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MIDWEST AREA BIOENERGY RESEARCH FORUM

Technology Mini-Presentations

Improvement of Biodiesel Performance
-Bryan Moser

Processes to Produce Biofuels from Agricultural Residues
-Badal Saha

Engineering the Biochemical Conversion for Biofuel Production
-Joe Rich

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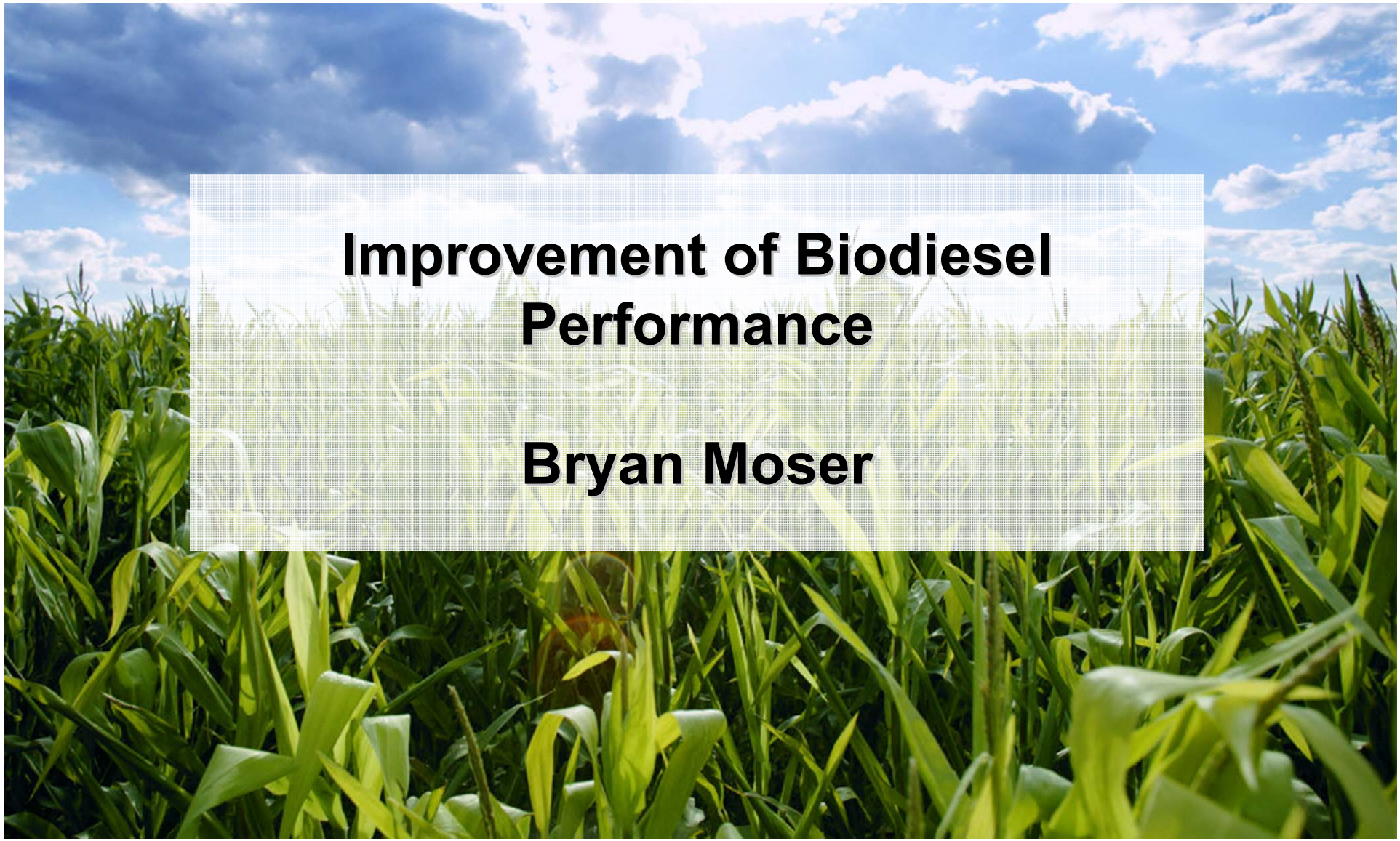


Improvement of Biodiesel Performance

Bryan Moser

BIODIESEL AT NCAUR

Bryan Moser, Ph.D.

Research Chemist

Food and Industrial Oils

National Center for Agricultural Utilization Research

Midwest Area

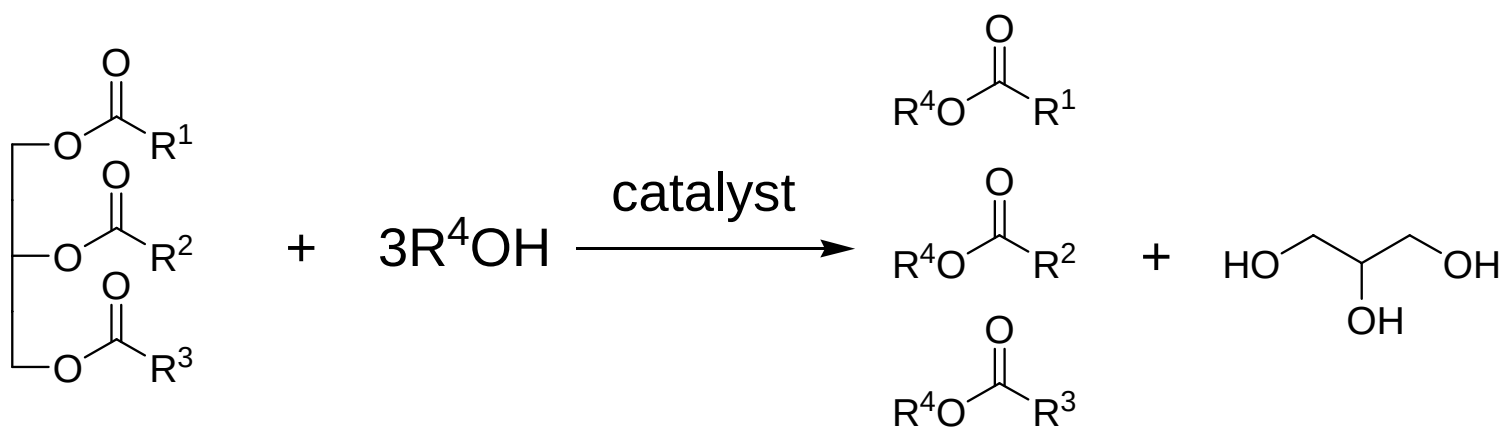
Agricultural Research Service

Please see Poster 11 for more information

WHAT IS BIODIESEL?

An alternative diesel fuel prepared from vegetable oils or animal fats

ASTM (American Society for Testing and Materials): A fuel composed of monoalkyl esters of long-chain fatty acids meeting the requirements of ASTM D 6751



Triacylglycerol Alcohol Biodiesel Glycerol

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BIODIESEL VERSUS PETRODIESEL

ADVANTAGES

Domestically produced
Contributes to energy security
Enhances agricultural economy
Biodegradable & non-toxic
Renewable
High flash point
Good lubricating characteristics
Reduces most regulated exhaust emissions

DISADVANTAGES

Low oxidative stability
Degradation during long-term storage
Poor low temperature properties
Increases NOx exhaust emissions
High feedstock cost

Please see Poster 11 for more information

BIODIESEL RESEARCH AT NCAUR

RESEARCHERS

Dr. Robert O. Dunn, Chemical Engineer
Dr. Gerhard Knothe, Research Chemist
Dr. Bryan R. Moser, Research Chemist
Dr. Shailesh Shah, Research Chemist
Dr. Sevim Z. Erhan, Research Leader

FOCUS OF RESEARCH

Improvement of fuel properties and performance
Improved analytical methods for production and use
Development of bio-based additives
Development of new uses and products for glycerol

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ROBERT O. DUNN, PhD

Research Focus

Effect of contaminants on cold flow properties
Effects of oxidative degradation on fuel quality
Dry fractionation to improve cold flow properties
Thermodynamic modeling to predict effect of fuel composition on cloud point

Representative Publications

Effect of temperature on oil stability index (OSI) of biodiesel, *Energy & Fuels* **2008**, *22*, 657-662

Effect of antioxidants on the oxidative stability of methyl soyate (biodiesel), *Fuel Proc. Technol.* **2005**, *86*, 1071-1085

Oxidative stability of soybean oil fatty acid methyl esters by oil stability index (OSI), *J. Am. Oil Chem. Soc.* **2005**, *82*, 381-387

Winterized methyl esters from soybean oil: An alternative diesel fuel with improved low-temperature flow properties, *SAE Trans. Sec. 4: J. Fuels Lubr.* **1997**, *106*, 640-649

Low temperature properties of triglyceride-based diesel fuels: Transesterified methyl esters and petroleum middle distillate/methyl ester blends, *J. Am. Oil Chem. Soc.* **1995**, *72*, 895-904

Robert.Dunn@ars.usda.gov

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GERHARD KNOTHE, PhD

Research Focus

Correlation of composition to fuel properties

Analytical methods for quality assessment

Effect of biodiesel on fuel blend lubricity

Combustion efficiency and reduction of NOx

Representative Publications

"Designer" biodiesel: Optimizing fatty ester composition to improve fuel properties, *Energy & Fuels* **2008**, *22*, 1358-1364

Analyzing biodiesel: Standards and other methods, *J. Am. Oil Chem. Soc.* **2006**, *83*, 823-833

Kinematic viscosity of biodiesel fuel components and related compounds. Influence of compound structure and comparison to petrodiesel fuel components, *Fuel* **2005**, *84*, 1059-1065

Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters, *Fuel Processing Technol.* **2005**, *86*, 1059-1070

Lubricity of components of biodiesel and petrodiesel. The origin of biodiesel lubricity, *Energy & Fuels* **2005**, *19*, 1192-1200

Gerhard.Knothe@ars.usda.gov

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BRYAN R. MOSER, PhD

Research Focus

Development of bio-based additives for:

- enhanced oxidative stability
- improved low temperature properties

Evaluation of alternative feedstocks

Representative Publications

Branched chain derivatives of alkyl oleates: Tribological, rheological, oxidation, and low temperature properties, *Fuel* **2008**, *87*, 2253-2257

Evaluation of castor and lesquerella oil derivatives as additives in biodiesel and ultra low sulfur diesel fuels, *Energy & Fuels* **2008**, *22*, 1349-1352

Diesters from oleic acid: Synthesis, low temperature properties, and oxidation stability, *J. Am. Oil Chem. Soc.* **2007**, *84*, 675-680

Preparation and evaluation of a series of α-hydroxy ethers from 9,10-epoxystearates, *Eur J. Lipid Sci. Technol.* **2007**, *109*, 206-213

Evaluation of partially hydrogenated methyl esters of soybean oil as biodiesel, *Eur J. Lipid Sci. Technol.* **2007**, *109*, 17-24

Bryan.Moser@ars.usda.gov

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SHAILESH SHAH, PhD

Research Focus

Development of new uses for glycerol
Development of new products using glycerol

(Will join research project in August)

Shailesh.Shah@ars.usda.gov
Please see Poster 11 for more information

MAJOR ACCOMPLISHMENTS

- Developed a rapid and accurate test method for determining oxidative stability
  - Pressurized differential scanning calorimetry (PDSC)
- Developed a fractionation method to improve cold flow properties
  - reduction in saturated fatty ester content
- Discovered the influence of contaminants on fuel properties
  - e.g., free fatty acids, monoacylglycerols, etc.
- Elucidated the optimum fatty ester composition for best fuel properties
  - e.g., methyl palmitoleate (C16:1) or methyl or ethyl decanoate (C10:0)
- Determined the influence of individual fatty esters on fuel properties
- Developed a library of bio-based additives
- Developed biodegradable mulching mats from glycerol

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IN-HOUSE CAPABILITIES

We possess expertise in the following methods:

- Biodiesel fuel property determination:
  - cloud point (CP) & pour point (PP)
  - cold filter plugging point (CFPP)
  - low temperature flow test (LTFT)
  - oxidative stability (OSI, Rancimat)
  - lubricity by HFRR (high frequency reciprocating rig)
- Spectroscopy: NMR, IR, NIR, UV-VIS
- Chromatography: HPLC, GC, GC-MS, IC, GPC
- Calorimetry: DSC, PDSC
- Synthesis: transesterification, organic synthesis
- Other methods: kinematic viscosity, RI, SG, surface tension, FP

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CONTACT INFORMATION

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Processes to Produce Biofuels from Agricultural Residues

Badal Saha

USDA - ARS - National Center for Agricultural Utilization Research

Processes to Produce Biofuels from Agricultural Residues

Badal C. Saha
Fermentation Biotechnology Research Unit

USDA-ARS- Midwest Area Bioenergy Research Forum, July 18, 2008

Process for Biofuel Production

Feedstock

↓

Pretreatment

↓

Enzymatic Saccharification

↓

Fermentation

↓

Biofuel Recovery (Ethanol, Butanol)

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Technological Constraints

- Costly pretreatment
- Fermentation inhibitors
  - Furfural, HMF, Phenolic Compounds, Aliphatic acids
- Inefficient enzymes
  - End product inhibition
- Fermentation of multiple sugars
  - New microbe
- Recovery of dilute biofuel (ethanol, butanol)
- Integration of process steps
  - Simultaneous saccharification and fermentation (SSF)
  - Simultaneous saccharification, fermentation and product recovery (SSFR)

Novel Enzymes for Lignocellulosic Biomass Saccharification

- Endo-glucanase (cellulase) from a newly isolated fungus active over a broad pH range
- Novel product and substrate tolerant β-glucosidase from a yeast (US Patent 5,747,320)
- Hemicellulases from two newly isolated fungi capable of saccharifying corn fiber complex heteroxylan
- Novel thermostable α-L-arabinofuranosidase from a yeast like fungus that releases arabinose from a variety of arabinoxylans

  (US Patent 5,882,905)

- Details : Badal Saha, Badal.Saha@ars.usda.gov

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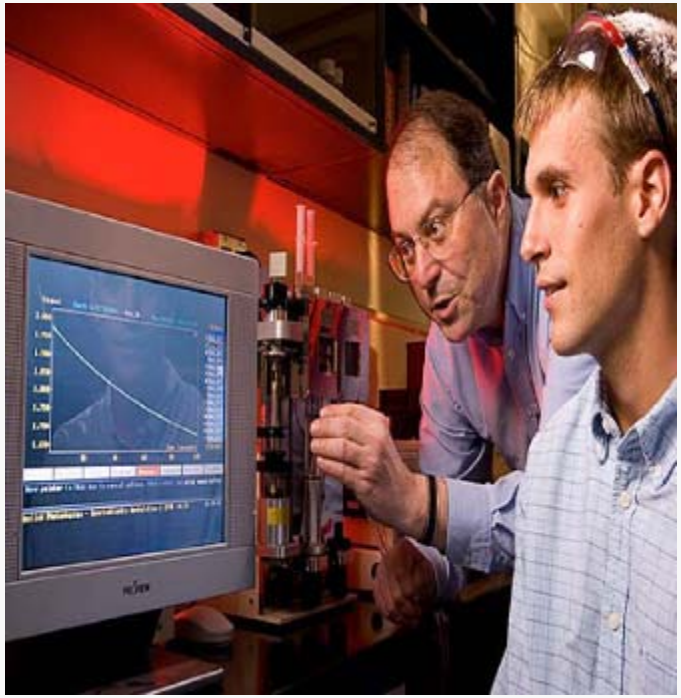
Novel Enzyme for Use in Hemicellulose Bioconversion

- β-Xylosidase from an anaerobic bacterium is the most active β-xylosidase known to date
- Very good stability properties
- Overproduced in *E. coli*: 30% of soluble proteins (1 L culture produces 5 g enzyme)
- US Patent Application on file (2007; Jordan et al.)
- Details: Douglas Jordan, Douglas.Jordan@ars.usda.gov (Poster # 10)



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Novel Enzyme Discovery

- Discovery and characterization of xylanase overproduced by a color variant strain of a yeast like fungus and application of the enzymes from this strain for saccharification of corn fiber and defatted corn germ (Details: Timothy Leaders,

  Tim.Leathers@ars.usda.gov

- A fungal strain isolated from corn kernel produces a full complement of enzymes capable of hydrolyzing xylans from lignocellulosic feedstocks (Details: Ken Bischoff,

  Kenneth.Bischoff@ars.usda.gov

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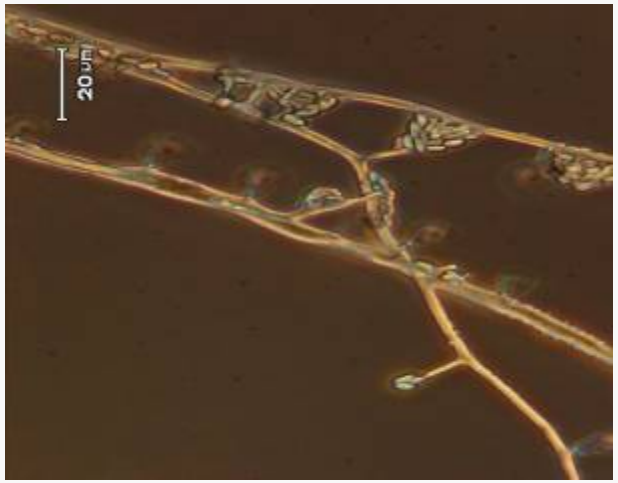
Bioabatement of Fermentation Inhibitors

- A new fungal isolate is capable of significantly depleting the toxicant levels of common fermentation inhibitors typically present in dilute acid hydrolyzates of lignocellulosic feedstocks
- Inexpensive and easy to integrate with fermentation process
- US Patent 7,067,303 (June 27, 2006; Nichols et al.) is available for licensing
- Details: Nancy Nichols, Nancy.Nichols@ars.usda.gov (Poster # 16)



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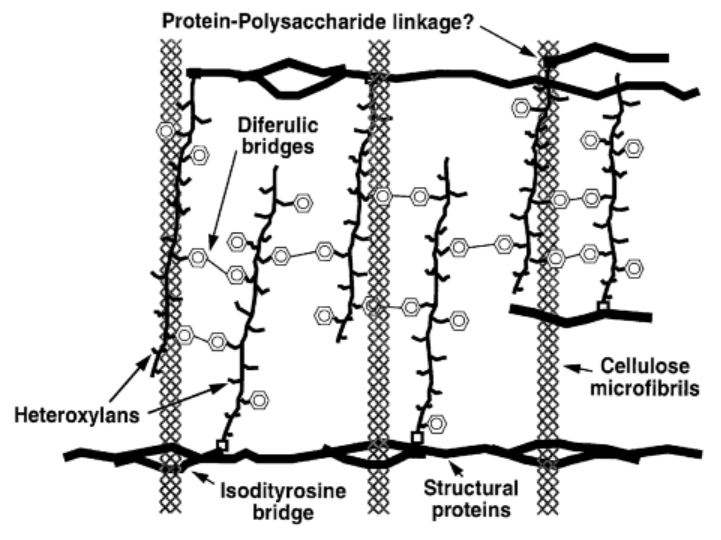
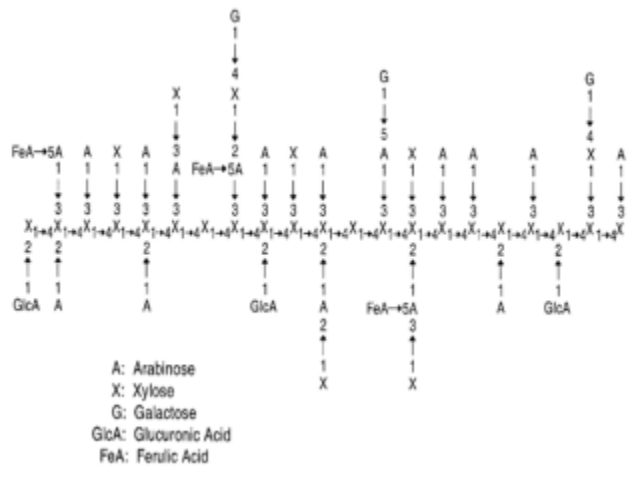
Fuel Ethanol from Corn Fiber

- Corn fiber contains 20% residual starch, 15% cellulose and 35% hemicellulose (total carbohydrate content, 70%)
- The structure of corn fiber hemicellulose is very complex and commercial hemicellulase enzymes are not effective to hydrolyze solubilized hemicellulose from corn fiber
- Dilute acid pretreatment of corn fiber at a moderate temperature followed by enzymatic saccharification of cellulose produces 85-100% of sugars without generating fermentation inhibitors
- Details: Badal Saha, badal.saha@ars.usda.gov





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Fuel Ethanol from Wheat Straw

- Wheat straw contains 45% cellulose and 25% hemicellulose (total carbohydrate content, 70%)
- Alkaline peroxide pretreatment of wheat straw followed by enzymatic saccharification produces highest sugar yield (near 100%) without generating common fermentation inhibitors
- Lime pretreatment is best in simplicity and cost (no inhibitors and easily fermentable)
- Simultaneous saccharification and fermentation (SSF) works well which allows process integration
- About 77 gallons of ethanol is produced per ton of wheat straw by lime pretreatment
- Similar success has been achieved with barley straw as feedstock
- Details: Badal Saha, Badal.Saha@ars.usda.gov (Poster # 18)

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Fuel Ethanol from Rice Hulls

- Rice hull is a complex feedstock with high lignin (15%) and ash (20%) contents. It contains 35% cellulose and 12% hemicellulose (total carbohydrate content, 47%)
- Alkaline peroxide pretreatment of rice hulls followed by enzymatic saccharification produces 95% of sugars without generating common fermentation inhibitors
- Silica is solubilized and easily recovered by this process. It can serve as value-added coproduct of rice hulls conversion to fuel ethanol
- About 56 gallons of ethanol is produced per ton of rice hulls
- Simultaneous saccharification and fermentation (SSF) works well which allows process integration
- Details: Badal Saha, Badal.Saha@ars.usda.gov (Poster # 18)

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Screening Switchgrass Cultivars for Ethanol Production Efficiency

- Tested the "Convertibility" to ethanol of over 100 different samples of switchgrass.
- The results show that there can be a wide spectrum of conversion yields from switchgrass and validate the need for further plant breeding of energy crops
- The data is being applied to develop a quick scanning near-infrared (NIR) model that will be used for breeding switchgrass cultivars for superior ethanol yield
- Details: Bruce Dien,

  Bruce.Dien@ars.usda.gov

  (Poster # 4)

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An Integrated Process for Bioconversion of Wheat Straw to Butanol

- Dilute acid pretreated wheat straw is simultaneously saccharified and fermented to butanol efficiently by an anaerobic bacterium without using any detoxification step
- Since the accumulation of butanol is toxic to the fermentative organism, it is successfully removed simultaneously by gas stripping
- The newly developed integrated process (simultaneous saccharification, fermentation and recovery, SSFR) reduces the production cost significantly
- Invention disclosure on file
- Details: Nasib Qureshi, Nasib.Qureshi@ars.usda.gov (Poster # 17)

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Commercialization Opportunity

- Need commercial partners to scale up and demonstrate each technology at pilot scale

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Engineering the Biochemical Conversion for Biofuel Production

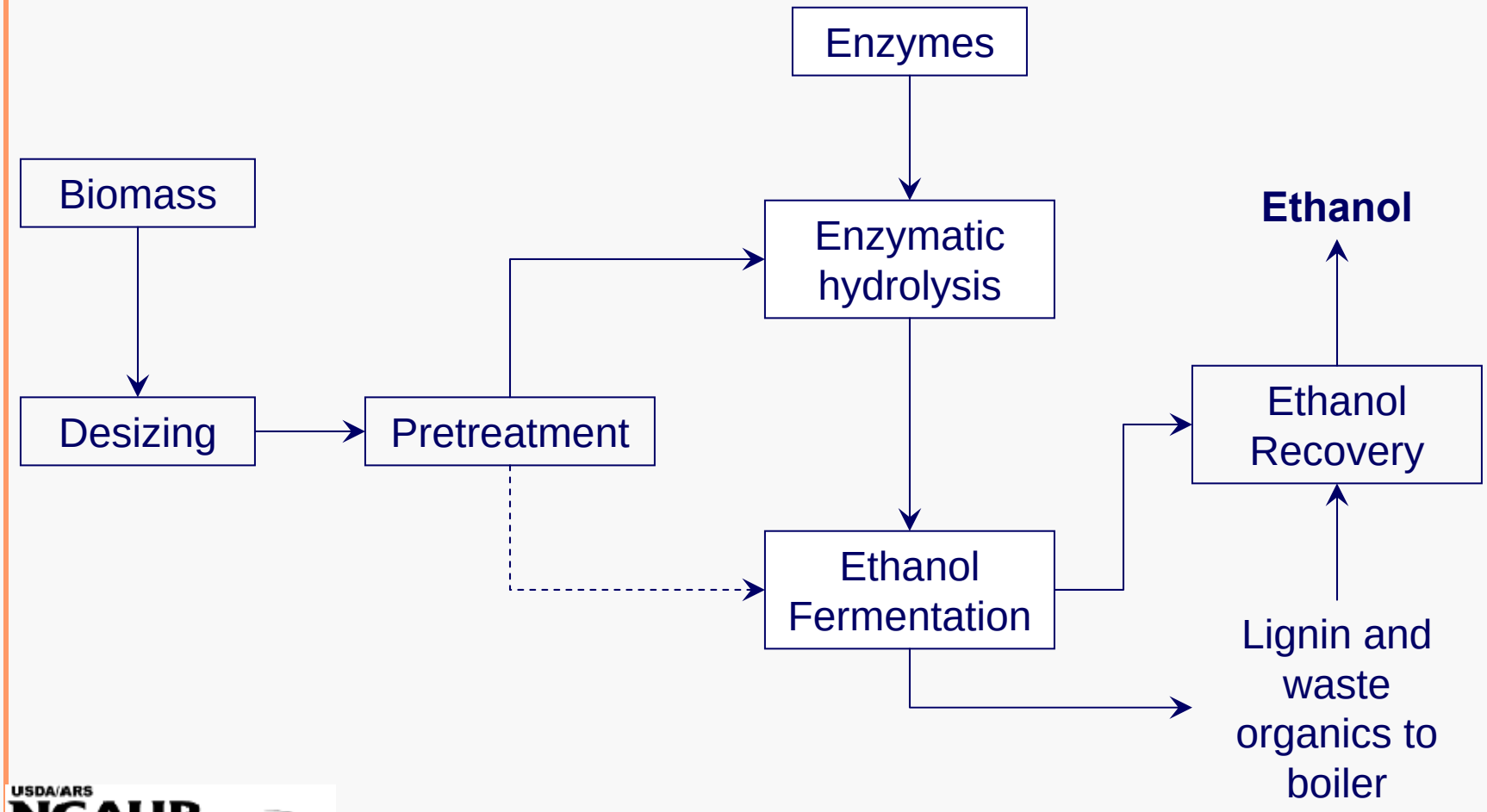
Joe Rich

USDA - ARS - National Center for Agricultural Utilization Research

Engineering biochemical conversion for biofuel production

Joseph O. Rich
Bioproducts & Biocatalysis

Bioethanol Process Overview



Adapted from Merino and Cherry (2007) *Adv Biochem Engin/Biotechnol* 108:95-120

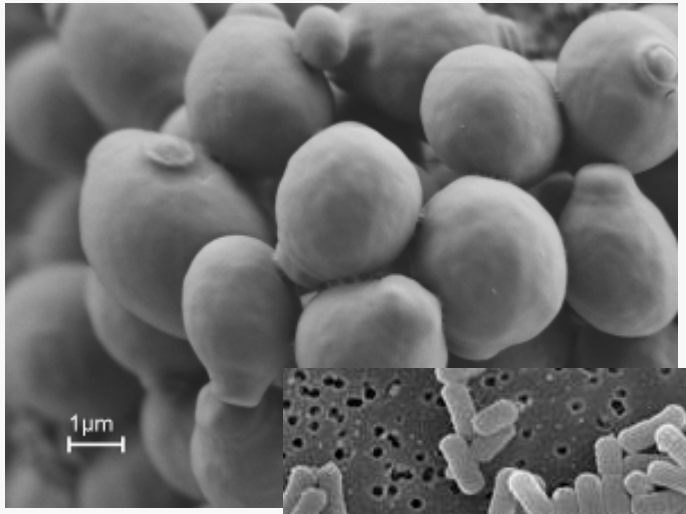
Challenges for Developing the Next Generation Bioconversion System

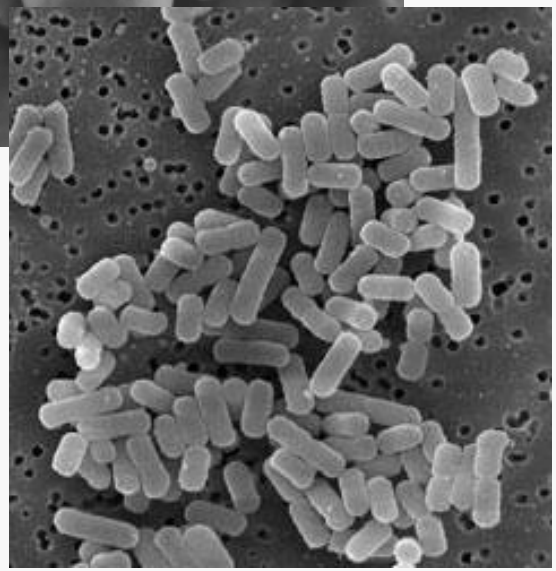
- Utilization of available sugars
- Biofuel productivity and yield
- Cost of hydrolytic enzymes
- Process robustness
  - Tolerance to
    - inhibitors formed during pretreatment
    - ethanol
  - Strain stability

Targets for Engineering Improved Ethanol Production

Known ethanologen

- Introduce and optimize pentose metabolism genes
  - Xylose isomerase
  - Xylulokinase
  - Xylose reductase / xylitol dehydrogenase
  - Xylose tranport mechanism
- Improve productivity





Known pentose utilizer

- Improve ethanol yields
  - Stress tolerance
  - Ethanol production (PET operon, ADH, PDC)

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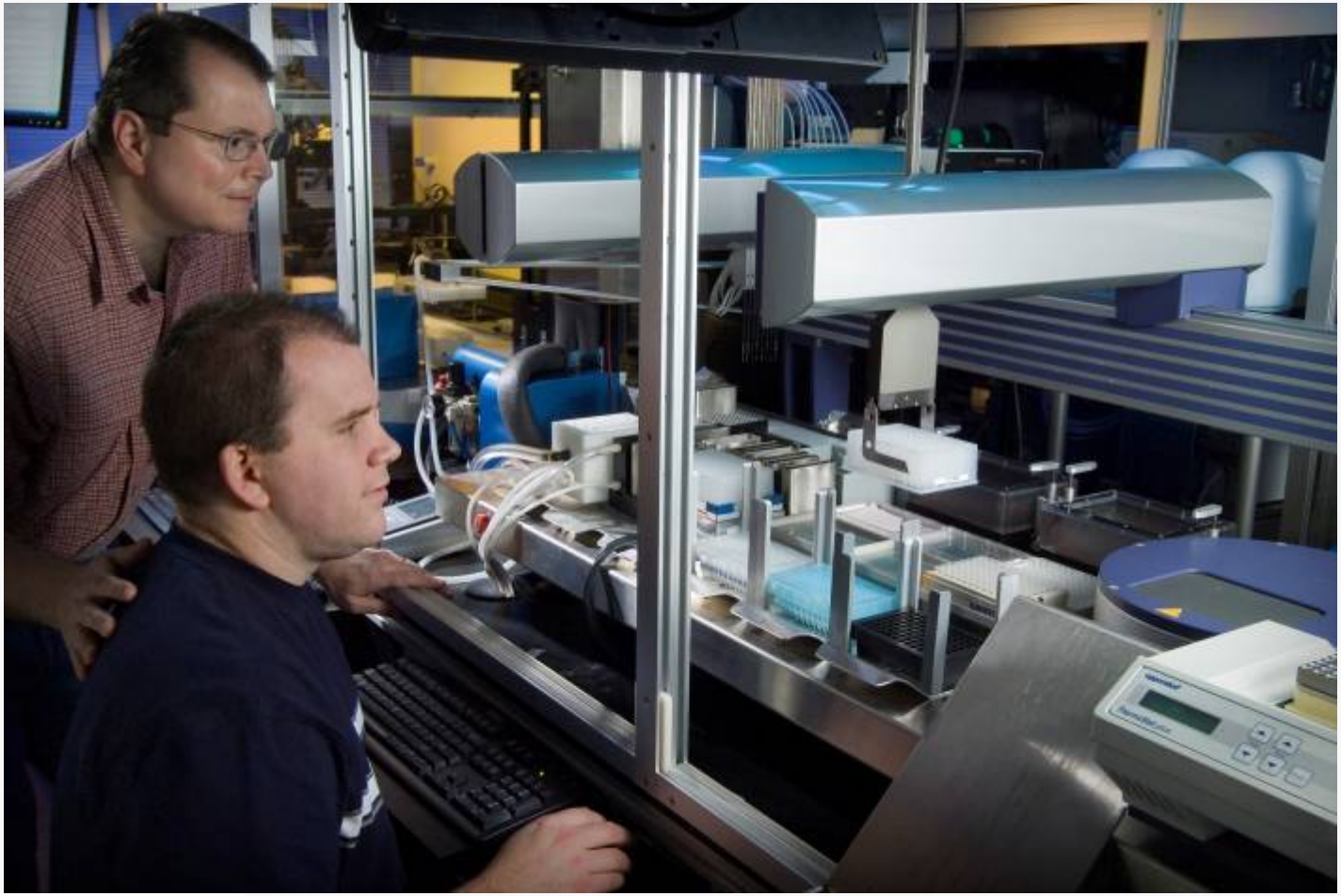
Lactobacillus image from QualitySilage.com (www.qualitysilage.com)

AUTOMATED PROTEOMIC WORKCELL

Technology to Enable High Throughput Molecular Biology



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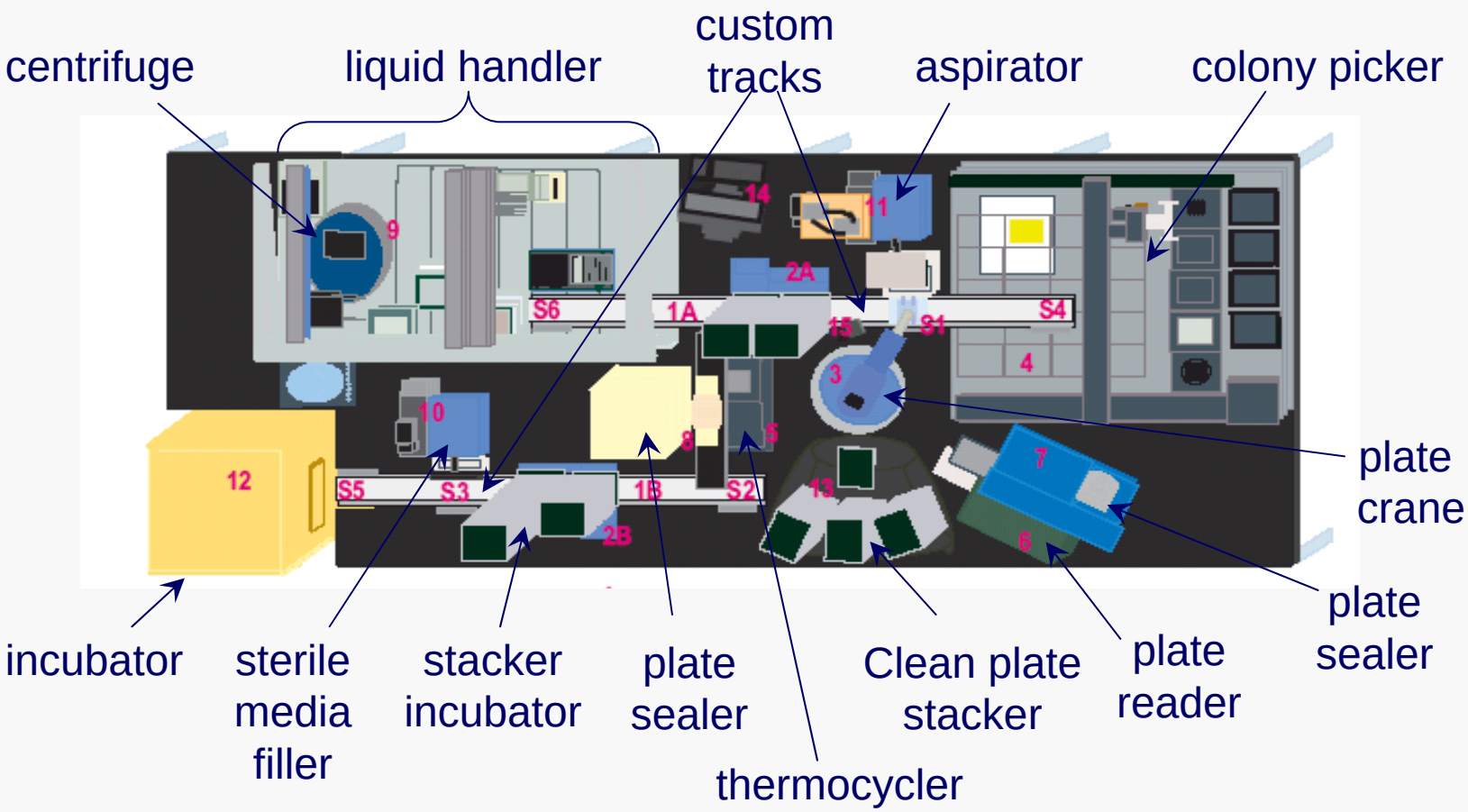
AUTOMATED PROTEOMIC WORKCELL

Integrated Technology Platform for Large Scale Molecular Biology



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Please visit 1106 to see the robot

Movie of Workcell in operation

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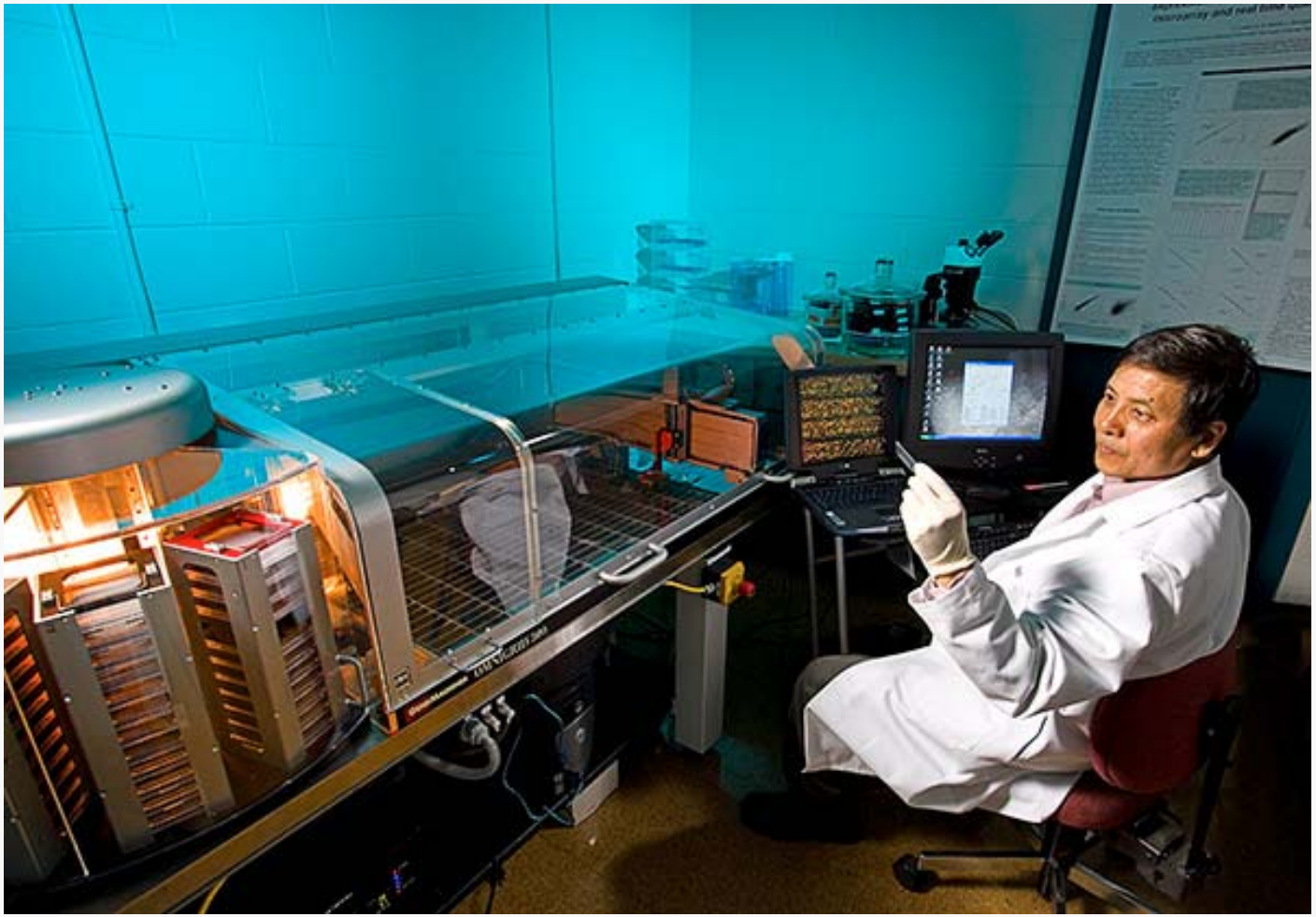
Robust Yeast that Detoxify Fermentation Inhibitors *and* Produce Ethanol



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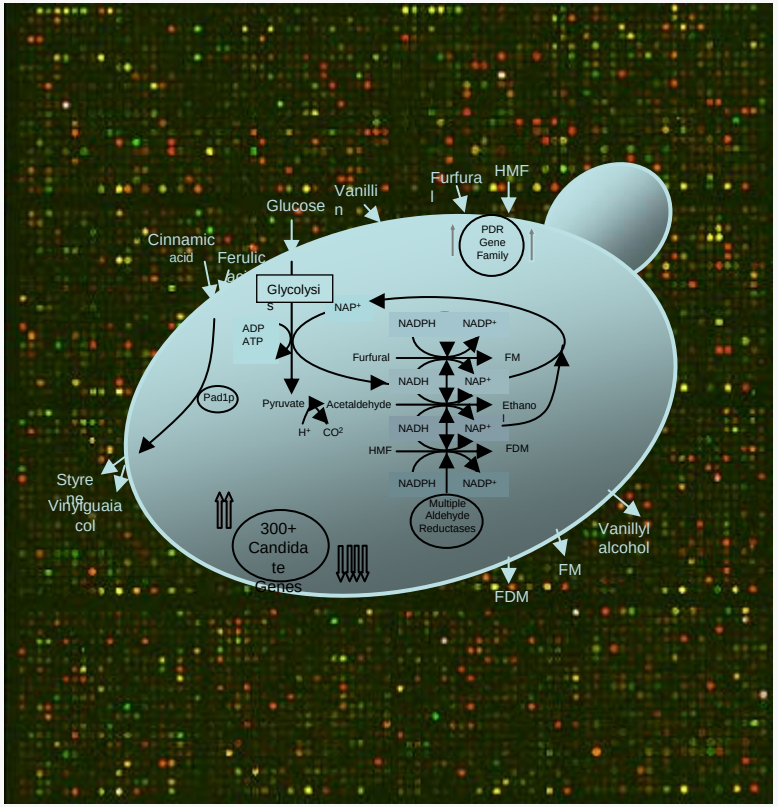
Stress Tolerant Yeast for Producing Ethanol from Biomass

- Inhibitor tolerant *S. cerevisiae* Y-50049 developed via directed adaptation
- Mechanism and novel genes identified for tolerance and *in situ* detoxification of inhibitors generated in common pretreatment processes
- Culture nutrition and process strategies developed for inhibitor tolerance and rapid mixed sugar fermentation to ethanol



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Core Capabilities

- **Microbiology**
  - Manipulation of MT plates
  - Media inoculation
  - Incubation
  - Harvesting cells
- **Molecular Biology**
  - Site-directed and scanning mutagenesis
  - Plasmid preparation
  - DNA sequencing
  - PCR
- **Functional Proteomics**
  - *In vitro* protein expression
  - Biochemical assay of enzyme activity
  - Protein-protein interactions (yeast 2-hybrid analysis)

NCAUR Bioconversion Research

- **Pentose-sugar utilization**
  - xylose transport into yeast – R. Hector (Poster 8)
  - xylose metabolism in yeast – S. Hughes (Poster 9)
- **Stress response of ethanologens**
  - bacteria – S. Liu (Poster 14)
  - yeast – L. Liu (Poster 13)
- **Coproducts from biorefinery**
  - protein and peptide expression – S. Hughes (Poster 9)
- **Novel Organisms for Producing Biofuels**
  - *Escherichia coli* FBR5 – B. Dien (Poster 4)
  - *Lactobacillus buchneri* – S. Liu (Poster 14)
  - *Clostridium beijerinckii* – N. Quershi (Poster 17)
  - *Pichia stipitis* – P. Slininger (Poster 20)

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Acknowledgments

Bioproducts and Biocatalysis

Joseph Rich
Ken Bischoff
Stephen Hughes
Tim Leathers
Siqing Liu

Crop Bioprotection

Patricia Slininger
ZLewis Liu

Fermentation Biotechnology

Michael Cotta
Mike Bowman
Bruce Dien
Doug Jordan
Jeff Mertens
Nancy Nichols
Nasib Qureshi
Badal Saha

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