USDA - ARS - National Center for Agricultural Utilization Research

Bioenergy Research at NCAUR

Michael A. Cotta
Acting Director


National Center for
Agricultural Utilization
Research
MIDWEST AREA
AGRICULTURAL RESEARCH SERVICE
U.S. Department of Agriculture
USDA

NCUAR

- 270,000 sq. ft.
- 270 FTE research staff
- 100 Ph.D. scientists
- 9 research units
- 36 CRIS projects
- $30M budget
- 140+ patents since 1980

Bioenergy Research at NCAUR (NP307)

- ***Fermentation Biotechnology Research*** (FBT)
- ***Bioproducts and Biocatalysis Research*** (BBC)
- ***Crop Bioprotection Research*** (CBP)
- Cereal Products and Food Science Research
- ***Food and Industrial Oil Research (FIO)***
- Microbial Genomics and Bioprocessing Research
- Mycotoxin Research
- New Crops and Processing Research
- Plant Polymer Research

Bioethanol, Biodiesel, and Hydrogen

Biodiesel Research at NCAUR

- Bioproducts and Biocatalysis Research
- Cereal Products and Food Science Research
- Crop Bioprotection Research
- Fermentation Biotechnology Research
- ***Food and Industrial Oil Research***
- Microbial Genomics and Bioprocessing Research
- Mycotoxin Research
- New Crops and Processing Research
- Plant Polymer Research

Research Leader: Dr. Sevim Erhan

Biodiesel is a fuel alternative which is technically fully competitive with petrodiesel

- Cost-competitive with petrodiesel
- Environmental benefits
- Renewable, domestic resource
- Low-level blends, some use in specialty markets
- Low-temperature and combustion properties require more research

Objective

Improve the fuel properties and combustion characteristics of vegetable oils (emphasizing soybean oil) and their derivatives as alternative fuels, extenders, and additives in the operation of compression-ignition (diesel) engines for on-road and off-road applications.

Research Focus

- Improvement of low temperature flow properties
- Reduction of harmful exhaust emissions (NOx)
- Improved analytical methods for production and fuel quality assessment
- Storage stability
- Lubricity
- Glycerol utilization

Exhaust emissions when using diesel fuel:

- CO_2, CO, NO_x
- VOCs: hydrocarbons, oxygenated species
- Particulates
- Polyaromatic hydrocarbons (PAHs)

Most emissions reduced with biodiesel

- Exception NO_x
- Cetane enhancement reduces NO_x emissions

Reduction of Exhaust Emissions

- Additives
- Influence of compound structure on emissions
- Investigate interaction of biodiesel with new emissions reduction technologies

Low-Temperature Properties of Methyl Esters

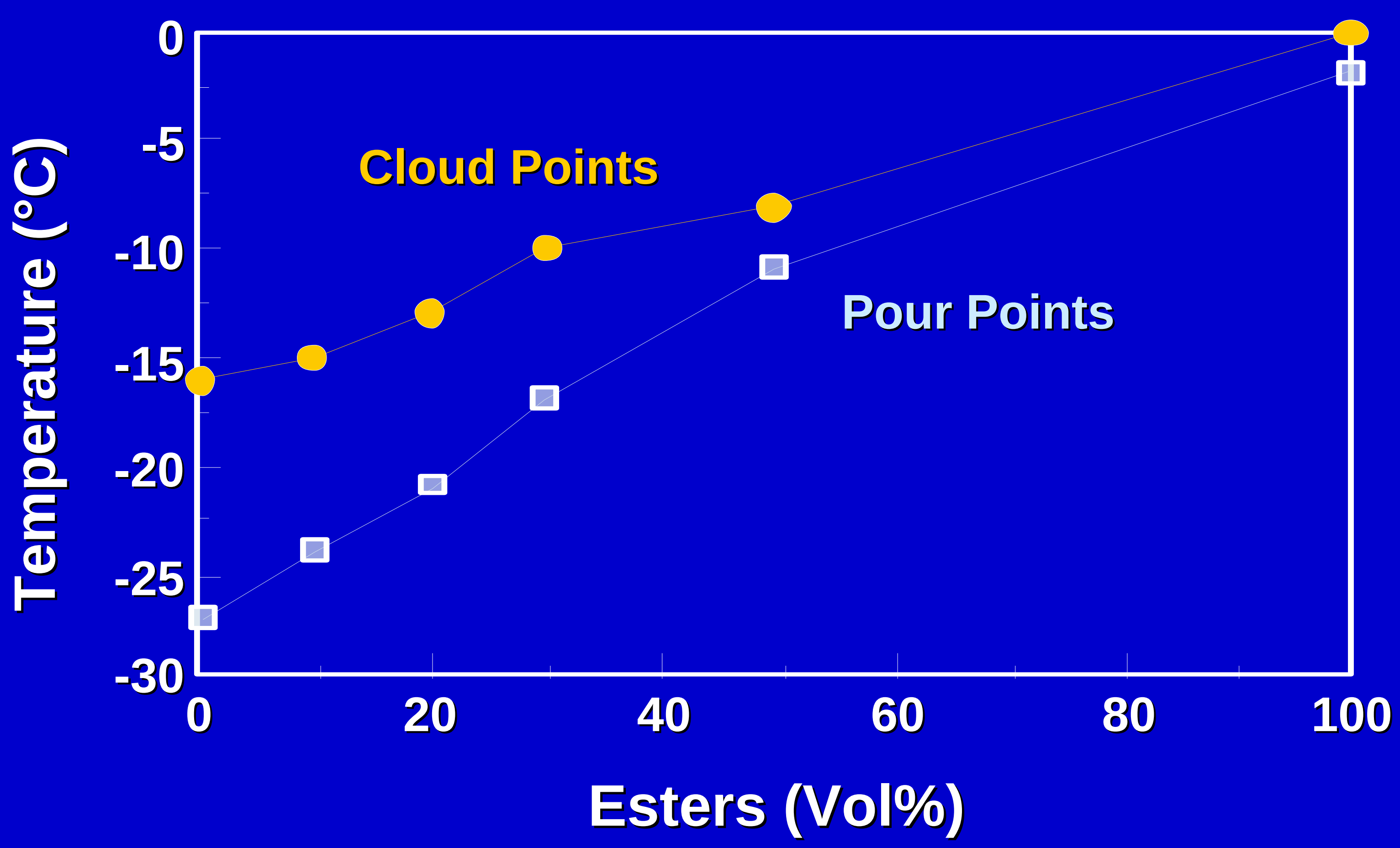
- Relatively high cloud and pour points
  - Limited use in cold weather
- Remedies:
  - Additives
  - Blending
  - Weatherization

Additive Approach

Develop and synthesize new additives

- Attach themselves to ester molecules
- Interact to inhibit crystal formation and reduce CP
- Hinder growth and prevent agglomeration of crystals

Ester/DF2 Blends



Other Approaches

Transesterification of vegetable oil with bulkier alkanol

- Isopropanol and isobutanol
- Use as either additive (co-fuel?) or as fuel

Winterization to remove saturates

- Cool to temperature slightly less than CP, then filter out solid saturates
- Feasibility use additives to decrease winterization temperature

Storage Stability

- Influence of compound structure: double bonds and (bis-)allylic methylenes.

- Storage conditions: Air, light, temperature, extraneous materials.

- Approaches to improving storage stability:
  - Antioxidants
  - Modified fuel composition.

Lubricity

- Biodiesel has inherent lubricity: Advantage over low-sulfur petrodiesel
- Low-level blends (B2) of biodiesel restore lubricity.
- Minor components (monoglycerides, fatty acids) in biodiesel enhance lubricity, especially of low-level blends.

Glycerol: A Versatile Substrate

- Domestically produced
- Renewable
  - derived from vegetable oils and animal fats
  - biodiesel co-product
- Highly functional
  - formation of aldehydes, esters, and ethers
- Replacement petrochemical feedstock
  - Non-toxic, Non-flammable

Food and Industrial Oils Research Projects/ Personnel

- *Improving the Performance of Alternative Fuels and Co-Products from Vegetable Oils*

Gerhard Knothe
Ronald Holser
Robert Dunn
Bryan Moser
Sevim Erhan

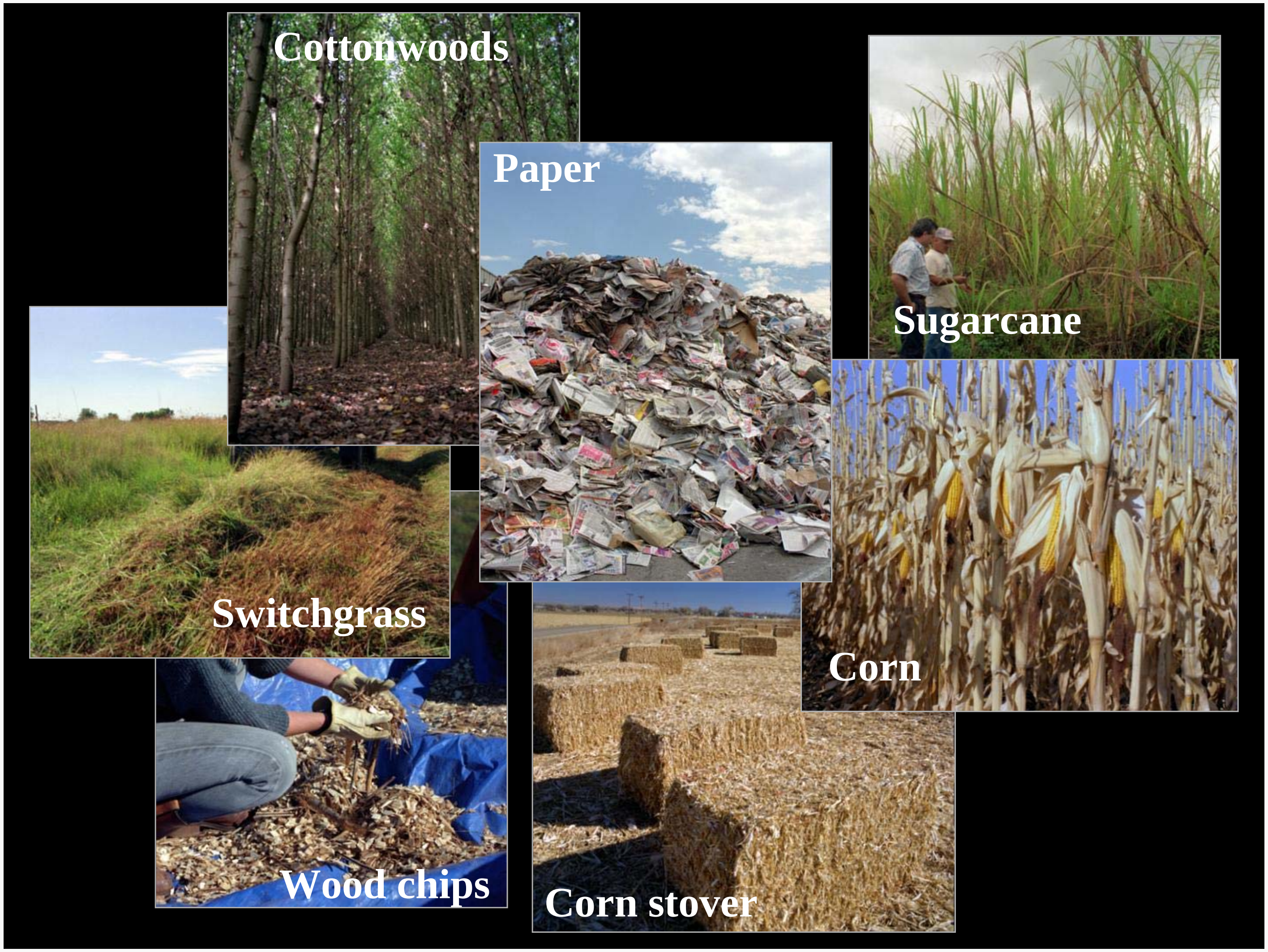
Bioethanol Research at NCAUR

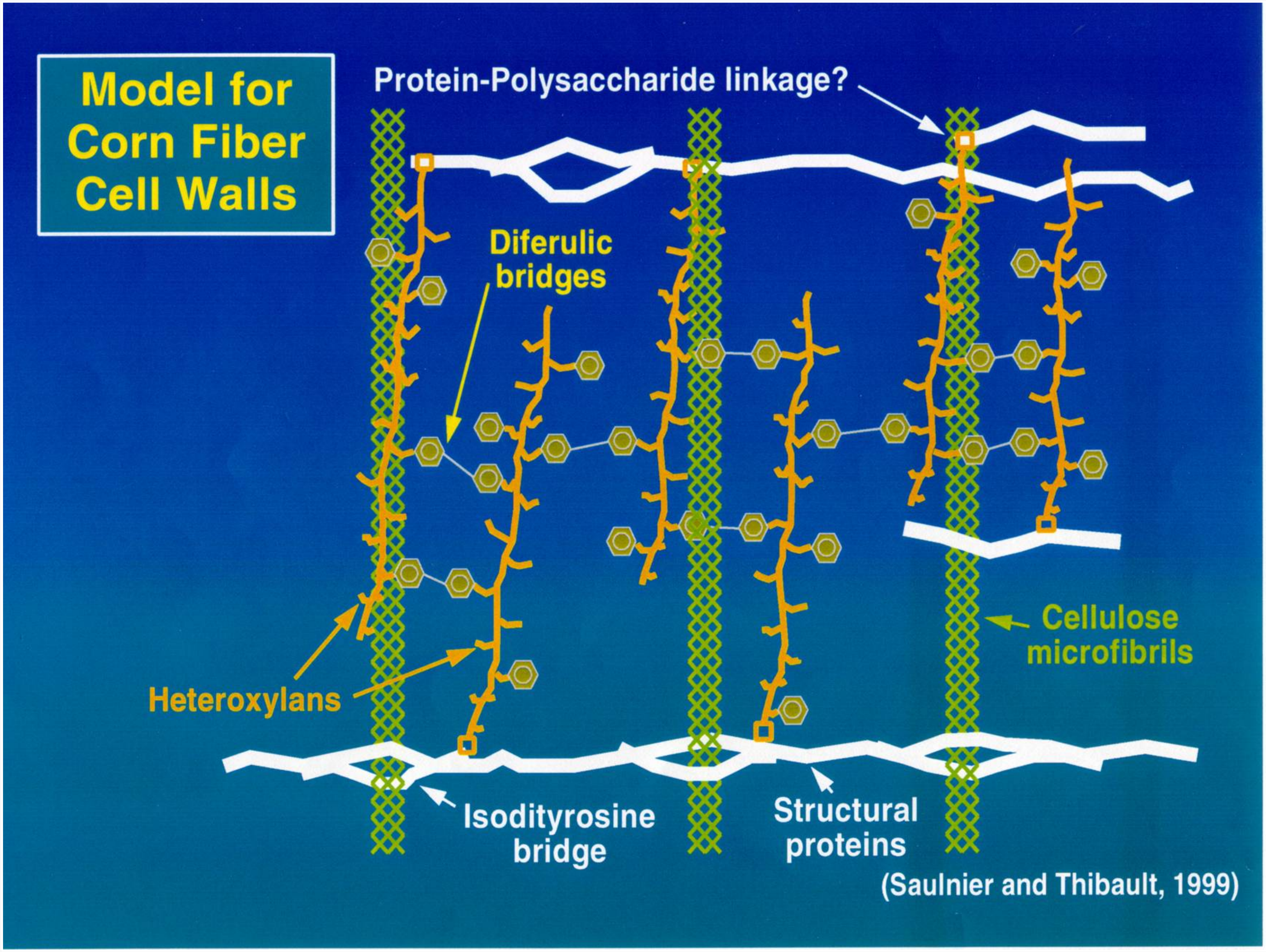
- ***Fermentation Biotechnology Research*** (FBT)
  - Mike Cotta, RL
- ***Bioproducts and Biocatalysis Research*** (BBC)
  - Tim Leathers, RL (acting)
  - Joseph Rich, RL [eod jan. 2007]
- ***Crop Bioprotection Research*** (CBP)
  - Pat Slininger, RL
- Cereal Products and Food Science Research
- Food and Industrial Oil Research
- Microbial Genomics and Bioprocessing Research
- Mycotoxin Research
- New Crops and Processing Research
- Plant Polymer Research

Corn to Ethanol Production, current [est. 2006]

- 5 billion gallons per year
- Approaching 2 billion bushels of corn
  - 56 million tons
- Corn production
  - 11-12 billion bushes per year
  - Ethanol production consumes 16-18%
- Mandate for 7.5 billion gallons per year by 2012
- Goal replace 30% of gasoline by 2030, or ~ 40 billion gallons
- Can we meet this goal?

potential substrates for ethanol production





Model for
Corn Fiber
Cell Walls
Protein-Polysaccharide linkage?
Diferulic
bridges
Cellulose
microfibrils
Heteroxylans
Isodityrosine
bridge
Structural
proteins
(Saulnier and Thibault, 1999)

Constraints to Bioconversion of Fibrous Biomass into Ethanol

- More severe pretreatment required to free sugars (physical, chemical, thermal)
- Lower sugar concentrations, pretreatment often limits final ethanol concentration
- Microbial inhibitors generated from side-reactions
- Hydrolyzing enzymes less efficient and more sensitive to end product inhibition
- Multiple sugars present in fermentation broth: glucose, xylose, arabinose, galactose...

NCAUR Research/ Biomass to Ethanol

- Feedstock/ Energy Crop development
- Pretreatment Strategies
- Enzymes for conversion of biomass polysaccharides to fermentable sugars
  - Enzyme expression systems
  - Discovery of new enzymes/ exploitation of novel sources
- New Biocatalysts for production of ethanol
  - Recombinant bacterial strains
  - Yeast strain improvement
  - Inhibitor resistance
- Bioprocess Engineering
  - Product recovery
  - Reactor design

♦ Feedstock/ Energy Crop development

Pretreatment

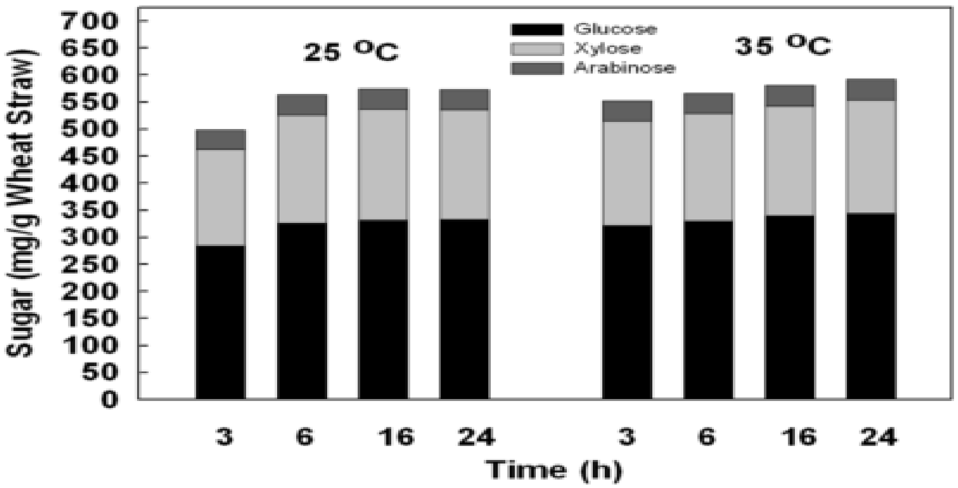
Selected Pretreatment Strategies

Acid

Pretreatment	Pentoses	Inhibitors
Strong Acid	+	++
Dilute Acid	+	++
Hot Water	-	+
AFEX	-	-
Alkaline Peroxide	-	-

Base

Effect of Duration of Alkaline Peroxide Pretreatment on Enzymatic Saccharification of Wheat Straw



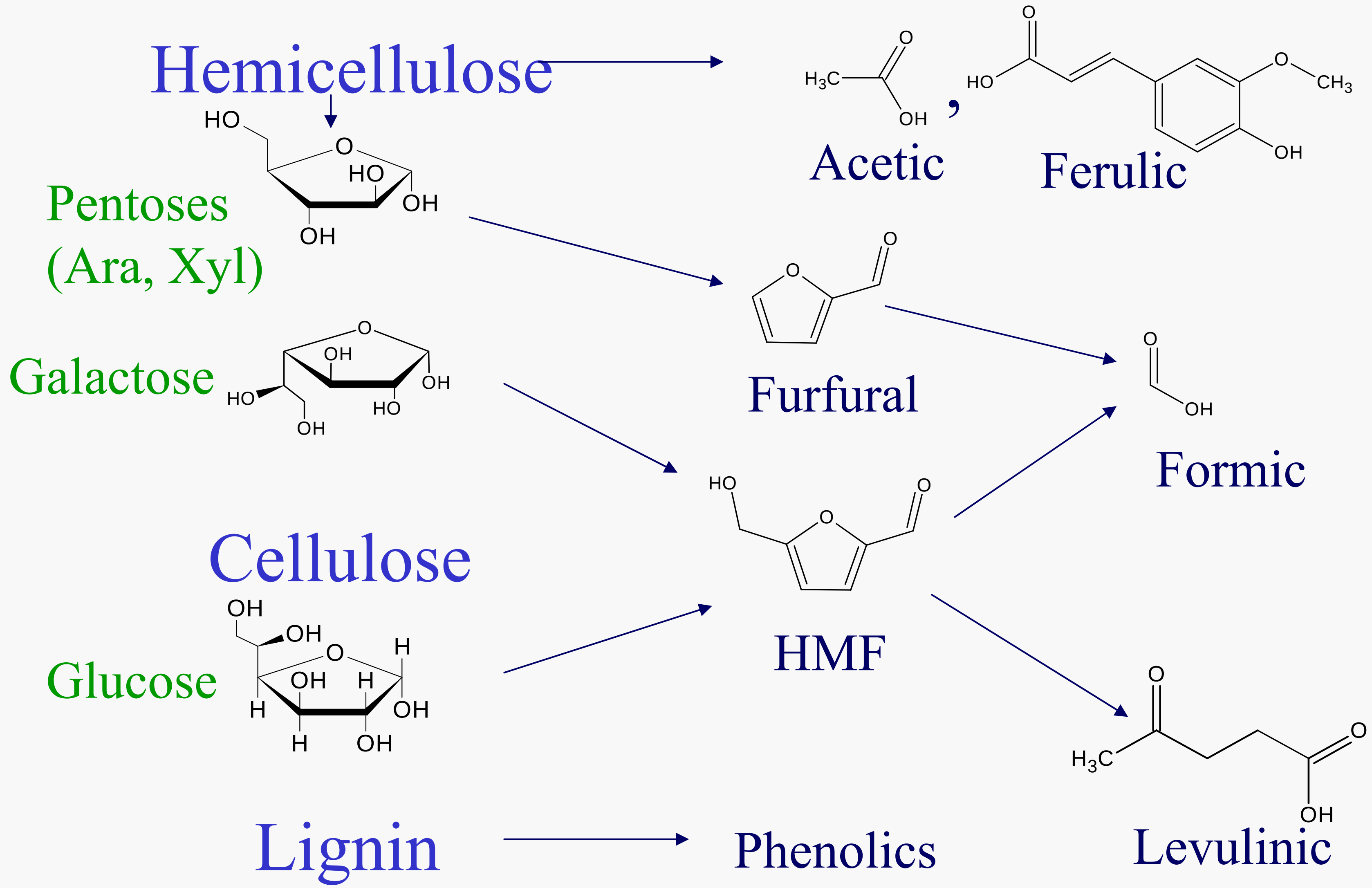
Alkaline Peroxide Pretreatment and Enzymatic Saccharification of Wheat Straw

	Sugar Composition mg/g straw
Glucose	387 ± 6
Xylose	256 ± 1
Arabinose	29 ± 2
Total Sugars	672 ± 4 (~98% yield)

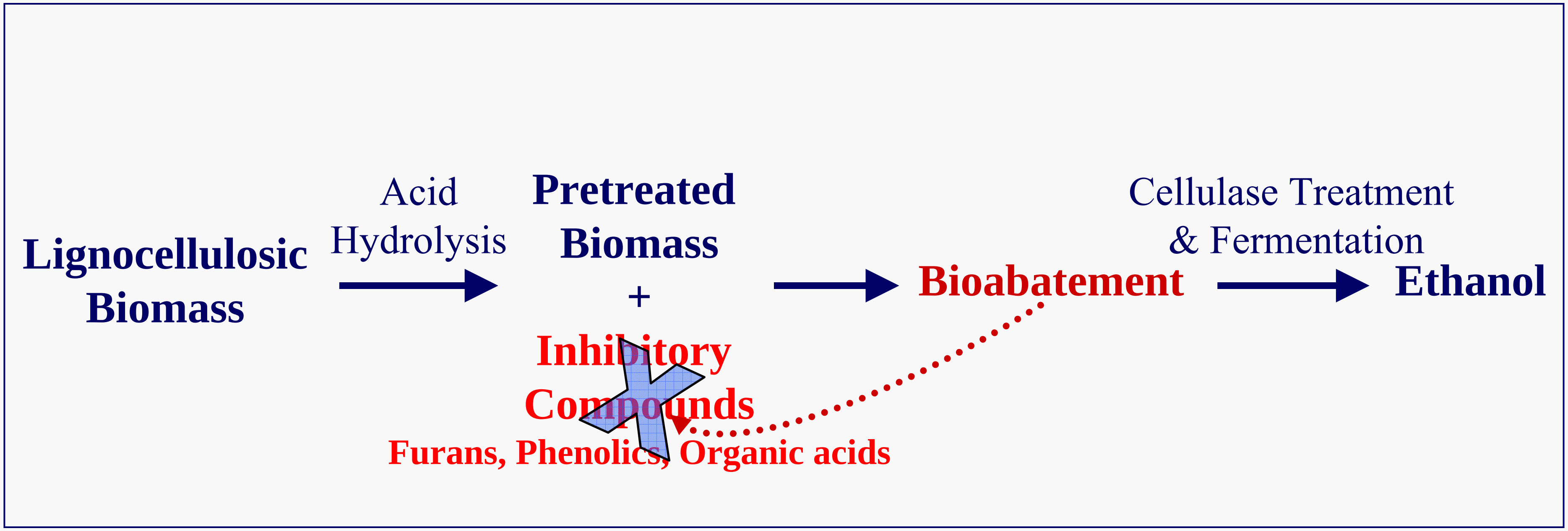
At pH 11.5, 2.15% H_2O_2, 35 ^oC, 24 h pretreatment. After pretreatment, pH was adjusted to 5.0 and enzyme cocktail was added. 120 h reaction time.

Inhibitor Abatement

Inhibitors Formed During Hydrolysis

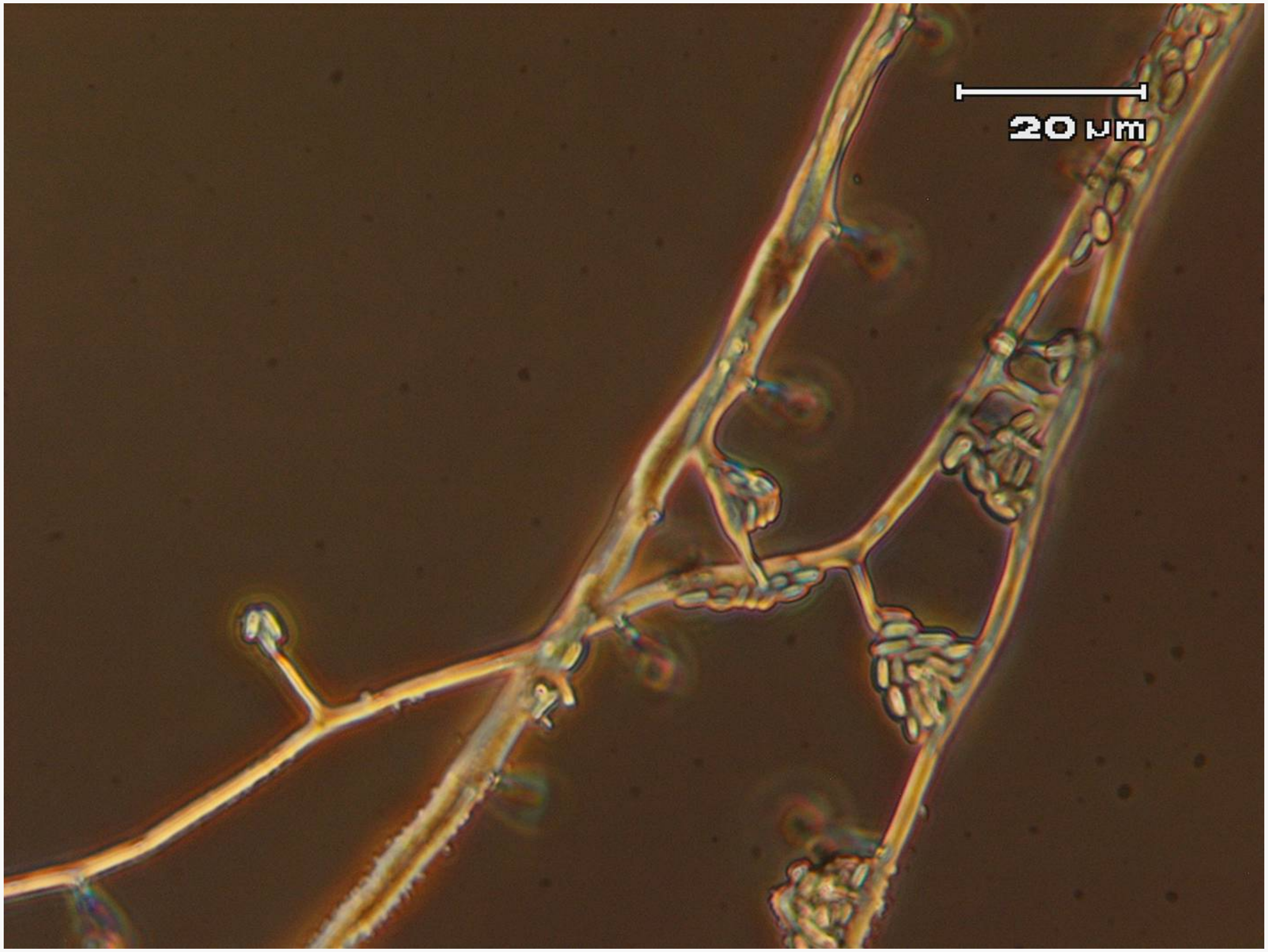


Bioabatement of Lignocellulosic Hydrolysates

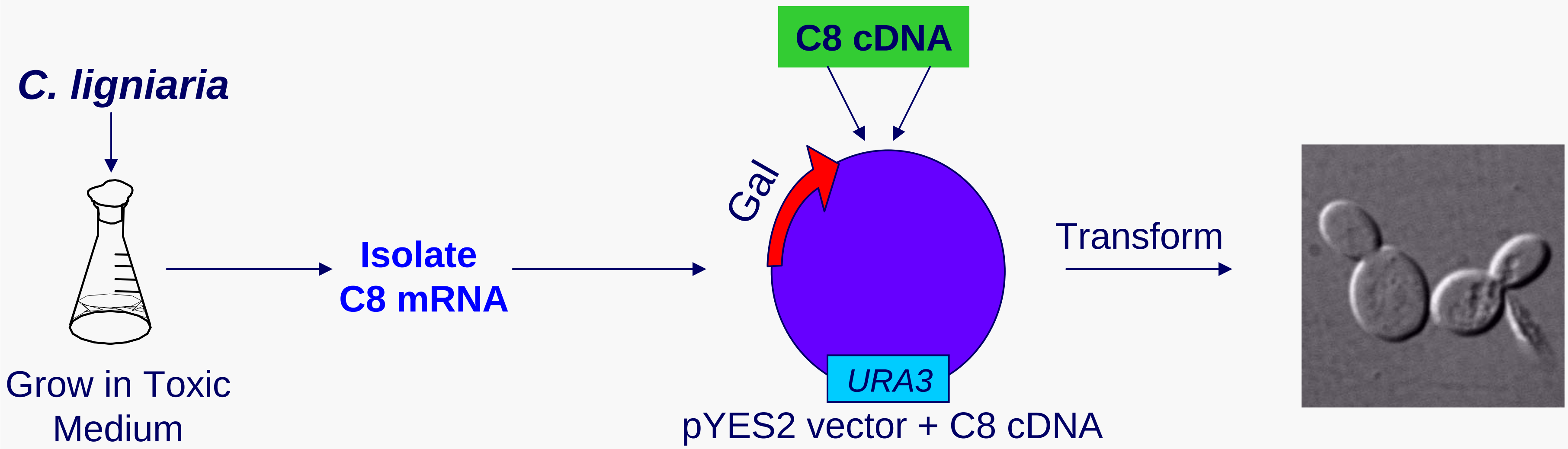


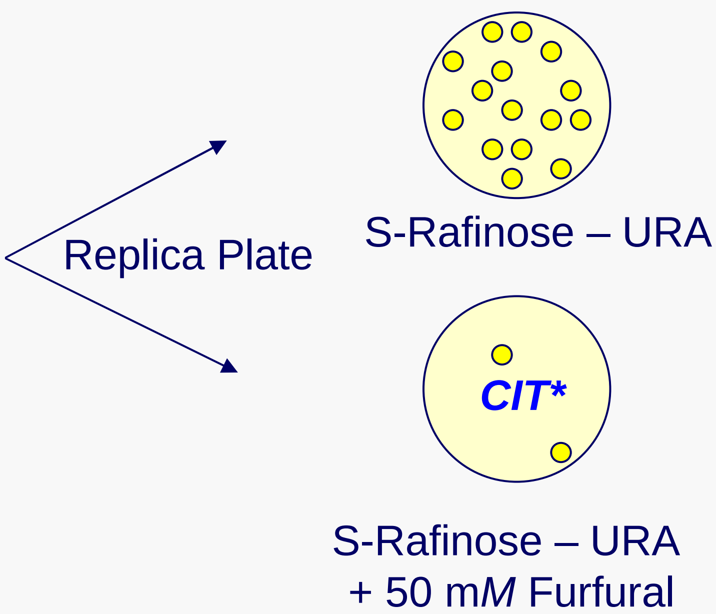
(Nichols, Lopez, & Dien 2002)

Coniochaeta ligniaria



Introduction of “resistance” genes



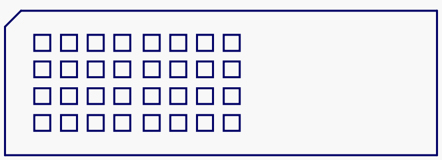


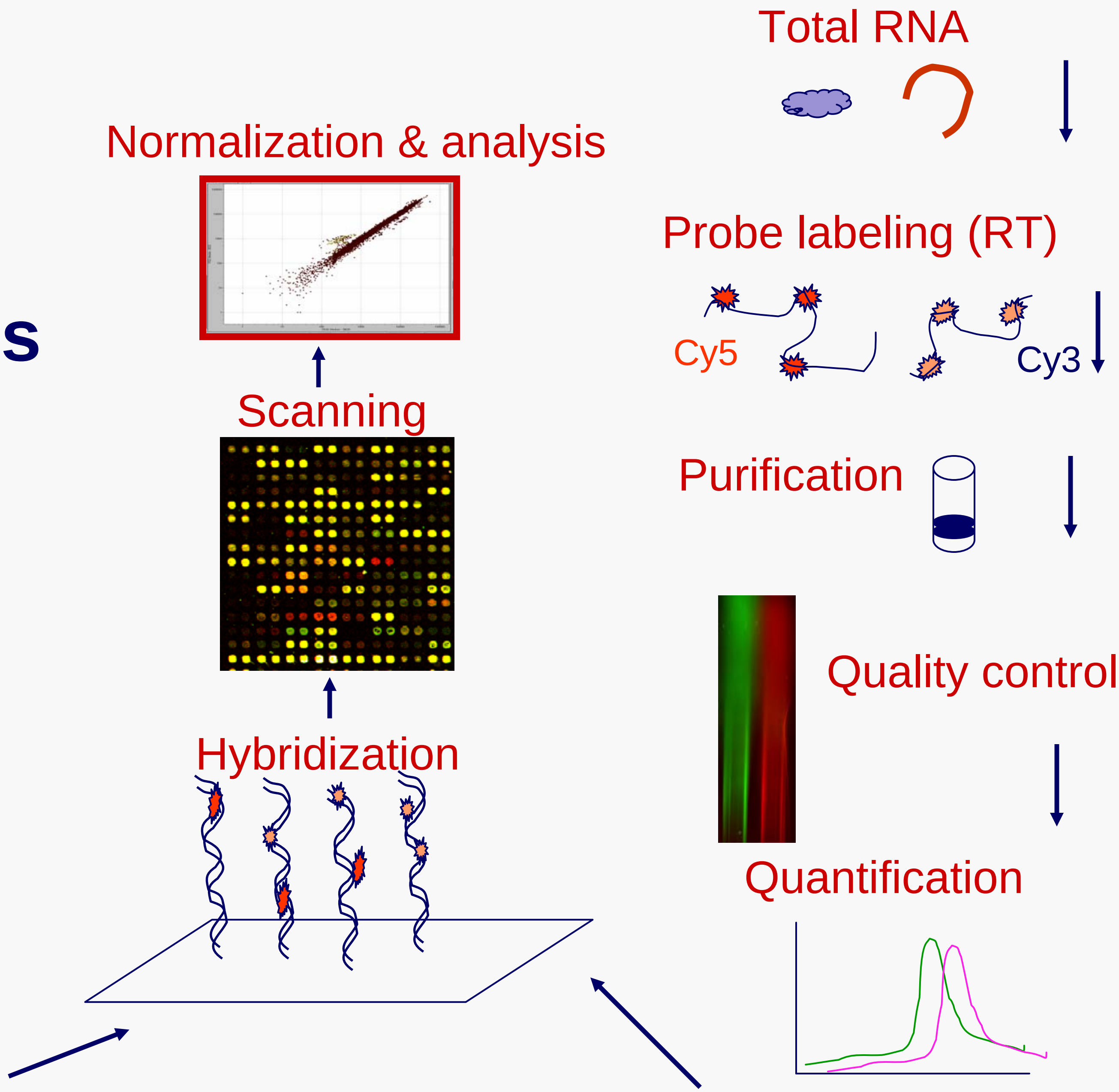
CFT Mutants

1. Transformants Verified on solid and liquid medium
2. cDNA isolated and compared
3. cDNA Sequenced and analyzed

DNA Microarray Experiments to Study Gene Expression

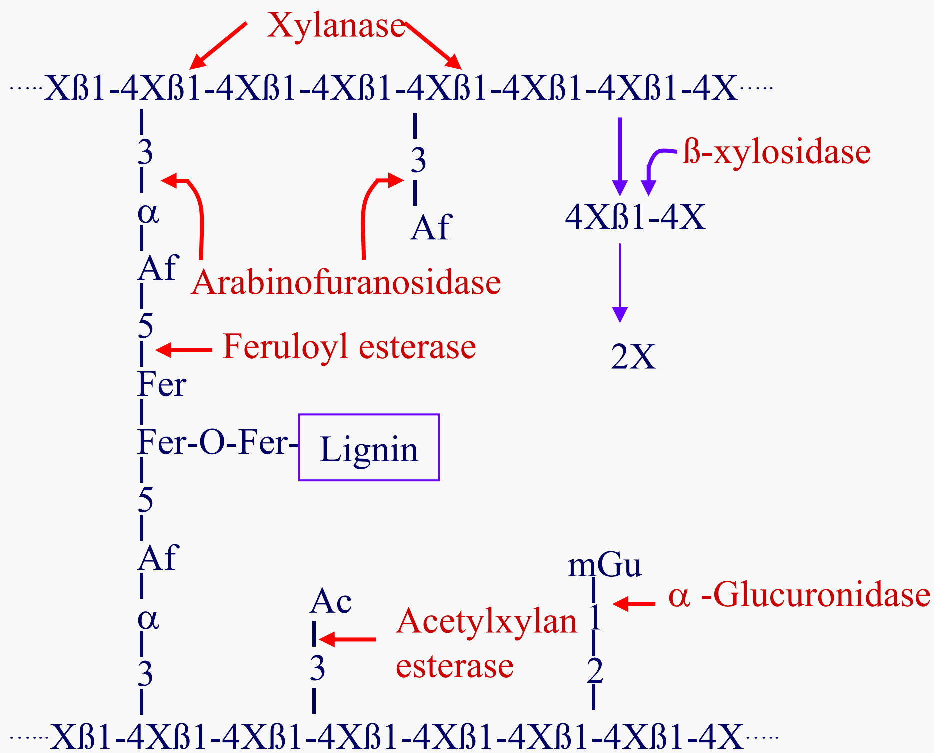
Printing oligos for each gene





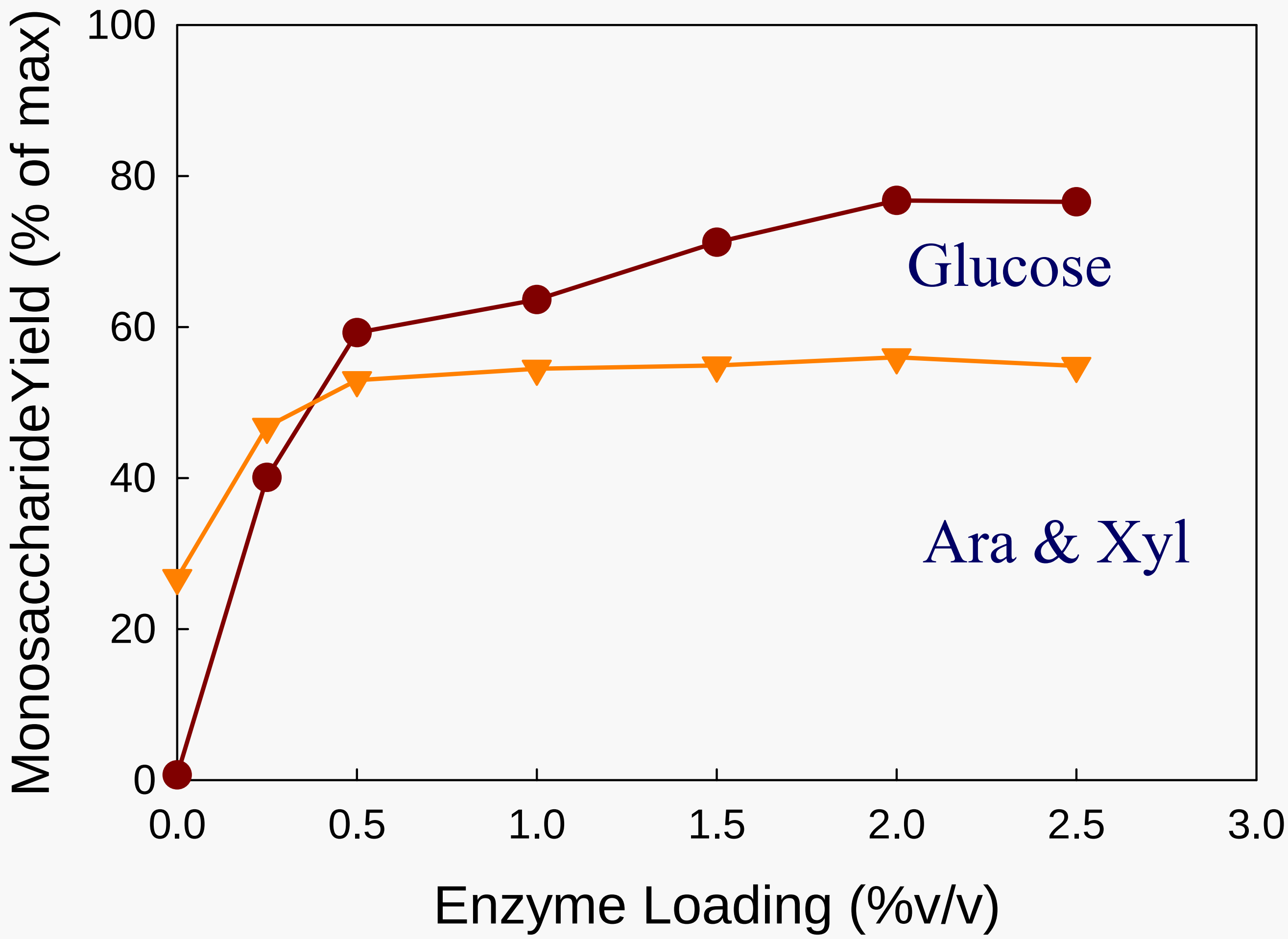
New Hydrolytic Enzymes

Complex Mixture of Enzymes Needed to Degrade Arabinoxylan



Selinger et al., 1996

Digesting hot-water treated hot-water treated corn fiber w/ commercial enzyme



Hydrolytic Enzymes Sequenced from *Orpinomyces* PC-2

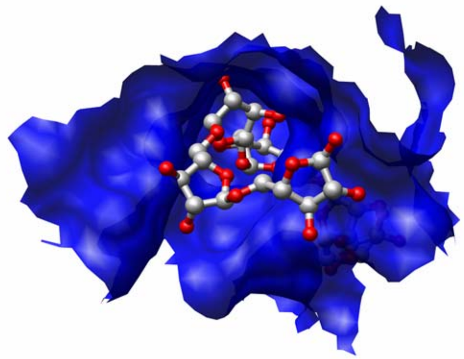
Enzyme[a]	Size (AA)	Binding[b] Sequence	Optimum pH Range	Best Temp. Range (°C)	Reference
CelA	459	NCDD	4.3-6.8	30-50	Li et al., 1997b
CelB	471	NCDD	4.8-7.6	30-50	Li et al., 1997a
CelC	449	NCDD	4.6-7.0	30-45	Li et al., 1997b
CelD	455	NCDD	4.5-7.0	30-50	Unpublished
CelE	477	NCDD	4.5-7.5	30-50	Chen et al., 1998
CelF	432	CBD	4.5-6.5	30-50	Chen et al., 2003
CelG	Partial	None	4.5-7.0	30-50	Unpublished
CelH	491	NCDD	ND	ND	Li et al., 2003
CelI	495	NCDD	ND	ND	Li et al., 2003
CelJ	Partial	NCDD	ND	ND	Unpublished
BglA	663	None	5.5-8.0	45-55	Ximenes et al., 2003
XynA	362	NCDD	5.5-7.5	50-60	Li et al., 1997a
LicA	245	None	4.5-8.0	35-55	Chen et al., 1997
ManA	574	CBD/NCDD	ND	ND	Ximenes et al., 2003
AxeA	313	None	7.0-8.5	30-45	Blum et al., 1999
FaeA	330	None	5.5-8.0	30-50	Blum et al., 2000a

[a]Cel, cellulase; Bgl, beta-glucosidase; Xyn, xylanase; Lic, beta-glucanase (lichenase); Man, mannanase; Axe, acetylxylan esterase; and Fae, feruloyl esterase.

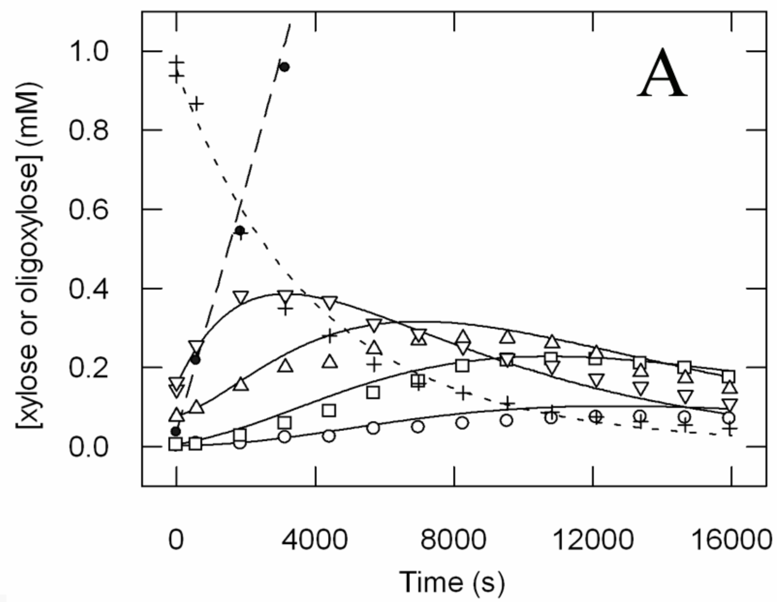
[b]NCDD, non-catalytic docking domain; CBD, cellulose binding domain.

Most Active β-Xylosidase Discovered

Why β-xylosidase? Commercial xylanases are limited in this activity and effective xylanases are needed for non-acidic catalyzed pretreatments.



Structure of Protein Binding Pocket



Enzyme breaking down X6

Versatile Biocatalyst

Two Major Strategies

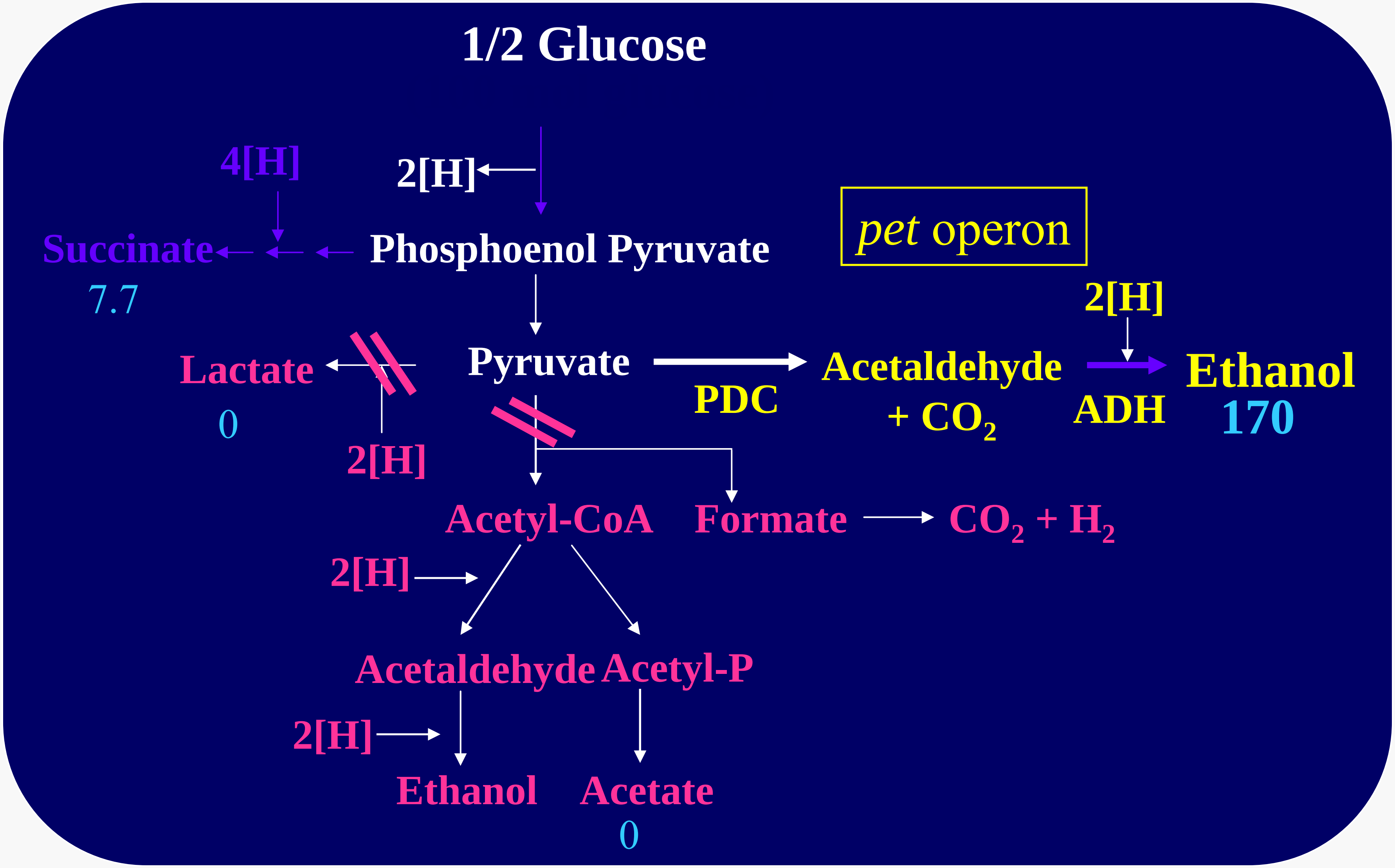
- Efficient ethanol producer

  Engineer to metabolize pentoses

- Able to use wide-spectrum of sugars

  → Engineer to only produce ethanol

Anaerobic selection for the plasmid-borne ethanol genes

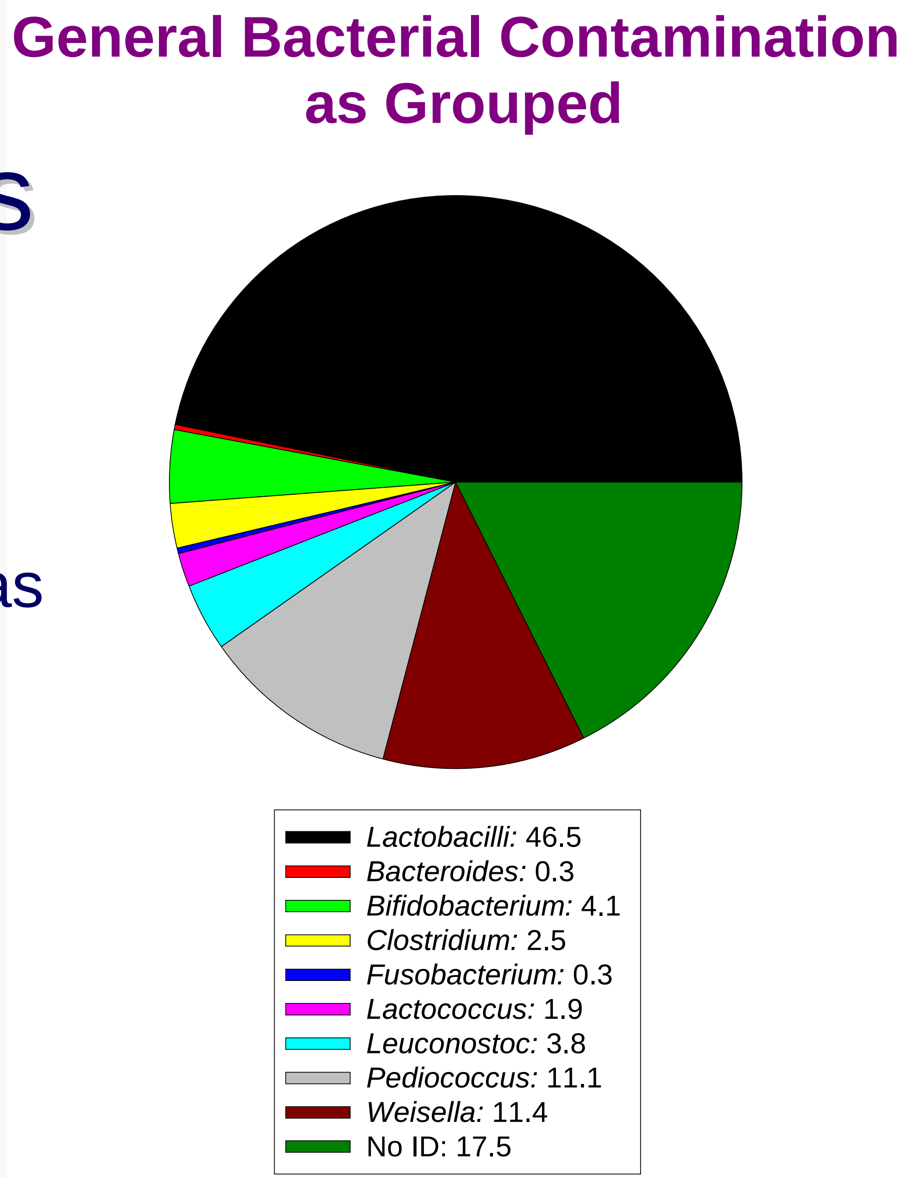


E. coli problems

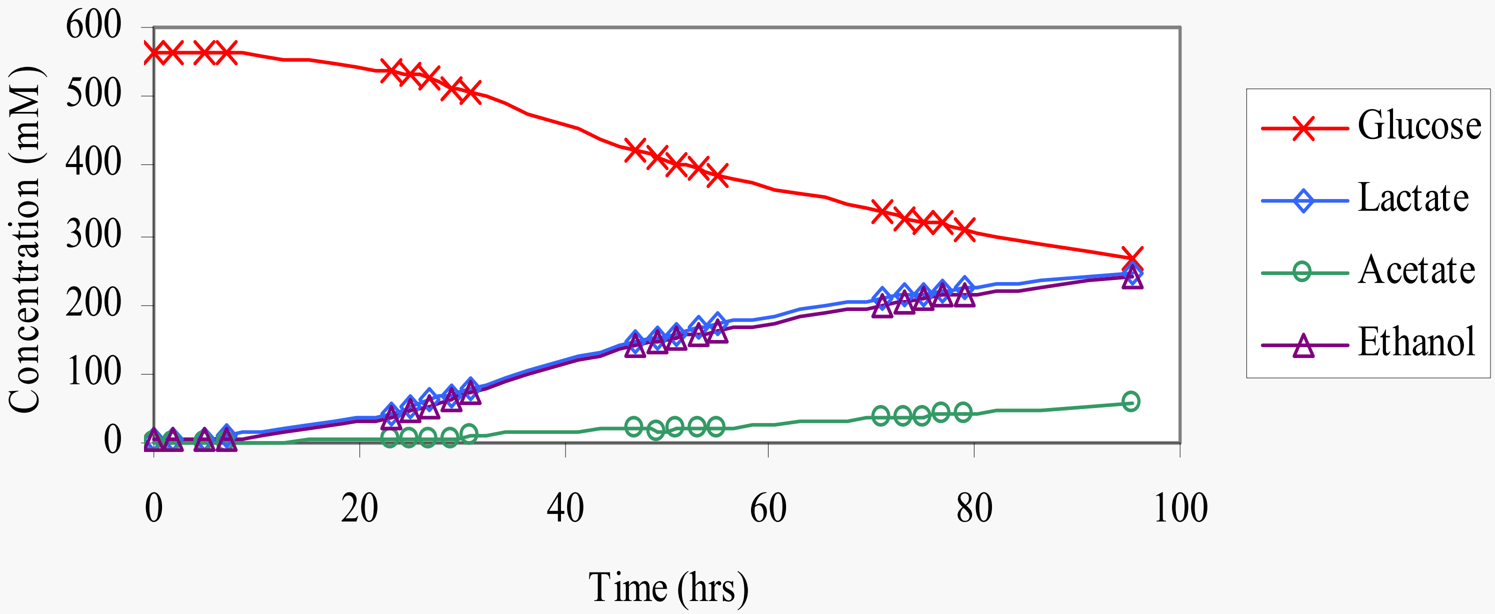
- Robustness
- pH range for fermentation
- Need to tolerate exposure to harsh environments (i.e. inhibitors, 8 < pH <4, T>45°C, osmotic pressure, desiccation)

Gram positives

- Synthetic pdc gene
- Lactic acid bacteria as ethanologens



Lactobacillus buchneri

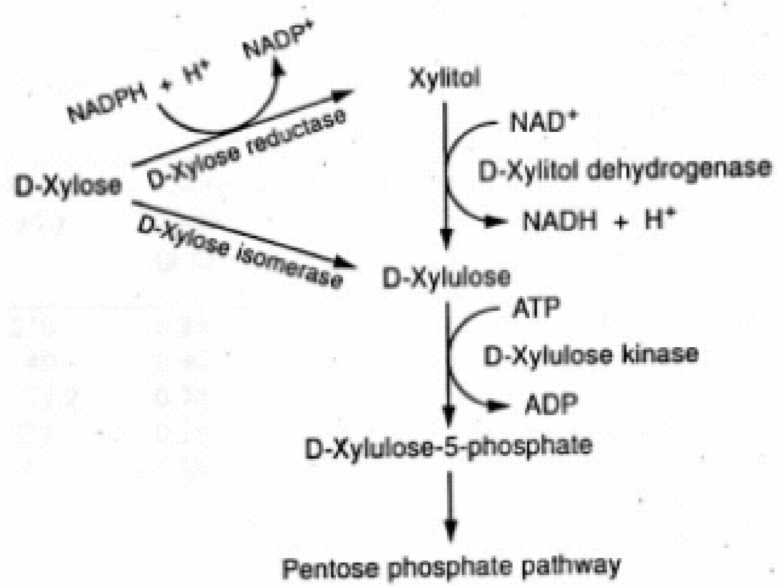


Glucose fermentation

Pathways For Microbial Xylose Utilization

Saccharomyces cerevisiae engineered to ferment glucose and xylose to ethanol

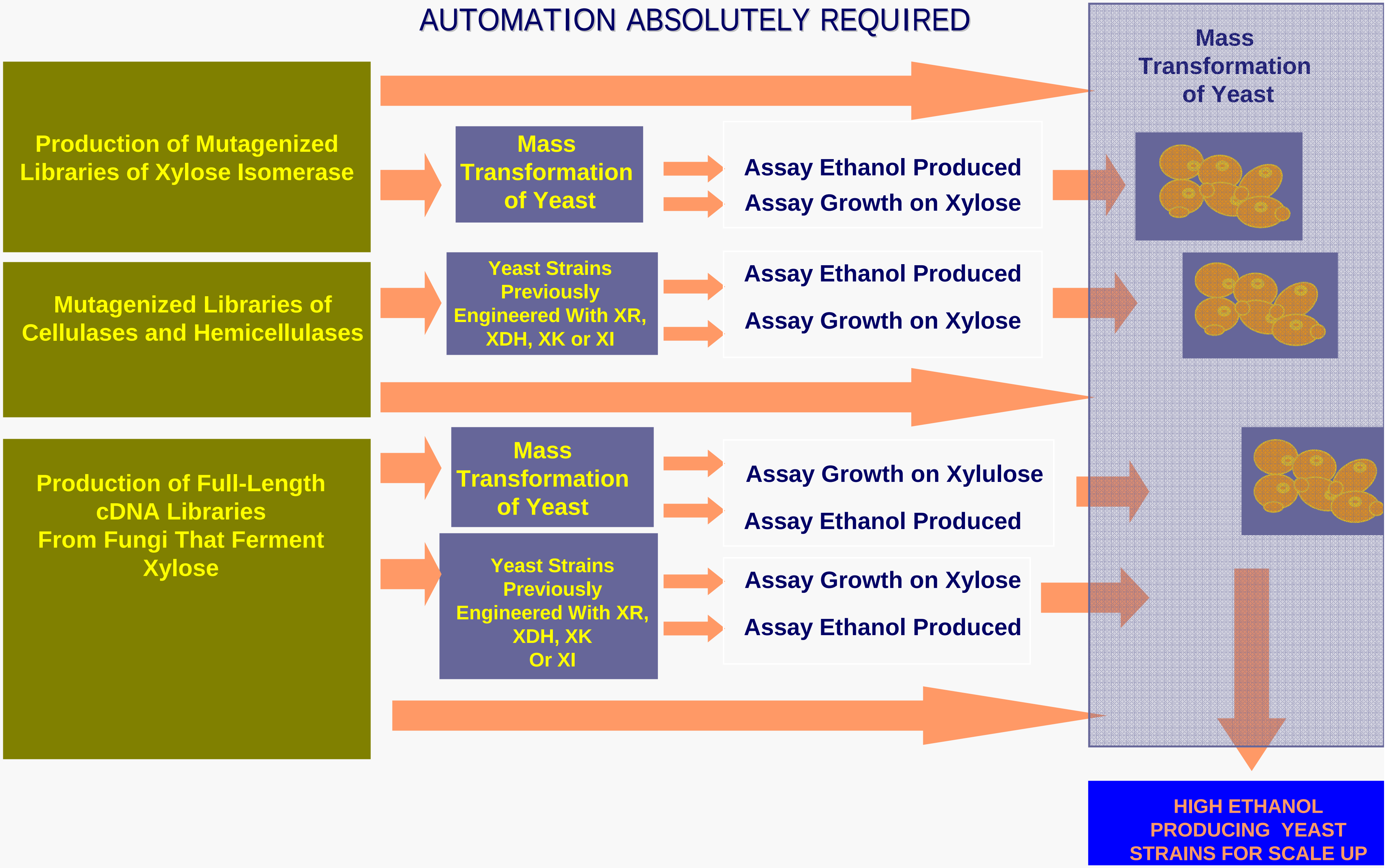
(Saha, 1997)



Aerobic Fungi use XR and XDH

Anaerobic Fungi and Bacteria use XI

AUTOMATION ABSOLUTELY REQUIRED



PLASMID-BASED FUNCTIONAL PROTEOMIC WORKCELL

Other Stuff?

Antimicrobial susceptibility of *Lactobacillus* species from ethanol plants

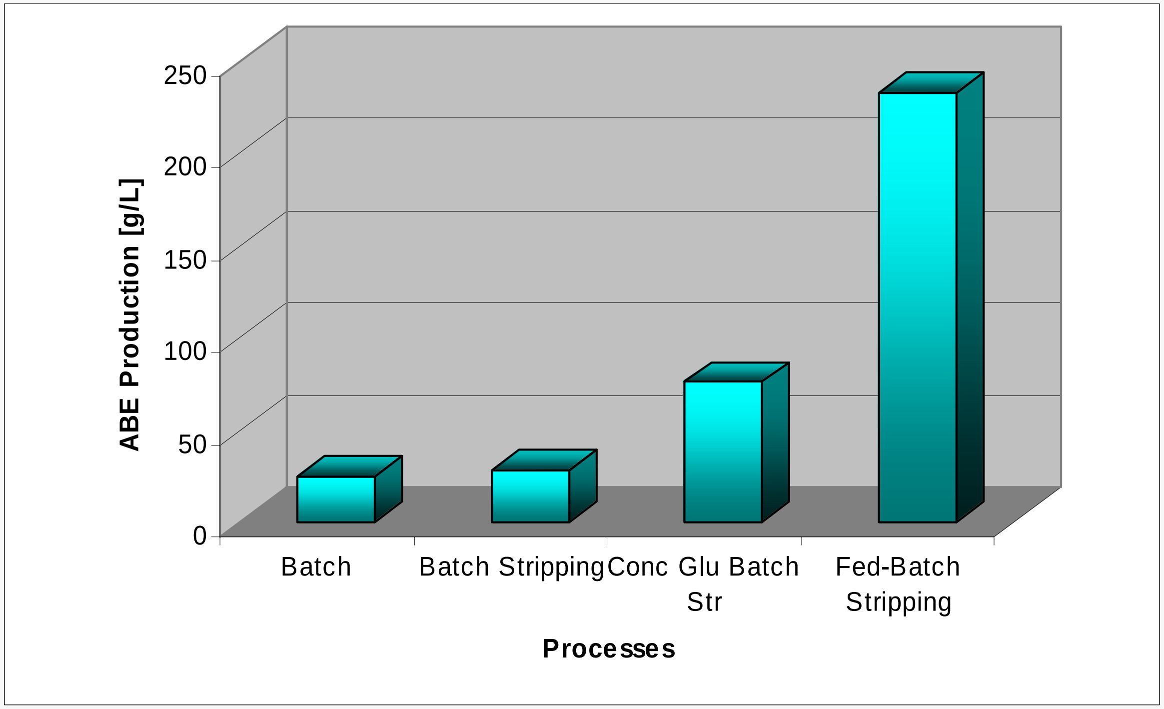
Antimicrobial	MIC_{50} (µg/ml)		MIC_{90} (µg/ml)		% Resistant[a]	
	Wet Mill	Dry Grind	Wet Mill	Dry Grind	Wet Mill	Dry Grind
AMP	1	2	1	> 8	8	69
CHL	≤ 2	≤ 2	4	> 16	0	21
PEN	1	8	2	> 8	0	64
TET	8	≤ 4	16	32	22	38
SYN	0.5	> 4	1	> 4	0	69
VIR	0.12	1	0.25	4	0	12

[a]Percentage of isolates with MICs equal to or greater than resistance breakpoints. Breakpoints used to interpret resistance were as follows: ampicillin (AMP), 2 µg/ml; chloramphenicol (CHL), 16 µg/ml; penicillin G (PEN), 4 µg/ml; tetracycline (TET), 16 µg/ml; synercid (SYN), 4 µg/ml; virginiamycin (VIR), 4 µg/ml.

Butanol

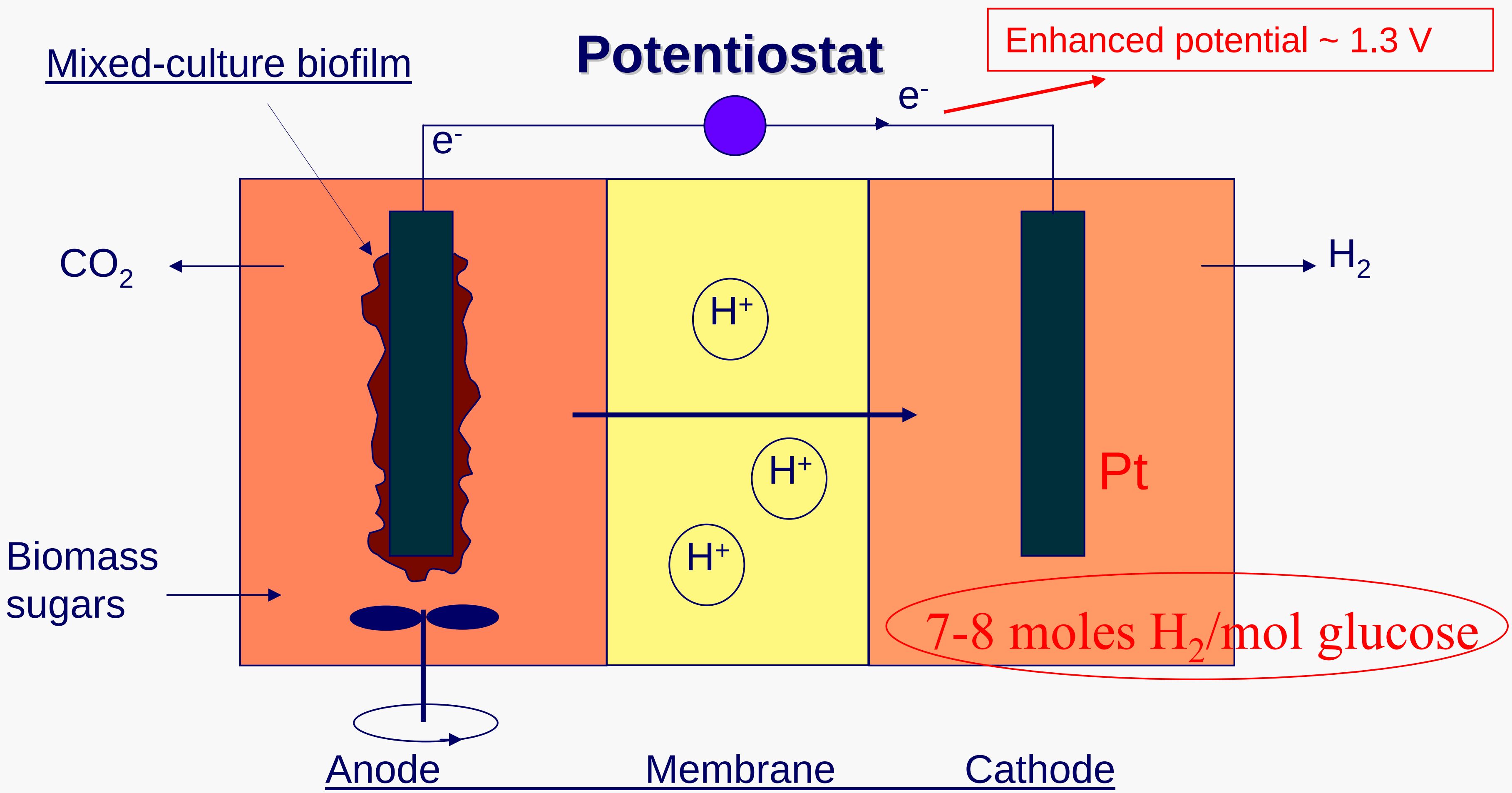
- Manufacture of:
  - dibutyl phthalate (as a precursor),
  - butyl acetate (as a precursor),
  - butyl acrylate (as a latex),
  - glycol ethers, and amine resins
- Other uses:
  - is an excellent fuel (it is miscible with gasoline and diesel fuel, has high calorific value, has a lower vapor pressure, and is less miscible with water);
  - used in plastic industry as a feedstock chemical;
  - food grade extractant;
  - a solvent in the manufacture of oil, pharmaceuticals, perfumes;
  - and as a solvency enhancer in the formation of nitrocellulose lacquers

Total ABE Production



In the four processes 24.8, 28.4, 75.9, and 233 g/L ABE were produced respectively.

Hydrogen production through an electrochemically assisted microbial fuel cell (MFC)



Liu, H., S. Grot, and B. E. Logan. 2005. Electrochemically assisted microbial production of hydrogen from acetate. Environ. Sci. Technol. 39:4317-4320; and Rozendal, R. A., H. V. M. Hamelers, G. J. W. Euverink, S. J. Metz, and C. J. N. Buisman. In Press, available online 2 February 2006. Principle and perspectives of hydrogen production through biocatalyzed electrolysis. Int. J. Hydrogen Energy.

What about Field Peas?

FBT Research Projects/ Personnel

•*Industrially Robust Enzymes and Microorganisms for Production of Sugars and Ethanol from Agricultural Biomass*

Bruce S. Dien
Nancy N. Nichols
Xin-Liang Li
Jeffrey A. Mertens
Douglas B. Jordan
Michael A. Cotta

•*Cost-effective Bioprocess Technologies for Production of Biofuels from Lignocellulosic Biomass*

Badal C. Saha
Nasib Qureshi
Michael A. Cotta
Ronald Hector
vacant- Carbohydrate Chemist

BBC Research Projects/ Personnel

•*Microbial Catalysts to Produce Fuel Ethanol and Value Added Products*

Kenneth Bischoff
Siqing Liu
Stephen Hughes
Joseph Rich, Research Leader [eod jan. 2007]

Crop Bioprotection Research Projects/ Personnel

•*Genomics and Engineering of Stress-tolerant Microbes for Lower Cost Production of Biofuels and Bioproducts*

Patricia J. Slininger
Z. Lewis Liu
Steven W. Gorsich

Collaborations of Note:

●Aventine Renewable Energy/Purdue University/U.S. DOE [NREL]: Hot water treatment of wet milled corn fiber to produce additional ethanol in a commercial ethanol plant.

●University of Illinois: Developing screening technologies for testing and identification of superior corn varieties for production of fuel ethanol.

●ARS-Midwest Energy Crops Working Group: Developing selection [genetic improvement] and conversion strategies for new dedicated energy crops for the U.S.

●National Corn to Ethanol Research Center/Illinois Corn Marketing Board/U. Illinois/Western Illinois University: Research to promote the commercialization of new methods in the conversion of corn to ethanol and add value to the dry grind to ethanol process.

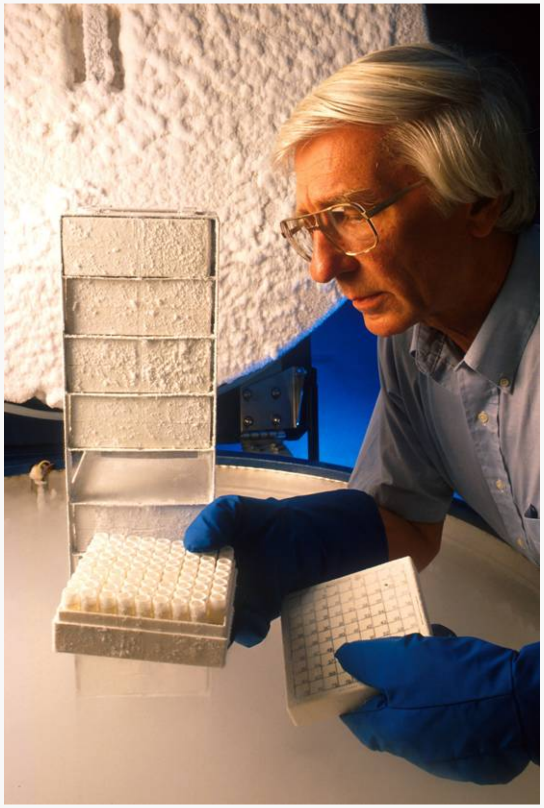
●ADM/U. Illinois: Demonstration of a commercial method for conversion of corn starch to butanol.

●Midwest Consortium for Sustainable Biobased Products [Purdue, U. Illinois, Michigan State University, Iowa State University, Argonne National Laboratory, Ames Laboratory]: Developing further the uses of Distillers Grains (DG) for production of alternative chemical and fuel products.

●North Dakota Dry Pea and Lentil Association: Application of field peas as a feedstock for fuel alcohol production.

Microbial Culture Collection

- More than 85,000 strains of microbes maintained at NCAUR
- Largest of its kind accessible to the public
- Widely considered to be the most useful in the world



Bioethanol research