Bacterial Biocatalysts for Fermentation of Biomass Sugars to Ethanol

K.T. Shanmugam and Lonnie O. Ingram

Dept. of Microbiology and Cell Science
University of Florida
Gainesville, Florida

Public Meeting of the Biomass Research and Development Technical Advisory Committee
Feb. 14, 2007

President Bush
State of the Union Address
Jan 23, 2007

"To reach this goal, we must increase the supply of alternative fuels, by setting a mandatory fuels standard to require *35 billion gallons of renewable and alternative fuels in 2017* -- and that is nearly five times the current target."

"We must continue investing in *new methods of producing ethanol using everything from wood chips to grasses, to agricultural wastes*."

VISION

FOR BIOENERGY AND BIOBASED PRODUCTS IN THE UNITED STATES

Bioeconomy for a Sustainable Future

2006

Biomass Research and Development Technical Advisory committee

BR&Di

Biomass Research and Development Initiative

Vision for Bioenergy and Biobased Products in the United States **Biofuels Goals**						
	2000	**2004**	**2010**	**2015**	**2020**	**2030**
Consumption of Biofuels (Billions Gasoline Gallon Equivalent)	**1.1**	**2.1**	**8.0**	**13**	**23**	**51**

Areas of Focus for R & D

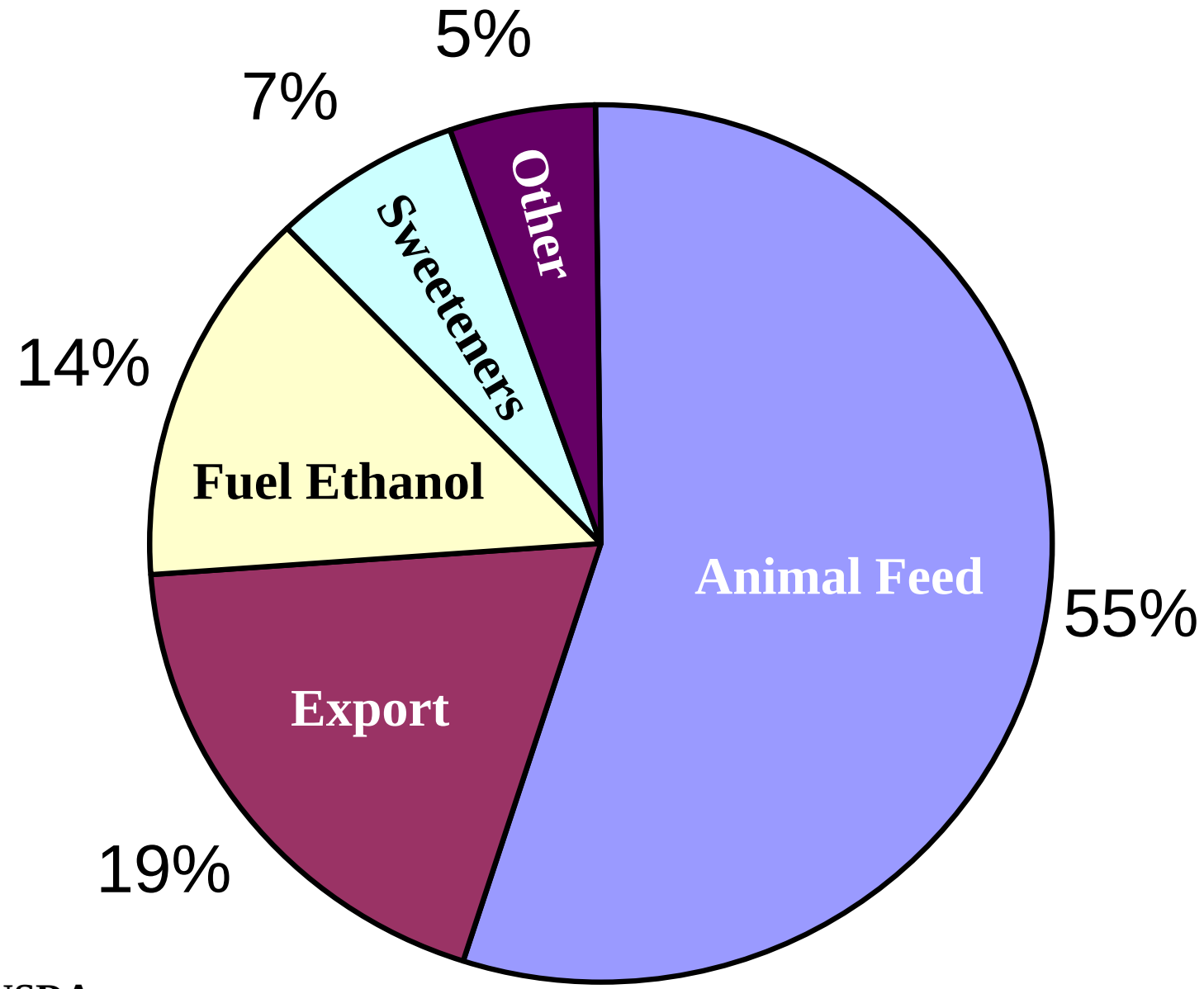
- **Reducing the cost of fermentation**
- **Enabling greater conversion of lignocellulosic biomass**

US Fuel Ethanol Production

Biorefineries (112)	**5.5 B Gallons / Yr**
Under Construction (77 + 7)	**6.2 B Gallons / Yr**
Anticipated Total	**11.7 B Gallons / Yr**

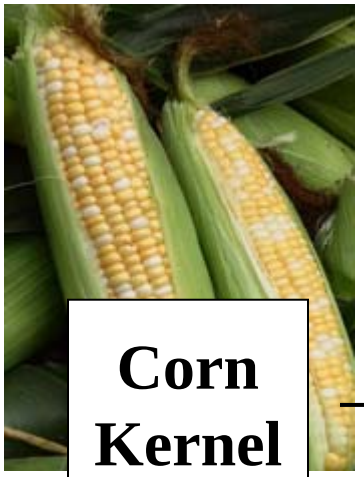
Source: Renewable Fuels Association - Jan. 29, 2007

USES OF CORN – 2005/2006

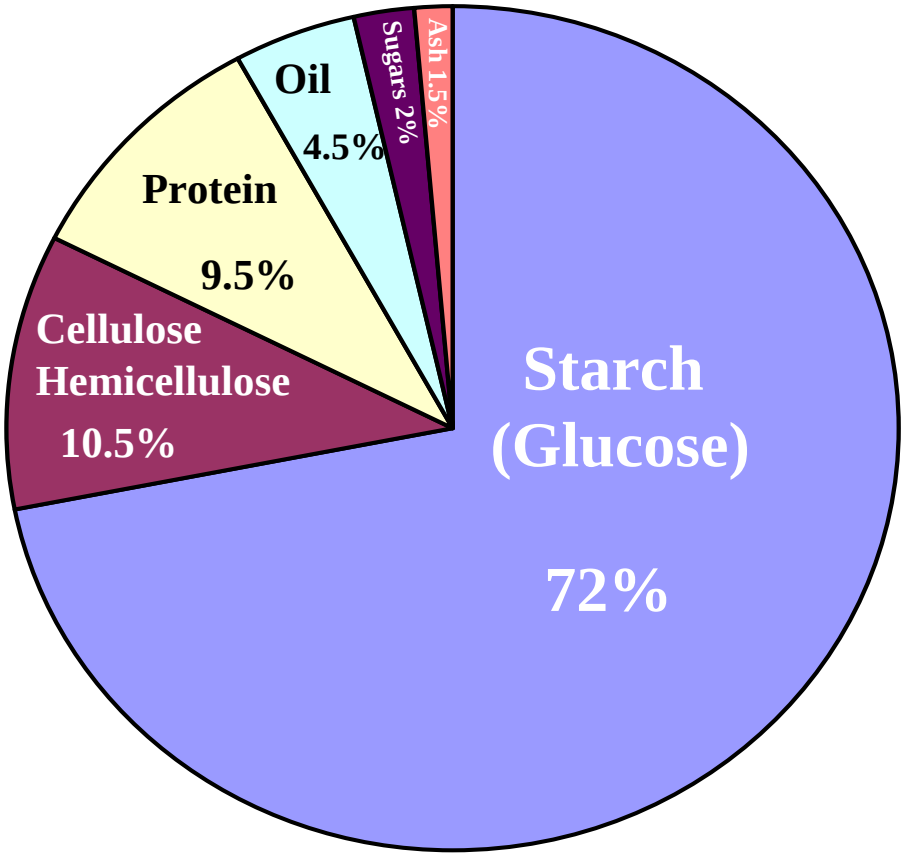


Source: USDA

Corn to Ethanol



Corn Kernel → Corn Meal —Enzymes→ Glucose → Fermentation (Yeast) → Purification → Ethanol



US Corn Production (2004)

US Total	**11.8 Billion Bushels**
Iowa	**2.24 BB**
Illinois	**2.09 BB**
Nebraska	**1.32 BB**
Minnesota	**1.12 BB**
Indiana	**0.93 BB**
Florida	**2.88 MB**

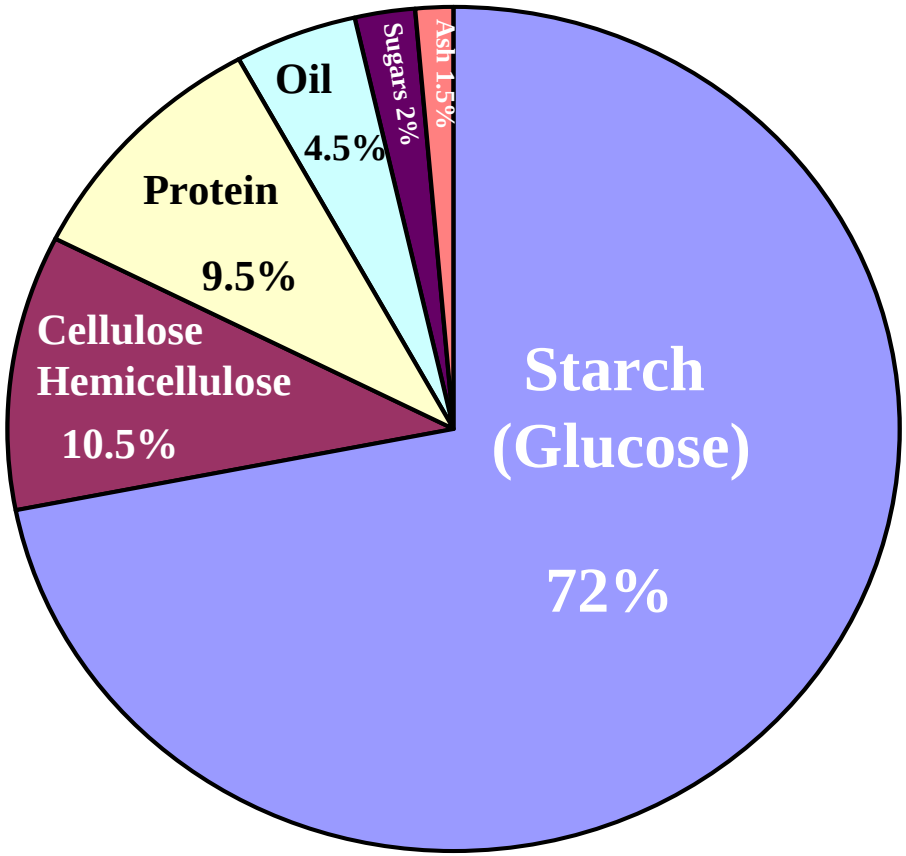
Source: USDA-ARS

Other Sources of Sugars

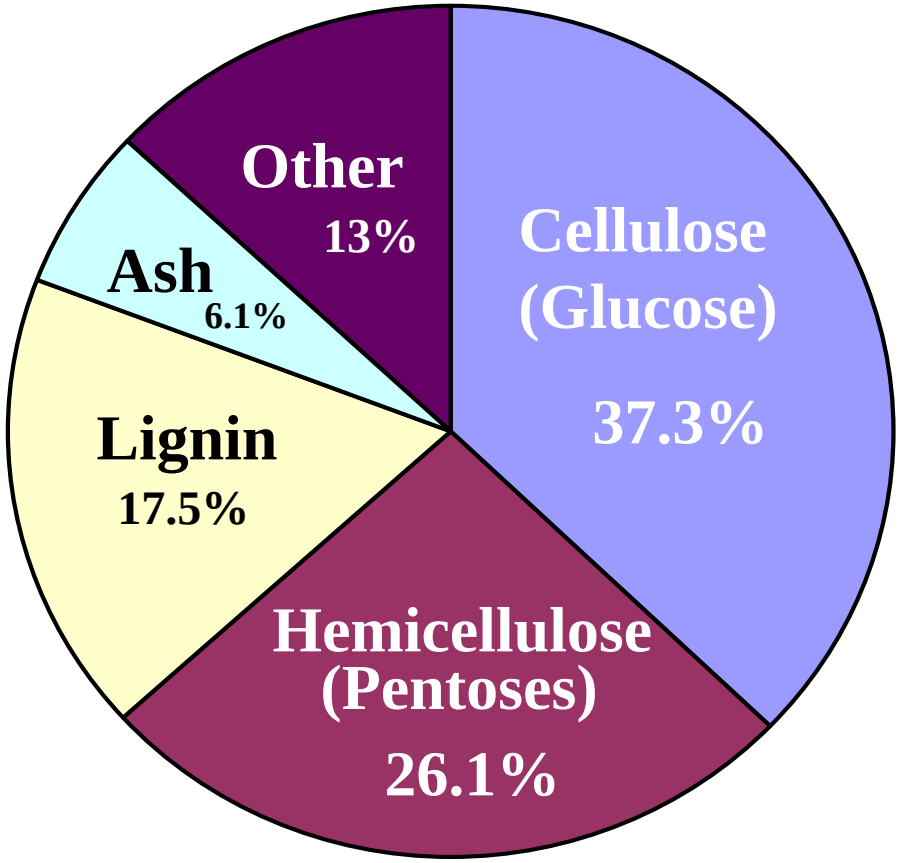
- **Crop Residues**
- **Energy Crops**
- **Forest Products**

Other Sources of Sugars

Corn Kernel



Corn Stover



Source: NREL

Sugar cane Bagasse – Biomass Residues

(South of Lake Okeechobee, Florida)

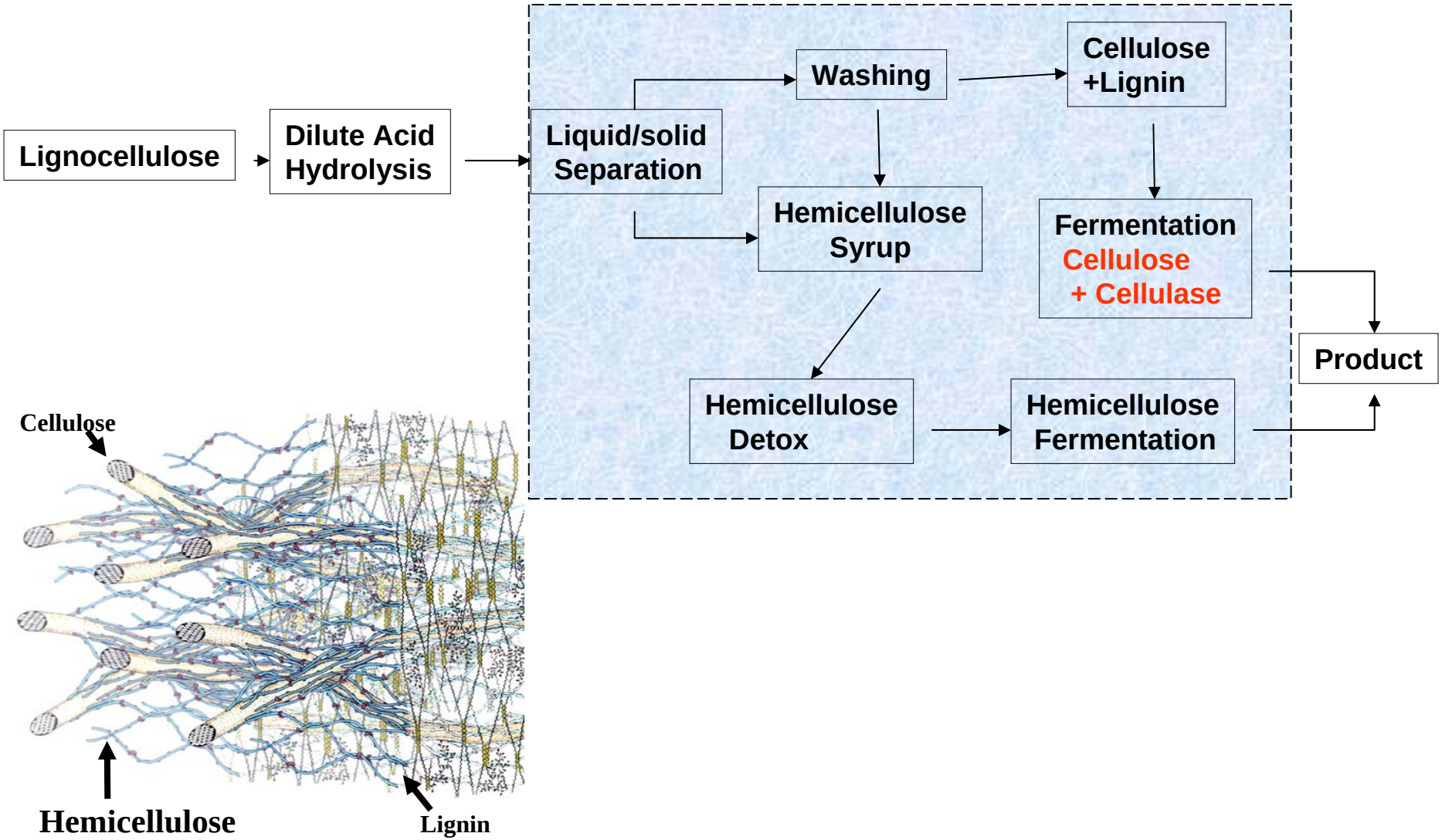
Energy Crop

Switch grass bales (1200 lb) from 5 year old field – Northeast South Dakota
Source: DOE Biofuels Joint Roadmap, June 2006

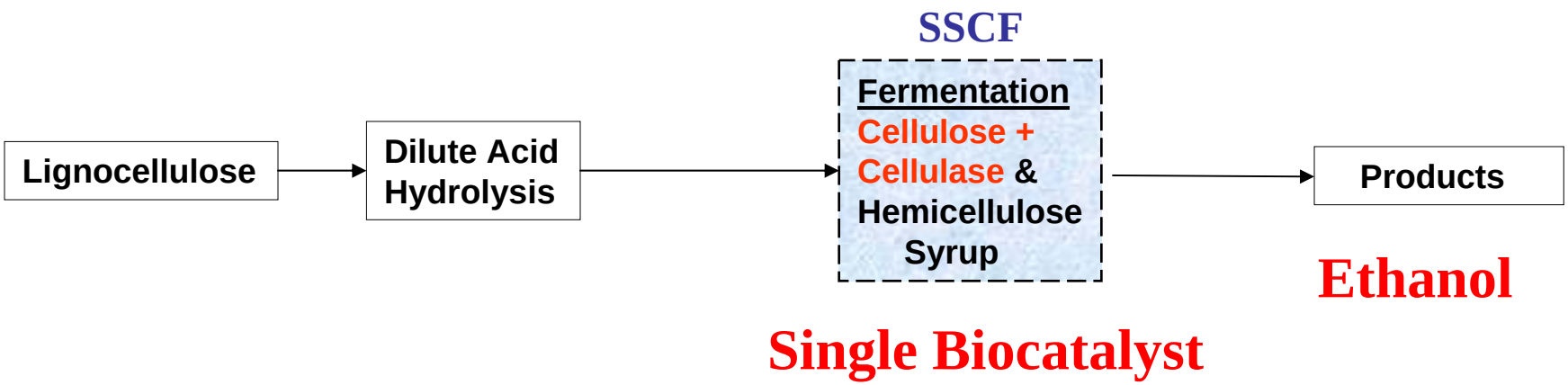
Hard Woods and Soft Woods

A rich tapestry of hardwood and softwood trees.
The old mill pond at the Aldridge Sawmill site, East Texas terrain.
Photo courtesy of USDA Forest Service

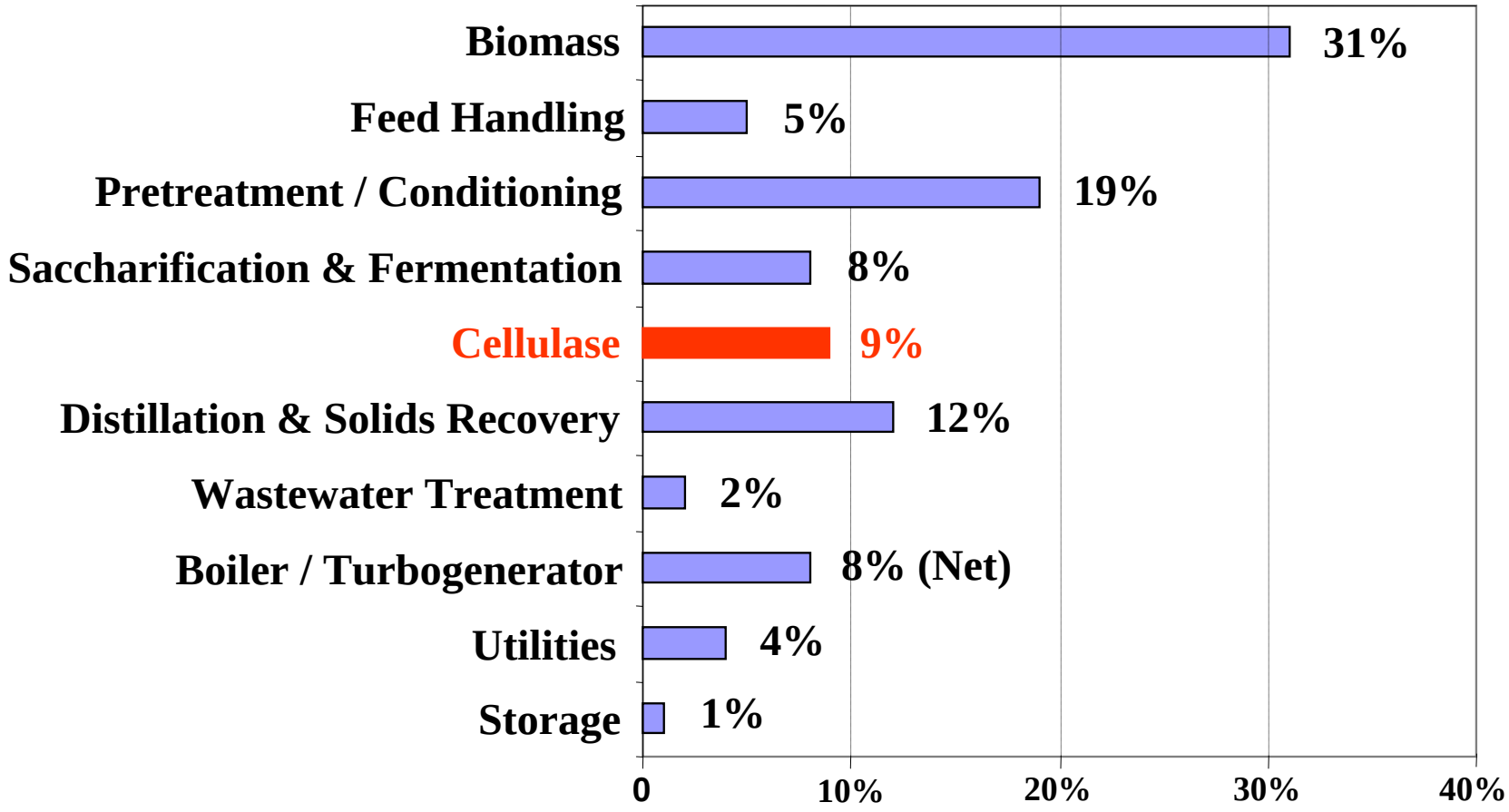
Conversion of Biomass to Fuel Ethanol & Chemicals



Process Simplification with Advanced Biocatalysts

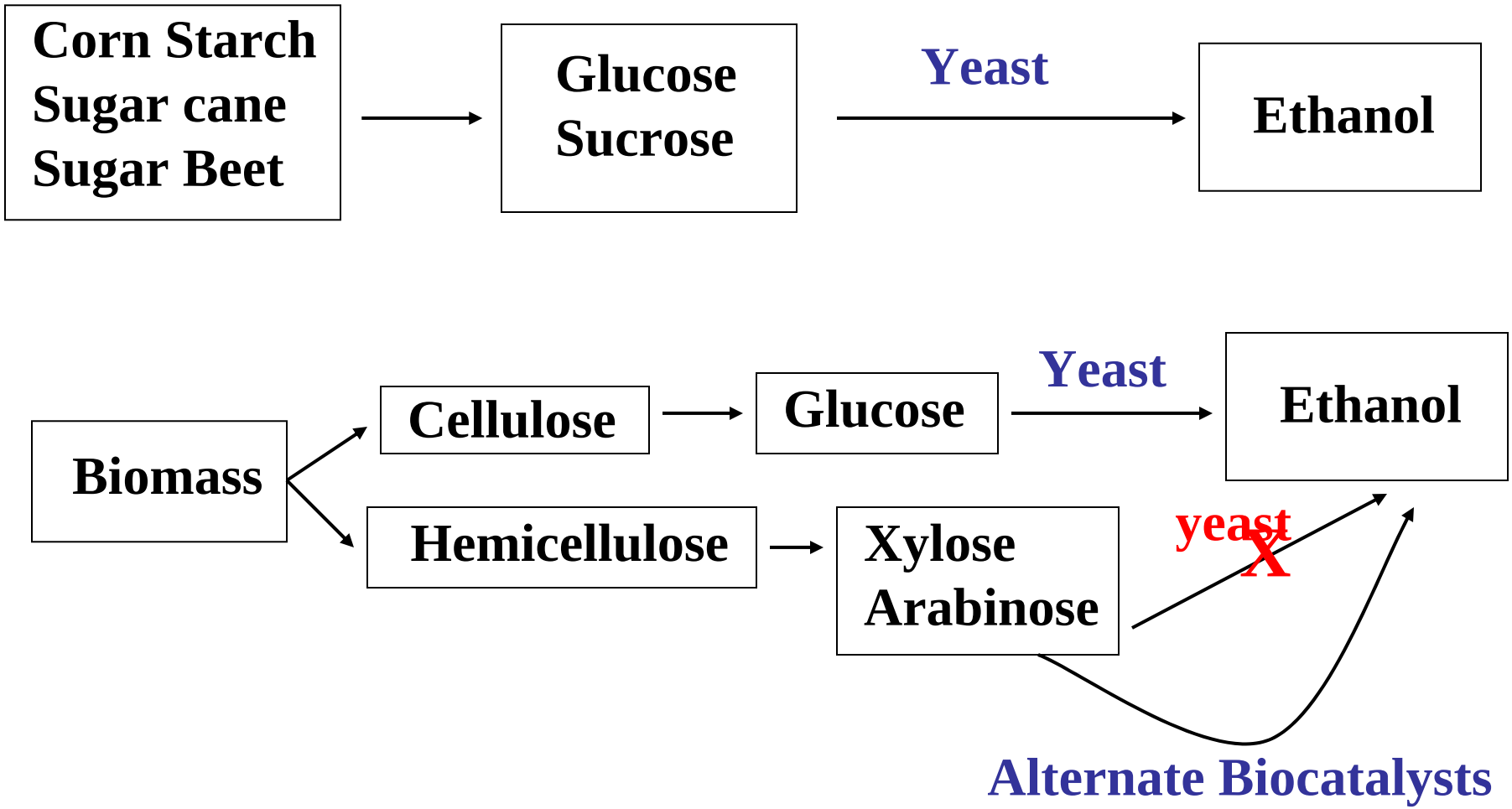


Cost Contribution from Each Process Area
(% of Ethanol Selling Price)



Source: NREL/TP-510-32438; June 2002

Yeast Fermentation Characteristics



Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda

A Research Roadmap Resulting from the Biomass to Biofuels Workshop Sponsored by the U.S. Department of Energy

December 7–9, 2005, Rockville, Maryland

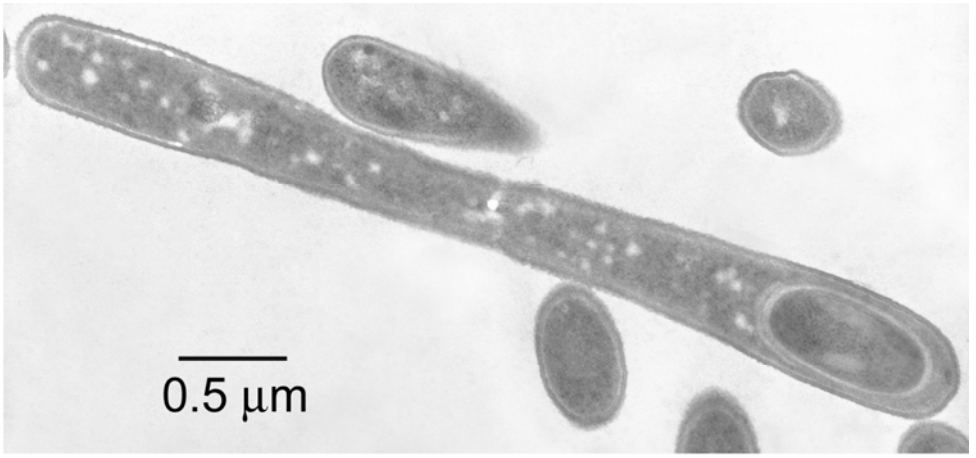
DOE/SC-0095, Publication Date: June 2006
Office of Science, Office of Biological and Environmental Research, Genomics:GTL Program
Office of Energy Efficiency and Renewable Energy, Office of the Biomass Program

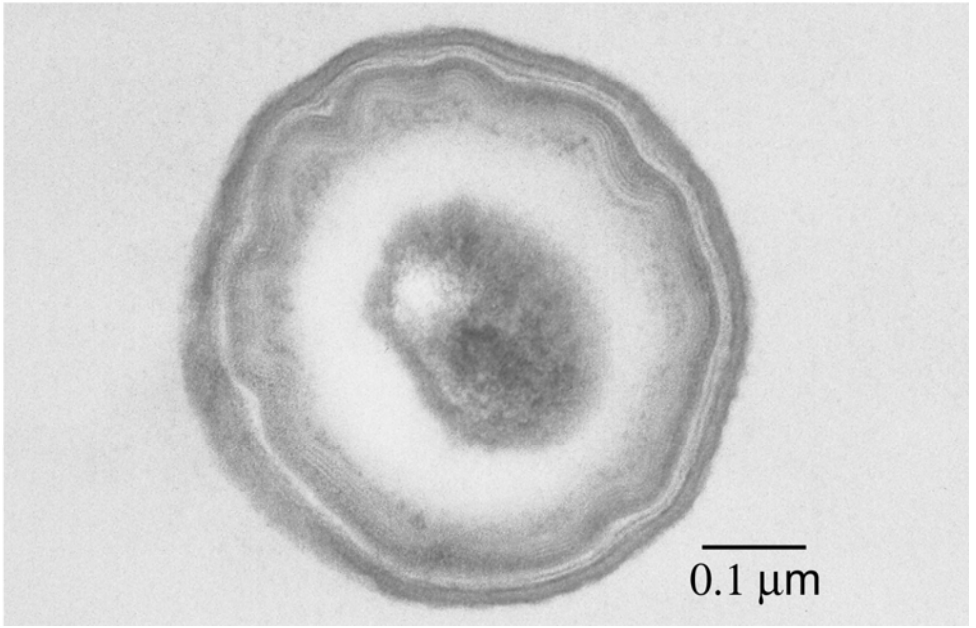
From DOE Research Roadmap, 2006

Technical Milestones
Within 5 years

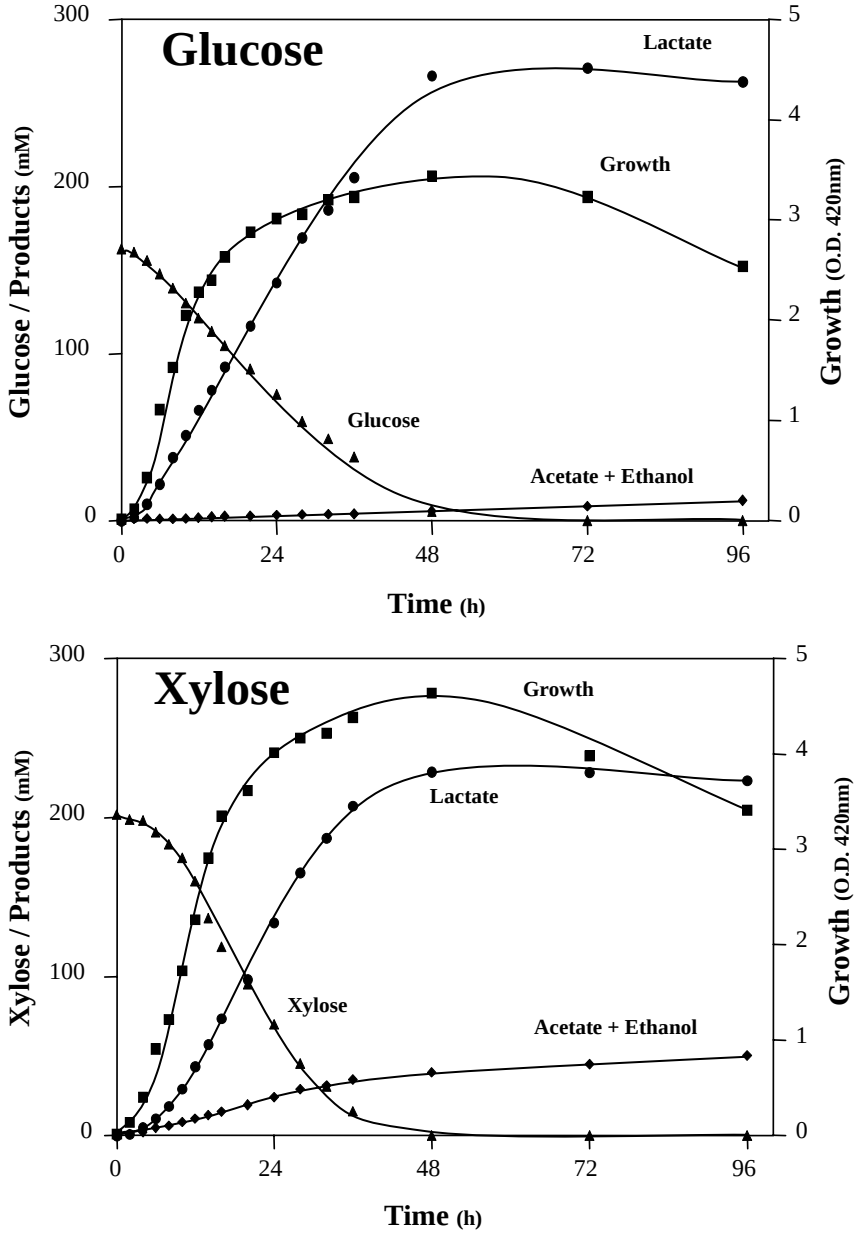
Candidate microbes such as *thermophilic ethanologens* compatible with desired cellulase enzyme optima. This allows process simplification to single-vessel fermentation with *efficient use of all biomass-derived sugars*

Bacillus coagulans, a potential Second Generation Biocatalyst



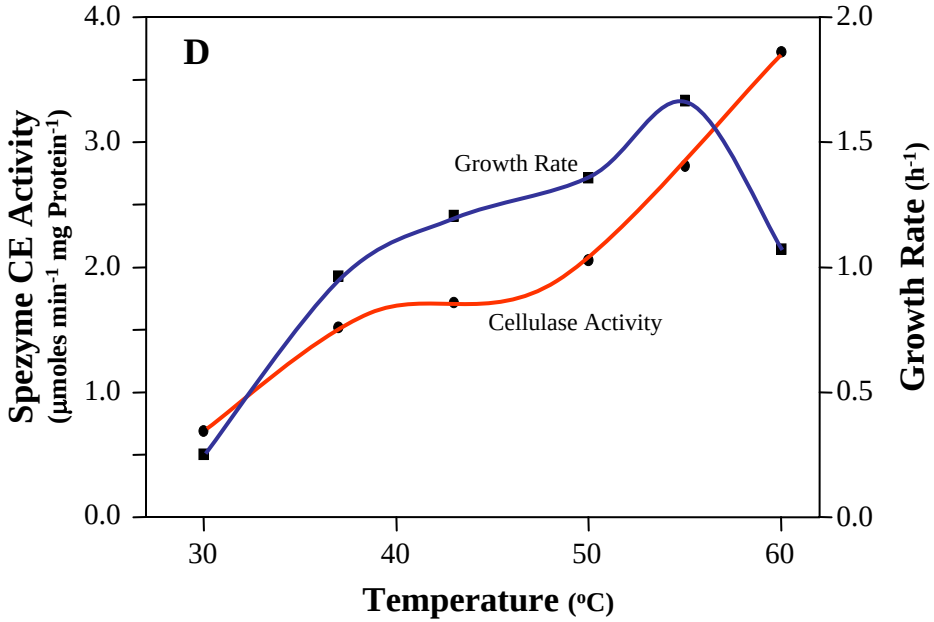


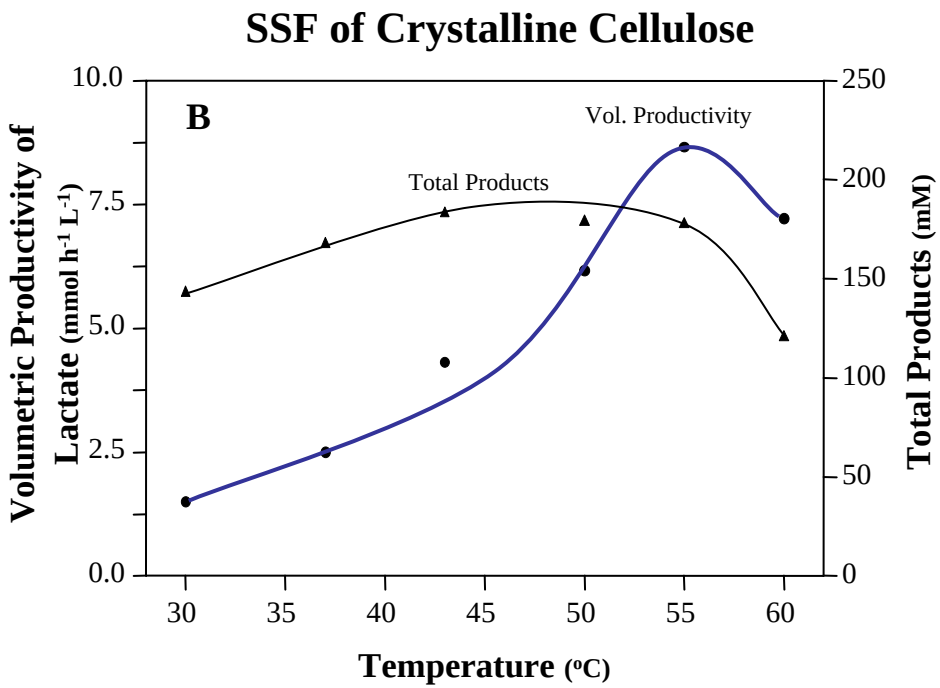
B. coagulans Ferments glucose and Xylose



Growth and Fermentation of *B. coagulans* matches that of Fungal Cellulase Activity

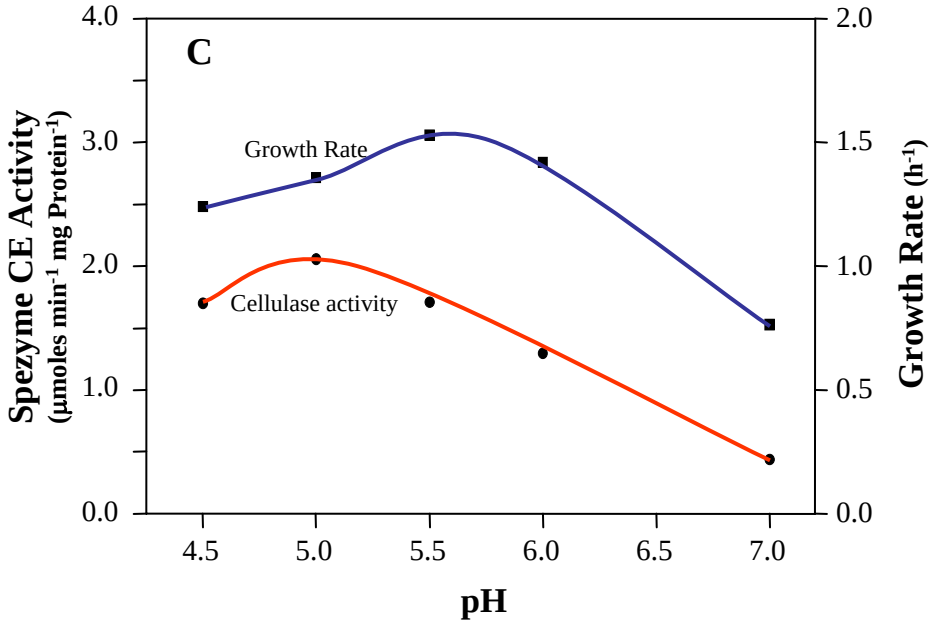
Effect of Temperature

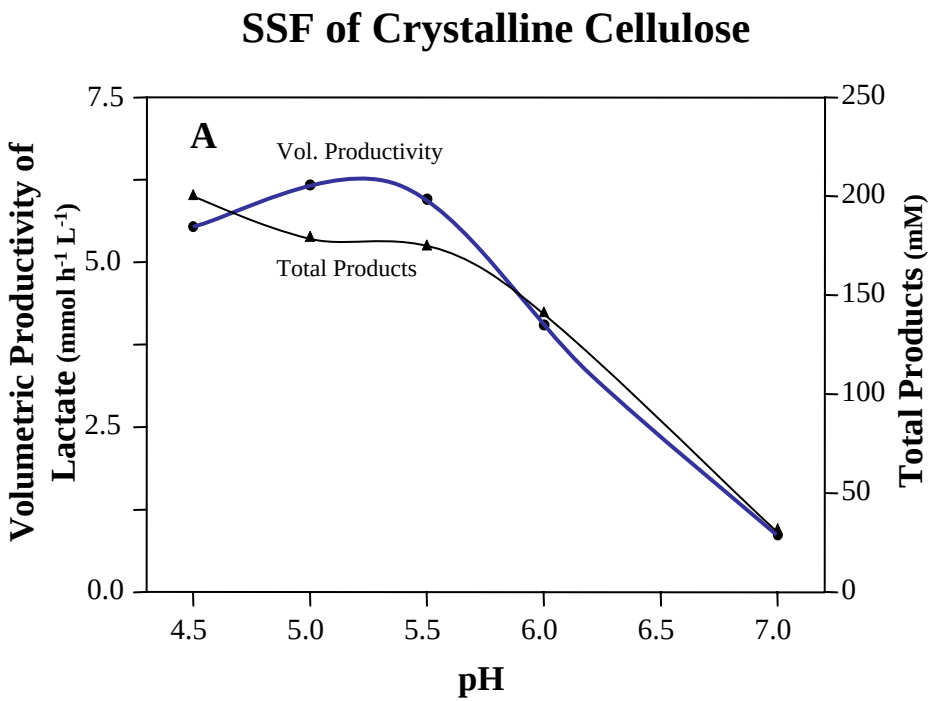




Growth and Fermentation of *B. coagulans* matches that of Fungal Cellulase Activity

Effect of pH

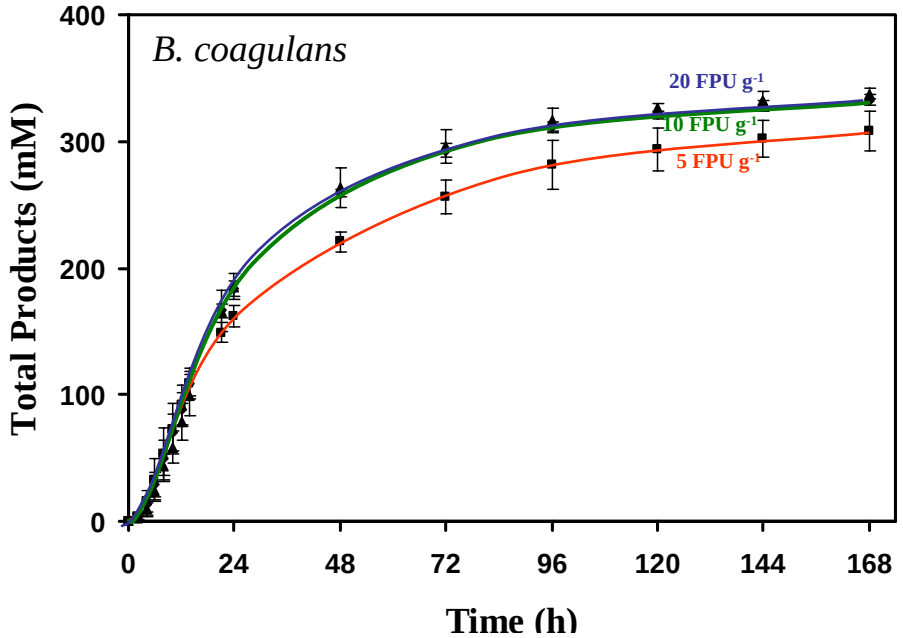




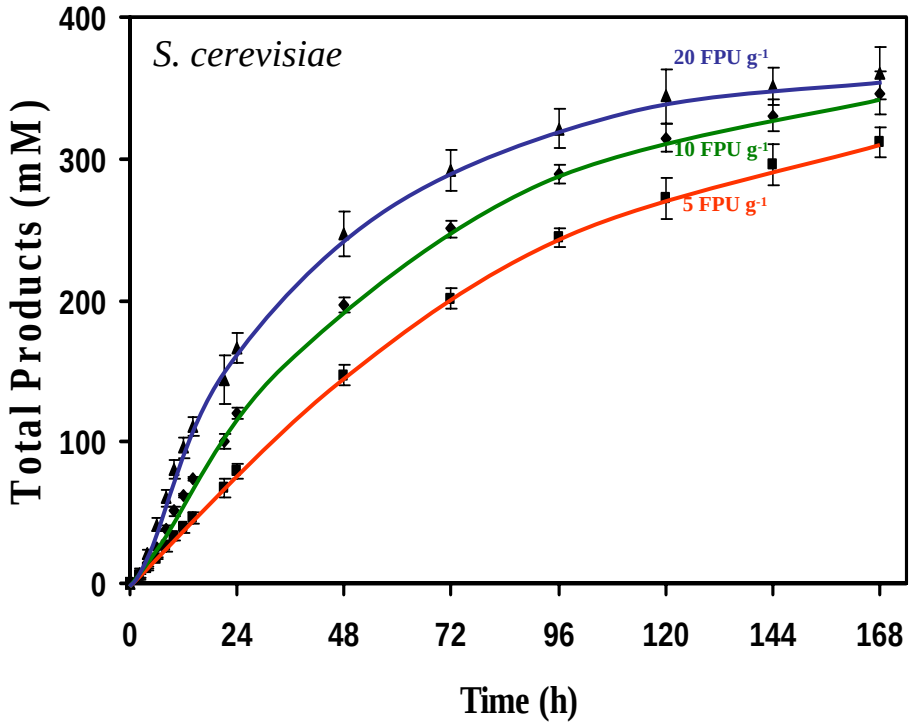
From DOE Research Roadmap, 2006

Technical Milestones
Within 15 years

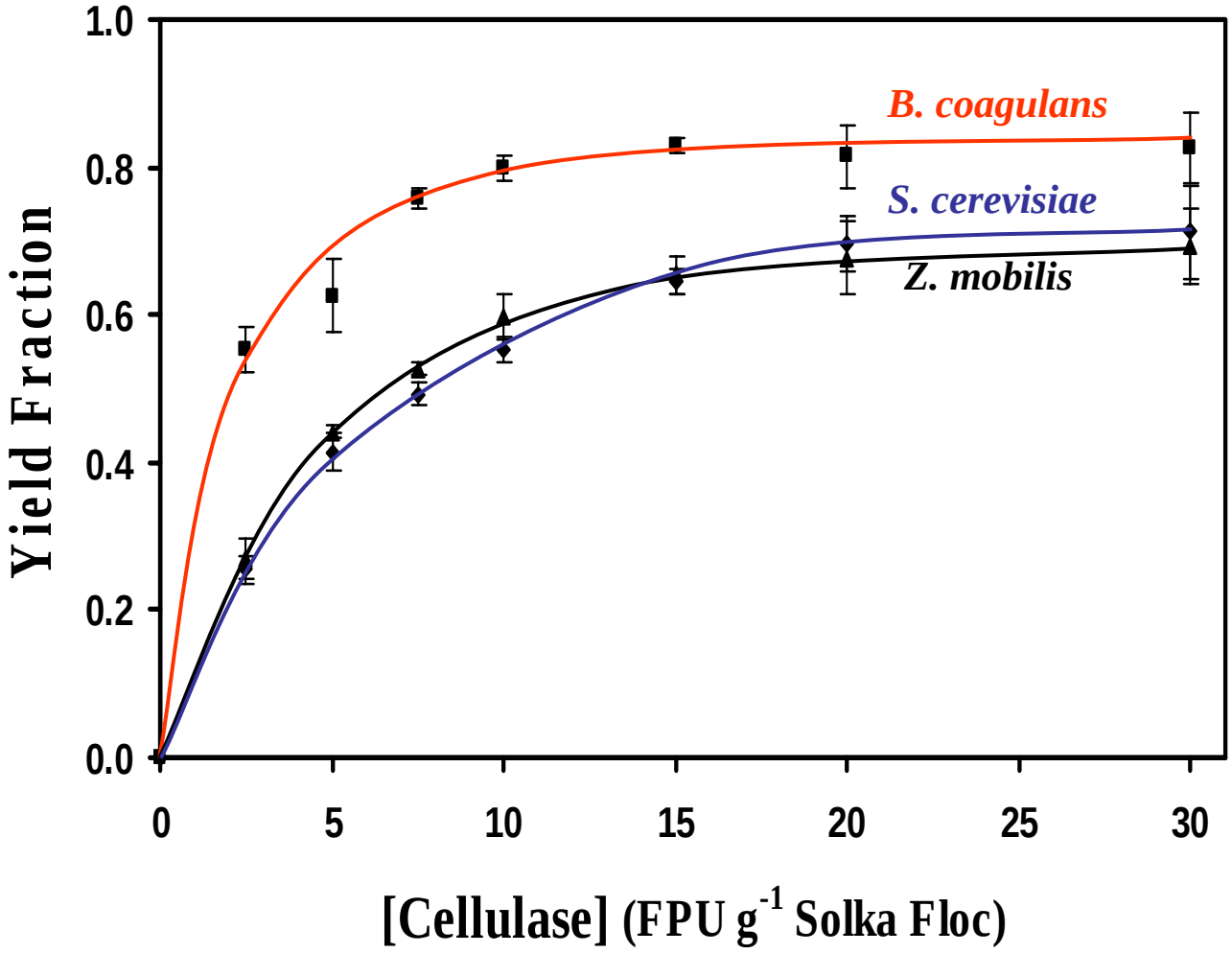
Thermophillic microbes demonstrated at scale to enable simultaneous saccharification and fermentation.



SSF of Cellulose

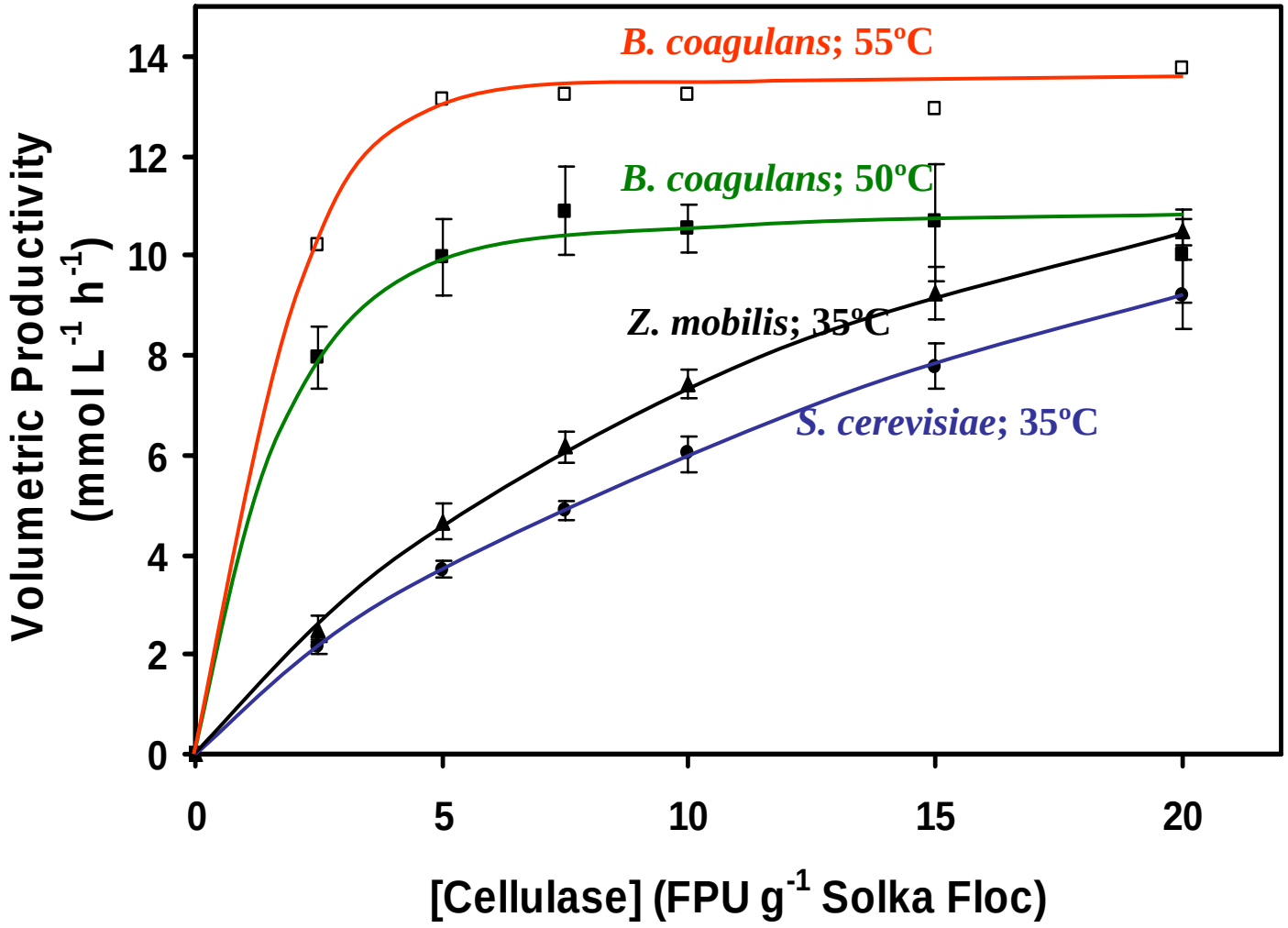


B. coagulans produces more product in shorter time

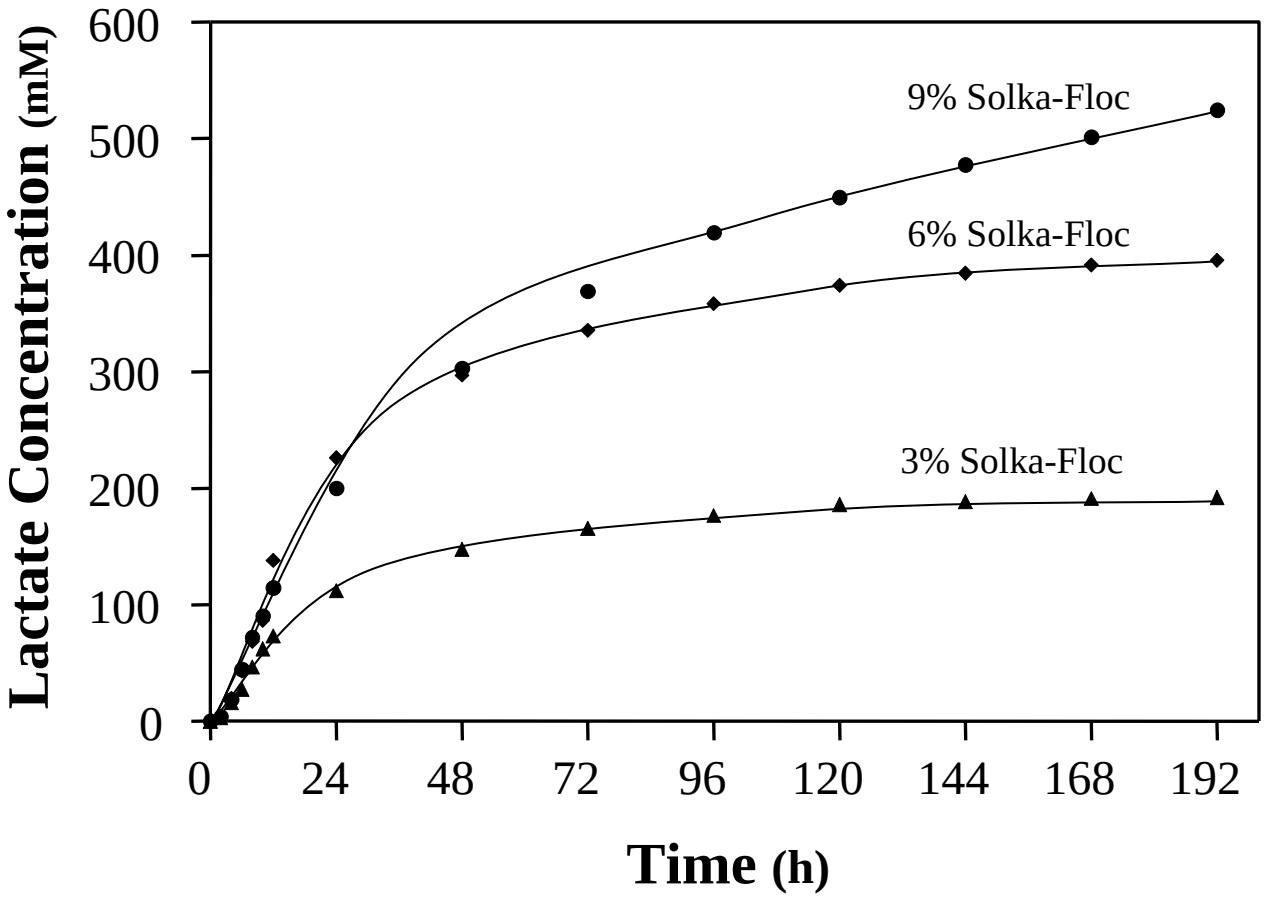


Fermentation time: 48 h

SSF with *B. coagulans* requires less enzyme than yeast



SSF of Cellulose with cellulase and *B. coagulans*



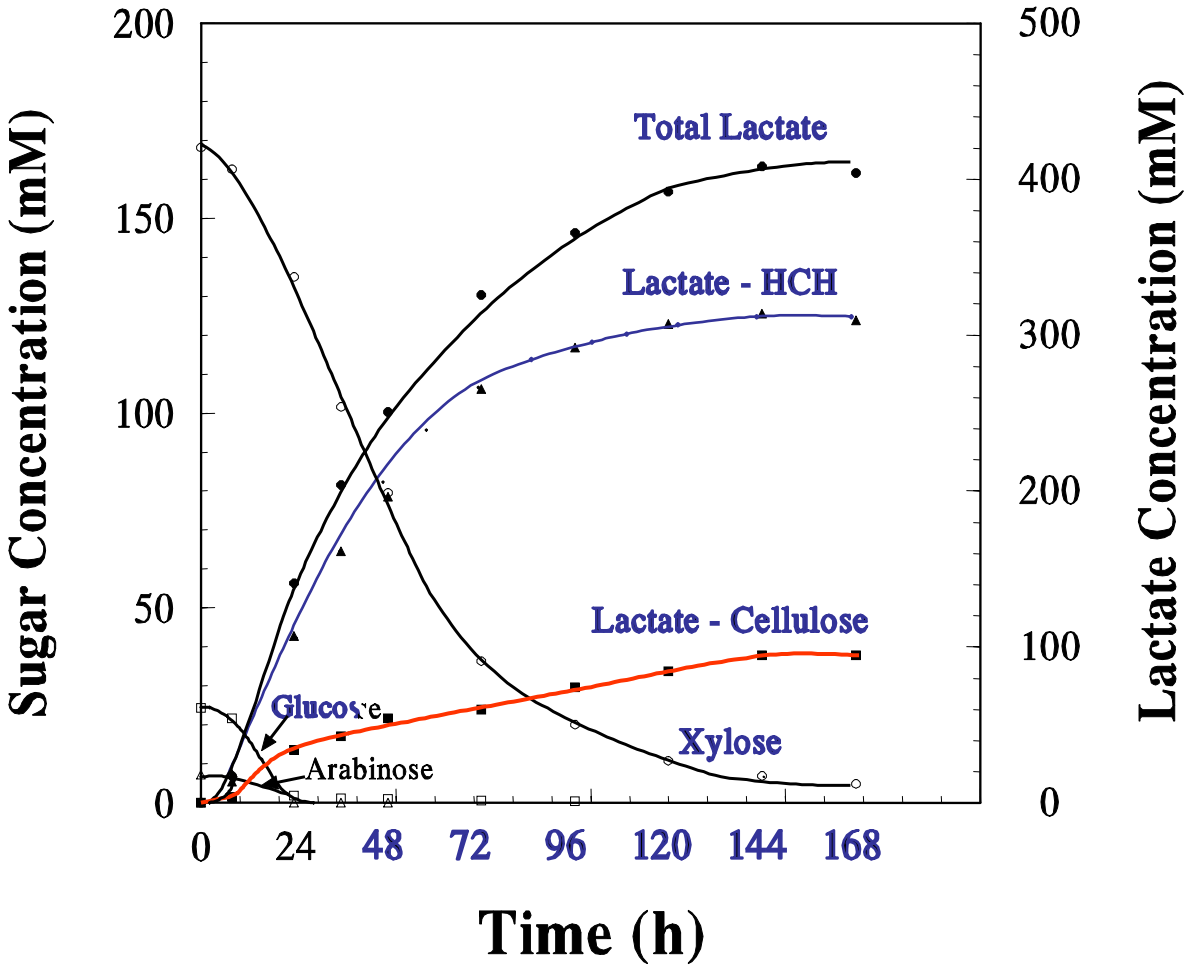
Genencor Spezyme CE, 10 FPU/ g cellulose

From DOE Research Roadmap, 2006

Technical Milestones
Within 15 years

Simultaneous saccharification and cofermentation (SSCF), in which hydrolysis is integrated with fermentation of both hexose and pentose sugars but with cellulase produced in a separate step. For example, development of thermophilic ethanol- producing organisms for use in SSCF could allow the consolidated process to run at higher temperatures, thus realizing significant savings by reducing cellulase requirements.

SSCF of Sugarcane Bagasse HCH with Cellulose by *B. coagulans*

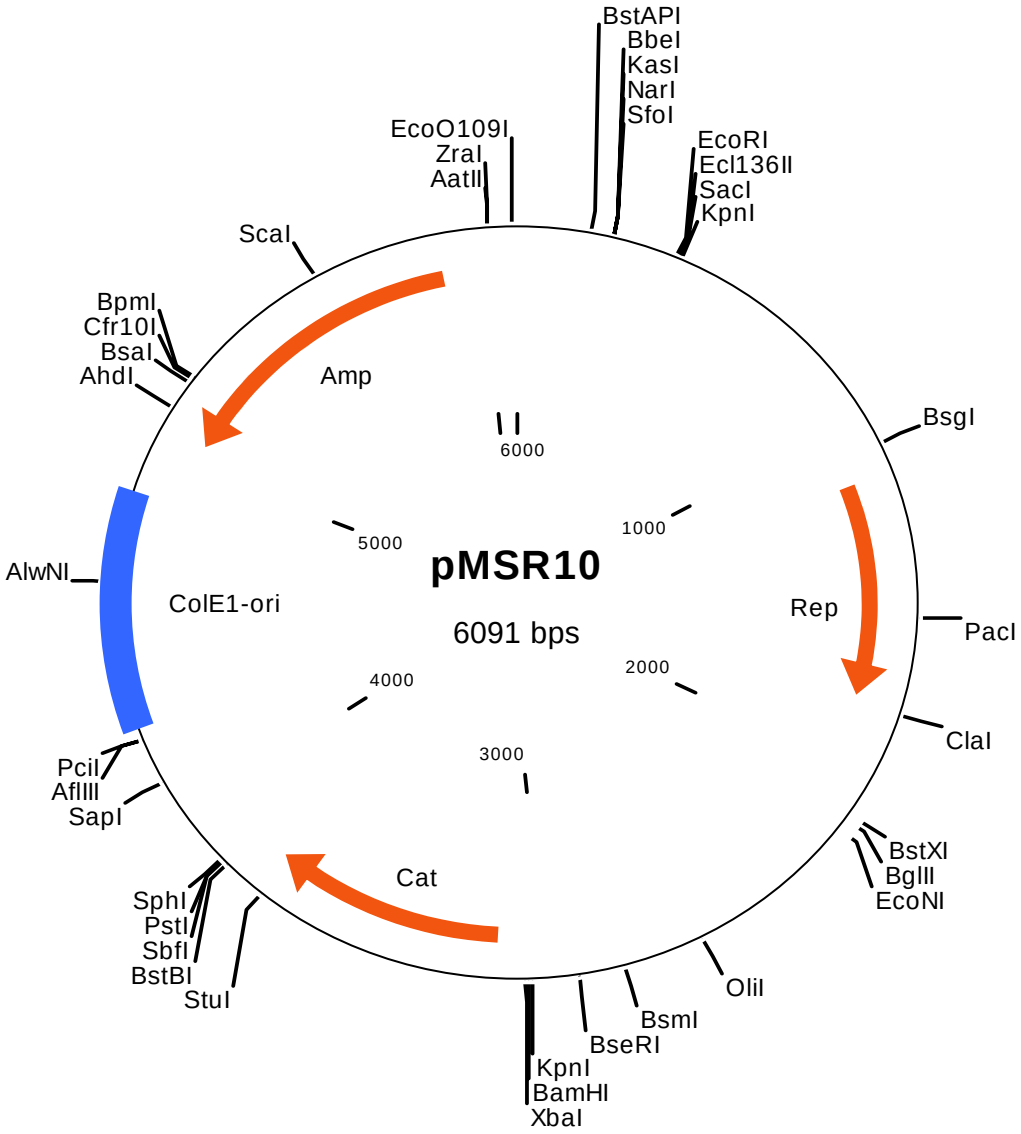


Metabolic Engineering of *B. coagulans* for Ethanol Production

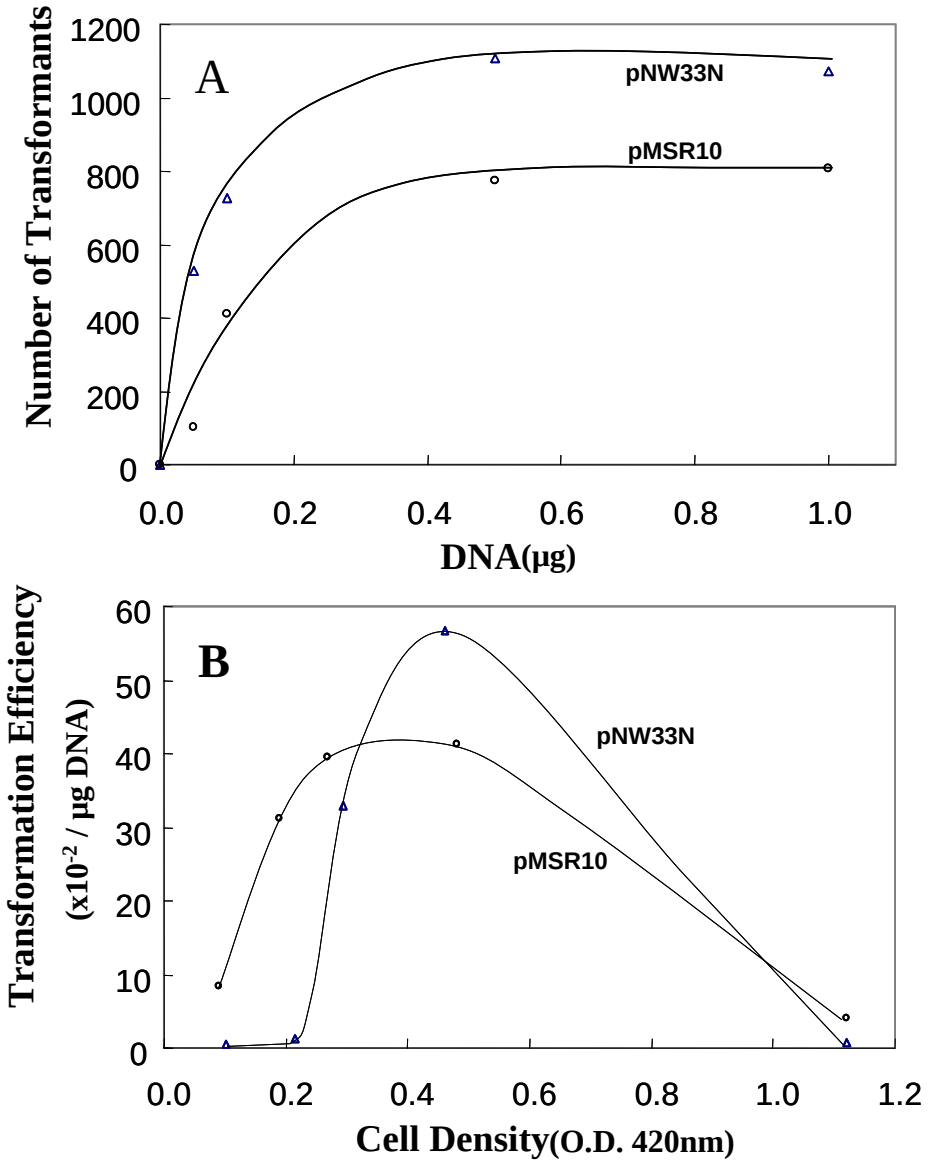
Current Research

1. Vector Plasmids
2. DNA Transfer
3. Source of Pyruvate decarboxylase
4. Source of Alcohol Dehydrogenase
5. Alternate Pathways for Ethanol Production

Thermophilic *B.coagulans* / *E. coli* Shuttle Vector

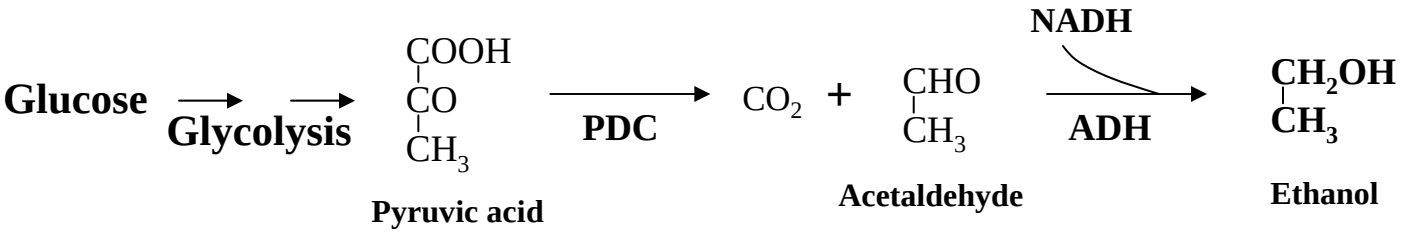


DNA Transfer into *B. coagulans*

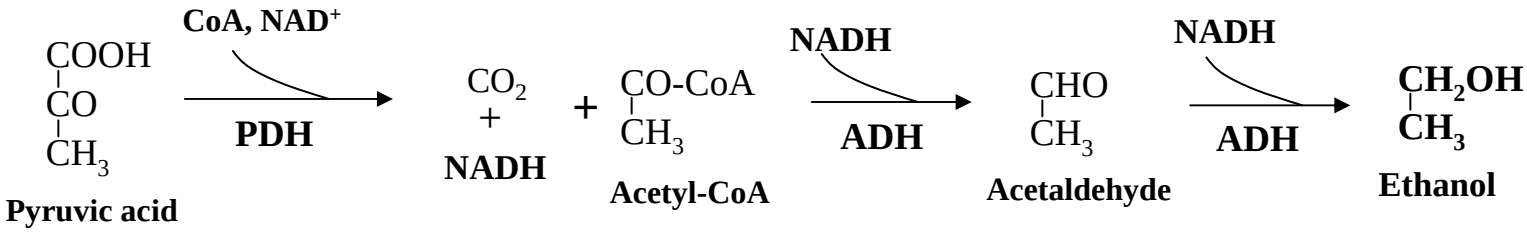


Pathways for Ethanol Production

Ethanologenic Organisms (yeast, *Zymomonas mobilis*, ethanologenic *E. coli*)



A Novel Pathway for Ethanol Production Developed in *E. coli*



Sources of Pyruvate Decarboxylase Gene

- ***Zymomonas mobilis***
- ***Acetobacter pasteurianus***
- ***Zymobacter palmae***
- ***Sarcina ventriculi***
- **Yeast**

pdc* genes from these organisms are available for metabolic engineering of *B. coagulans

Genome Sequence of *B. coagulans*

Incomplete Draft Sequence (DOE-JGI)

~ 2.9 x 10^6 bp
2,675 Putative ORFs
G+C % - 46.2

~600 ORFs unique to the organism
~100 ORFs shared with Lactobacillus
~2000 ORFs shared with *Bacillus subtilis*

Seven ORFs encoding Alcohol Dehydrogenase like Enzymes

Bacillus coagulans, a Second Generation Biocatalyst for Biomass to Ethanol Fermentation

- **Growth and Fermentation temperature matches that of fungal cellulases**
- **Requires less fungal cellulases for SSF of cellulose compared to yeast**
- **All the sugars in biomass are rapidly fermented**
- **Effective SSCF of hemicellulose hydrolysate (overlimed) and cellulose**
- **Gene transfer system has been established**
- **Genome sequence is available for metabolic engineering**

Research Support

US Department of Energy

State of Florida / University of Florida
Agricultural Experiment Station