betaine or dimethylsulfoniopropionate), indicating that the apparent need for complex nutrients initially observed reflected a need for increased production of osmoprotectants.

Presentation SpT B-01

Reducing the Cost of Lignocellulose Conversion to Ethanol

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Although the cost of making ethanol from biomass has dropped significantly over the last several decades, biomass-derived ethanol is still too expensive to compete with fossil fuels without subsidies. Therefore, the key challenge is to reduce the major operating costs of biomass conversion processes, primarily pretreatment and enzymes. We believe that the integration of a reduced cost pretreatment with an ethanol-producing microorganism capable of utilizing oligomeric carbohydrates would represent a major step towards that goal.

Prof. Ingram and colleagues have developed microbial technology that is capable of simultaneous hydrolysis and fermentation of amorphous cellulose without added enzymes. The microorganism, Klebsiella oxytoca, can utilize dimers and trimers of glucose and xylose, and can metabolize the five major sugars of lignocellulosic feedstocks. This strain, designated SZ21, also secretes two synergistic endoglucanases to sufficient levels to completely hydrolyze amorphous cellulose. Thus, this organism represents an advanced ethanol producer for SSF processes and has been shown to reduce the amount of enzyme required by up to 60%.

This same approach is now being implemented to address the simultaneous hydrolysis and fermentation of hemicellulose oligomers produced during low or no added acid pretreatment. Previous work has shown that ethanol-producing *Escherchia coli* can co-produce xylanases during fermentation of hemicellulose hydrolysates from dilute acid pretreatment. Prof. Ingram et.al. have developed a new strain that can secrete xylanase activity in sufficient quantities to hydrolyze xylo-oligomers produced by low or no added acid pretreatment of bagasse.

Previous work with low or no added acid pretreatment processes has resulted in relatively low yields of monomeric sugars (relative to standard dilute acid processes) and low solubilization of hemicellulose. Although significant cost savings can be realized in no added acid processes, the low yields were not economically viable for conventional ethanol-producing organisms. With the development of advanced organisms that can utilize oligomeric substrates, there is no longer a need for high monomer yields. Thus, low or no added acid processes can now be further developed.

The integration of low or no added acid pretreatments with advanced ethanol-producing microorganisms represents a significant step towards lowering the cost of biomass conversion processes.

Presentation SpT B-02

The Lignol Approach to Commercializing Biomass-to-Ethanol and Chemicals

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It is generally agreed that the cost of ethanol manufacture from cellulosic biomass is presently greater than that from starch-based or sugar-based feedstocks. However, cellulosic materials are regarded as preferred raw materials for ethanol production because of the much larger potential volume from these sources. Major efforts are now being made to reduce the costs of ethanol production from cellulosic biomass through strategies such as reductions in cellulase enzyme cost and the fermentation of pentose sugars from the hemicellulose component. An alternative, but not incompatible approach favored by Lignol Innovations is to increase revenues through the co-production of valuable chemicals from the biomass feedstock. Many other ethanol-from-biomass technologies view the non-cellulosic fractions of biomass as low value solid fuel at best, or an effluent requiring treatment at worst. Using organosolv-based biorefining processing of the raw feedstock, Lignol recovers valuable chemicals such as organosolv lignin, acetic acid, furfural, xylose and “extractives” from the process. The added revenues from these chemicals make even relatively small ethanol-from-biomass facilities in the range of 100 tpd of dry biomass input economically viable. Furthermore, the organosolv processing generates a cellulose fraction having a very high susceptibility to enzymatic saccharification and fermentation. Results of recent studies and their impact on total process economics and near-term commercial viability will be discussed.

Presentation SpT B-03

Process Development and Pilot Plant Demonstration of Biomass Ethanol Production

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Abengoa Bioenergy, a business unit of Abengoa (Seville, Spain), is the largest grain ethanol producer in Europe and 5th largest in the U.S. Significant growth potential for ethanol exists in the EU and US. The growth could be realized through improvement or expansion of current grain ethanol production facilities, new plants, or through conversion of non-starch carbohydrates (such as grain fiber, corn stover and straw). Abengoa Bioenergy is evaluating bioconversion processes and coordinating R&D efforts in improving current grain ethanol technologies and developing competitive technologies for converting agricultural residues to ethanol. An overview of technical issues related to process development and demonstration of enzyme-based biomass ethanol production will be presented.

Presentation SpT B-04

Current State of Lignocellulosic Fuel Ethanol Commercialization A Pilot Plant for Ethanol from Wood Waste

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In Sweden the company Etek Etanolteknik AB has made a process design of a pilot plant or Process Development Unit (PDU) with a capacity of about 400-500 liter ethanol/day or a feedstock input of 2 ton of dry substance/day. The plant is designed for development of both a two-step dilute acid hydrolysis process and a combination with enzymatic hydrolysis. The reactor in the second dilute acid hydrolysis step is a countercurrent reactor, which has a good potential to increase the yield and reduce the amount of byproducts. The main feedstock is softwood but other raw materials like hardwood and annual crops like straw and reed canary grass will also be tested.

The pilot plant will be open for cooperation with partners all over Europe and may be other countries. It will be located in Ornskoldsvik in the northern part of Sweden, close to an existing sulfite pulp ethanol plant.

The construction and erection has started and the pilot plant is planed to be in operation in the end of 2003. The plant is linked by ownership to the three Universities in the region, The Univ. of Umeå, Mid Sweden Univ. and The Technical Univ. of Luleå. Other Universities in Sweden are represented in the scientific board supporting the management in the development of the plant and the process. Etek Etanolteknik AB, owned by regional Energy companies, will be responsible for the construction and operation of the plant.

The investment costs is about 16 million EURO and the annual running cost is about 1,3-2,0 million EURO depending on the research program. The Swedish National Energy Administration will be the main financier of the plant with 12 million EURO.

The main objective with this paper will be to present the process in the plant.

Presentation SpT B-05

Overcoming Barriers to the Commercializing of Bioethanol Production

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The creation of a viable, commercial-scale bioethanol industry will rely to a large degree on movement from discrete process elements that work effectively at the laboratory- or pilot-scale, to integrated processes that are suitable to the political and ecological conditions under which the process must operate. The most significant barriers to a viable industry will involve both technical and socio-economic variables. The current study will examine the availability of biological substrate for energy production, the efficiency and scalability of process elements, and the economic viability of co products from the process, and will link these variables to current socio-economic conditions in a 'roadmapping' exercise for two case studies. The case studies employed in this study are chosen as representative of a number of parallel business models, and can be described by their position along a number of descriptive axes. One axis is size; there are large established organizations as well as small entrepreneurial start-ups actively seeking viable bioethanol production processes. Another axis describes business interests; some groups have an existing, vested interest in one or more elements of the bioethanol production process, while others seek to develop process elements and integrated solutions for licensing or commercial development. The final axis describes political considerations; some models must operate under North American political structures that include large subsidies for existing fossil fuels, while others operate under European policies that have much lower subsidies and may be more proactive in developing a bioethanol industry. The ongoing research study is currently collecting data and liaising with IEA partners, and will submit a final report in December of 2003. By the end of the program, the research team will be able to inform the program partners on a number of relevant issues for bioethanol commercialization.

Presentation SpT B-06

Prospects and Progress Toward Biomass Hydrolysis Commercialization

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There has been substantial progress towards the commercialization of biomass hydrolysis for ethanol and other chemicals. The last five years has seen several major changes:

- Industrial interest is at an all time high as measured by the number and size of companies investing in biomass hydrolysis technology
- Enzymatic process focus has taken over by a wide margin as measured by the dollars invested in R&D
- Large investment in R&D by U.S. government has created a sufficient pool of expertise to support the technology development

Currently the focus is on the big names that have jumped into the biomass hydrolysis arena including Shell Global Solutions, DuPont, Abengoa, and Cargill Dow. The efforts of these companies, in many cases in collaboration with the U.S. government, will cause the following progress in the next 5 years:

- Start of construction on a large demonstration or a commercial scale plant before early 2008
- Cellulase enzymes will become commercially available at costs that begin to make the biomass hydrolysis technology attractive
- More large scale efforts will be seen, particularly out of the EU
- More incentives to drive commercialization will become available, particularly in the EU and Asia

By 2010 the world will see a few commercial biomass hydrolysis facilities in operation producing products such as ethanol, and polylactide polymers. By 2015 several additional facilities will be built and opened and the early adopter's technology development will be complete. This presentation will provide the rationale and evidence for the conclusions stated above.

Presentation SpT B-07

The United Kingdom Lignocellulosic Bioethanol Challenge

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In November 2002 the UK Government published its intent to support bioethanol and in particular would talk further with stakeholders to discuss further support for Lignocellulosics. What should the stakeholders tell the UK Treasury? Is the industry ready? Will the Government listen and respond in the April 2003 budget statement?

Presentation SpT B-08

Current State of Lignocellulosic Fuel Ethanol Commercialization

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The commercialization of fuel ethanol from lignocellulosics is still far away. We are at the stage where competing technologies are under development with only one company, Iogen, working on a demonstration plant scale.

There are several barriers to commercialization including the need to operate at high throughput rates to make a reasonable profit. An intermediate barrier is the need to construct a demonstration plant that is too small to generate a profit before we build a large scale profitable plant. Only project owners with deep pockets and access to large quantities of suitable biomass can get past the obstacle of cost.

Other barriers include:

- Low petroleum selling price.
- Competitive processes based on corn.
- Competition for lignocellulosic raw materials.
- Complex energy intensive process designs.

Presentation SpT B-09

Current Research Projects on Lignocellulosics-to-Ethanol in Japan

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Due to global warming caused by excessive use of fossil resources, renewable biomass resources will become more important in the future as alternatives to fossil resources. In addition, according to our recent investigation, about 370×10^6 tons of biomass resources such as lignocellulosics are generated annually in Japan, of which 77×10^6 tons are not used efficiently. Therefore, technologies that can convert them to valuable liquid fuels and chemicals will be important for solving our energy and environmental problems. Particularly, the conversion of lignocellulosics to ethanol is one of the main concerns in bioenergy research and development in Japan to fulfill the target in Kyoto Protocol for the reduction of greenhouse gas emissions.

The several research projects on lignocellulosics-to-ethanol are undergoing in Japan for industrial applications such as the concentrated sulfuric acid process from Arkenol Inc. and the dilute sulfuric acid process from BC International Corp., both in the NEDO technology development projects for bio-energy conversion study. On the other hand, for one of the academic applications, supercritical water (>374 , >22.1 MPa) technology has recently received increasing attentions. In our laboratory, therefore, supercritical water treatment was made with lignocellulosics to obtain ethanol and useful chemicals.

For those research projects and related research activities in Japan, the current progress and achievements will be introduced in industrial and academic applications.

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