Recent Research and Perspectives on Lignocellulose Conversion into Ethanol

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Fuel Ethanol Dates Back to First Cars

Some Milestones in Ethanol Production Research at NCAUR

- Patented process for corn cob conversion to ethanol and furfural
- Alkali Peroxide pretreatment agricultural biomass
- Pentose fermenting yeast for ethanol
- Characterization of enzymes for biomass hydrolysis
- Recombinant ethanol producing bacteria

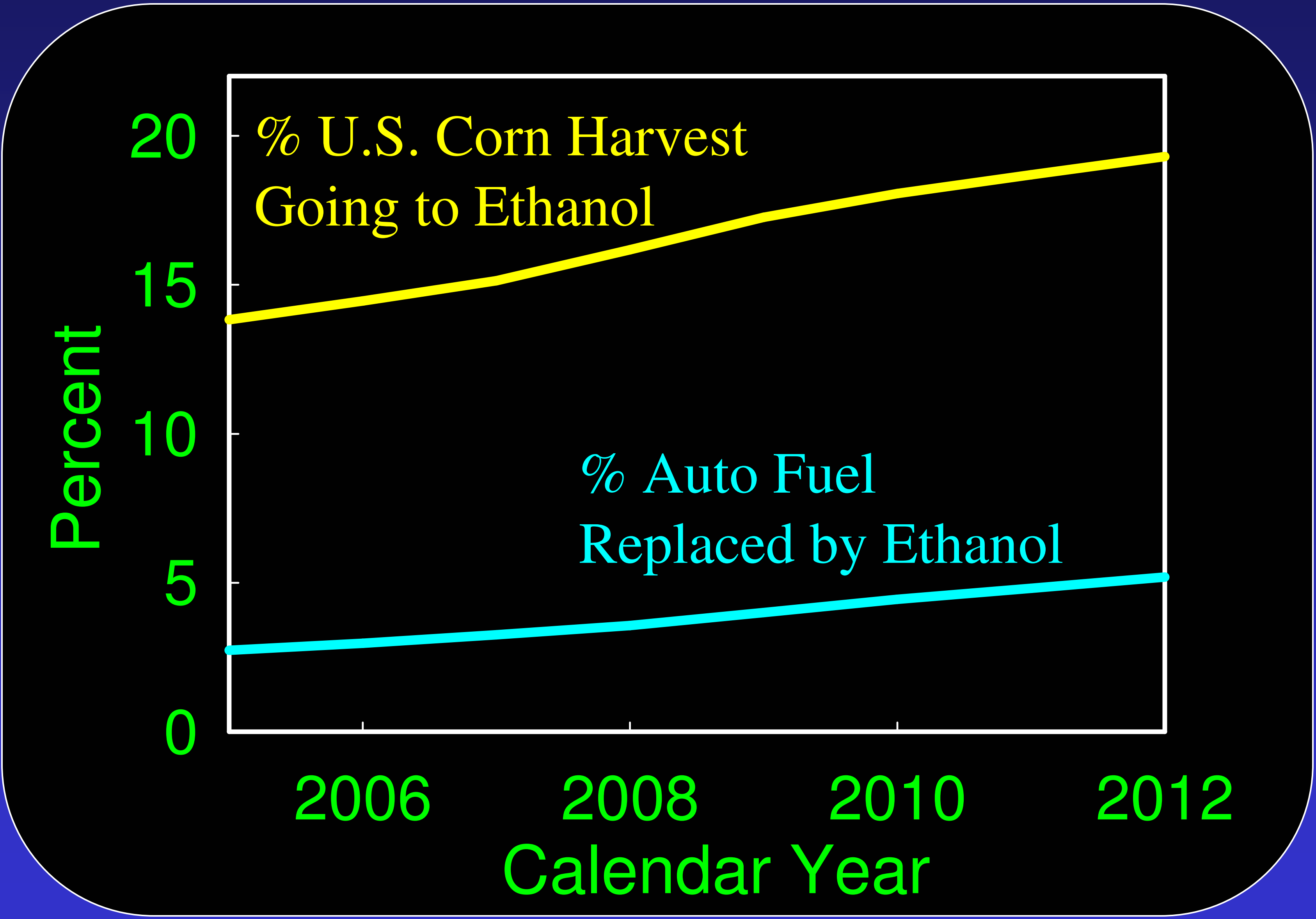
Benefits of Fuel Ethanol

- Lowers dependence on imported oil (170 million barrels in 2005).
- Lowers pollutants and greenhouse gas emissions, including CO, CO2, and VOC.
- Is an environmentally friendly replacement for oxygenate MTBE.
- Creates a market for corn (13% of U.S. corn harvest in 2005).
- Increased farm income by $4.5 billion and led to creation of 200,000 jobs (1994).

Annual Production: 3.9 B gal (2005)

Renewable Fuel Standard: 7.5 B gal (2012)

Potential of corn to replace oil for U.S. market



(RFA & NCGA, 2006)

Potential of lignocellulosic biomass to replace oil for U.S. market

Feedstocks	**Million dry ton per yr**	**Billion gal of ethanol per yr**
Agricultural Land (selected)		
Corn Stover & Wheat Straw	**75**	**5.22**
Corn Fiber & DDGS	**13**	**0.83**
CRP Biomass	**18**	**1.08**
Perennial Crops	**156**	**9.36**
Forestlands (selected)		
Logging & Processing Residues	**134**	**8.04**
Total:	**4,907**	**24.4**

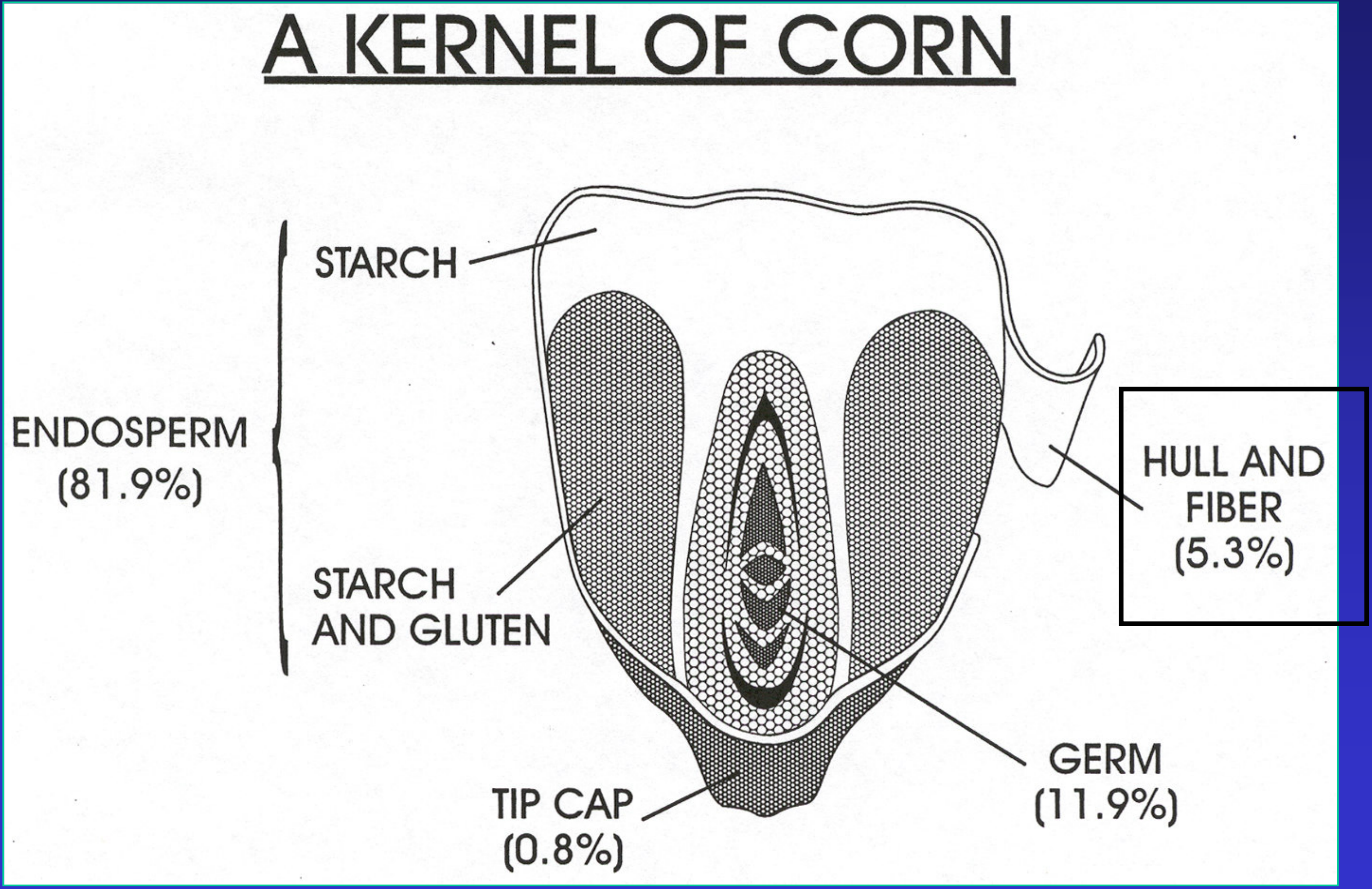
This is 17% of our total oil needs.

Notes: (1) 60 gal/ton ethanol yield; (2) source: http://feedstockreview.ornl.gov/pdf/billion_ton_vision.pdf

Corn Fiber: lignocellulosic biomass conversion to ethanol.

- Dilute-acid pretreated corn fiber
- Buffered hot-water pretreated corn fiber

Structure of corn kernel



Why Corn fiber?

- Could be a first step leading to other sources of lignocellulose
- Easy to digest and ferment because it contains no lignin
- Centrally located, so no collection fees
- Lower capital & labor costs, usually located at pre-existing ethanol fermentation facility

Ethanol Yield from a Bushel of Corn

Product	Ethanol Yield (gallons)
Starch (32 lbs)	2.5 - 2.7
Fiber* (4.5 lbs)	0.30

One bushel of corn weighs 56 lbs
One gallon of ethanol = 3.785 L = 6.58 lbs
*contained in DDGS; ref. Gulati et al., 1996

Fibrous Biomass vs. Corn Compositions

From:	Starch	Cellulose	Hemicellulose		Total
	%w/w	%w/w	%w/w		%w/w
Sugar	Glucose	Glucose	Arabinose	Xylose	
Corn Fiber	20	14	11	18	70
DDG	3	27	9	12	51
Corn Stover	none	38	3	18	62
Corn	78	na	na	na	78

Data: corn fiber Grohmann and Bothast, 1997; corn stover Wiselogel et al., 1996)

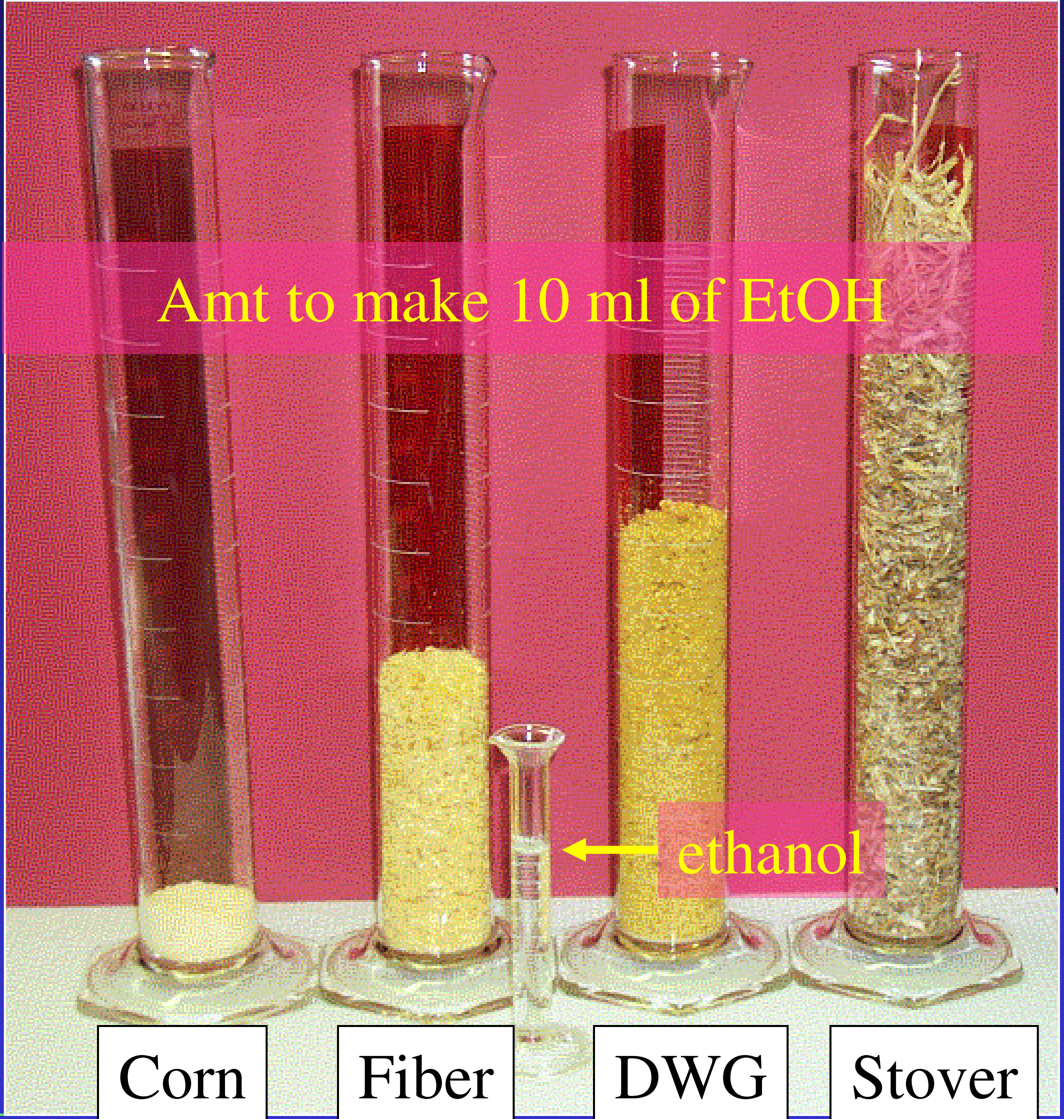
Challenges to processing fibrous biomass compared to grains

❖High bulk mat'l (wood less so)

❖2-phase reactions (β-glucan insoluble for > 10 d.p.)

❖Complex cell wall structure & lignin (e.g. storage vs. structural CHO's)

❖Xylan related sugars not fermented by *Saccharomyces*



	Corn	Fiber	DWG	Stover
Moisture	15%	46%	64%	5%

Designing Process

- Select pretreatment
  - Hydrolyze hemicellulose
  - Prepare cellulose for enzymatic digestion
- Select Hydrolytic enzymes
  - Hemicellulases
  - Cellulases
- Select ethanol producing biocatalyst
  - *Saccharomyces* does not ferment xylose!

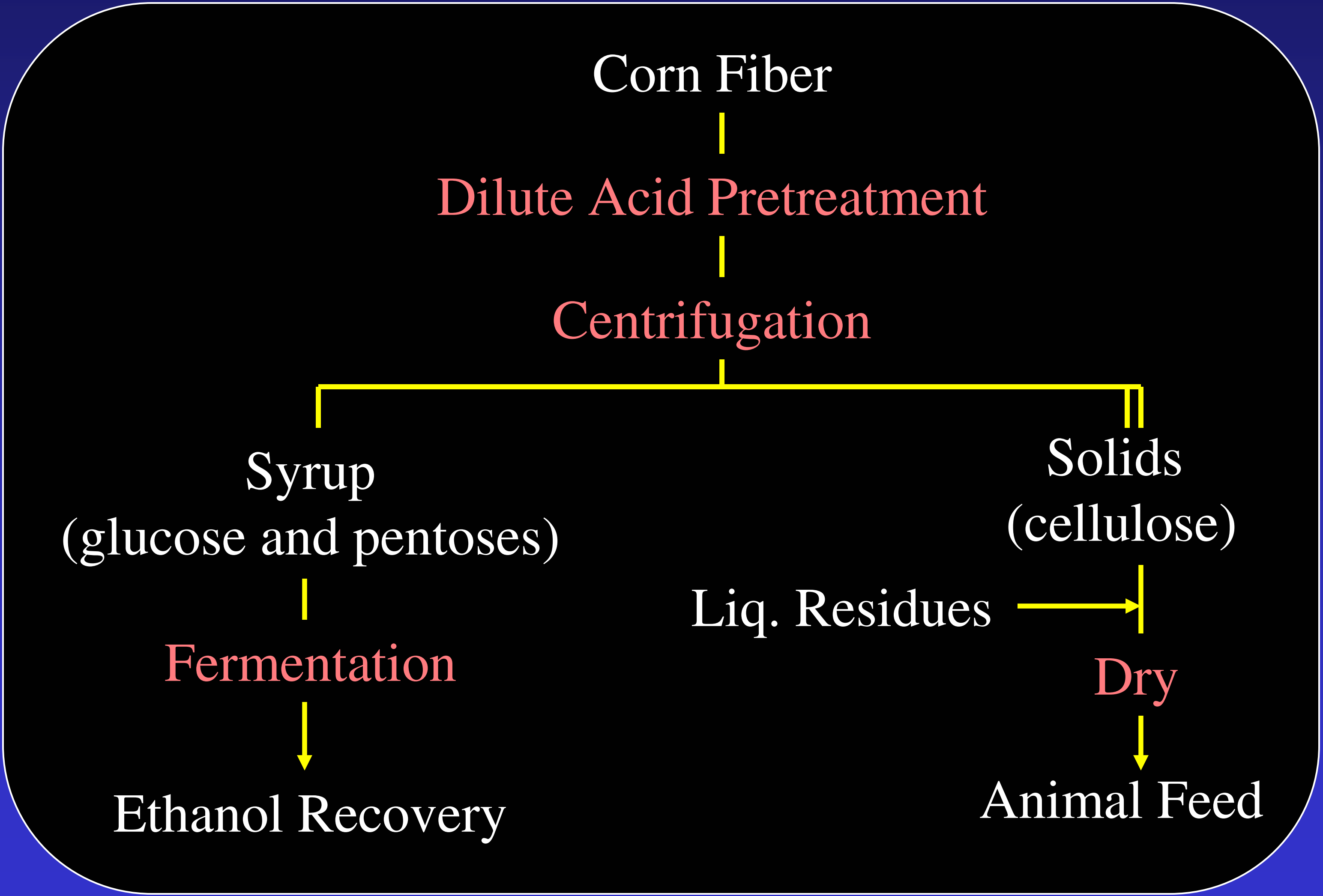
Selected Pretreatment Strategies

Acid

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

Base

Corn Fiber to Ethanol Process



Corn Fiber

Dilute Acid Pretreatment

Centrifugation

Syrup
(glucose and pentoses)

Fermentation

Ethanol Recovery

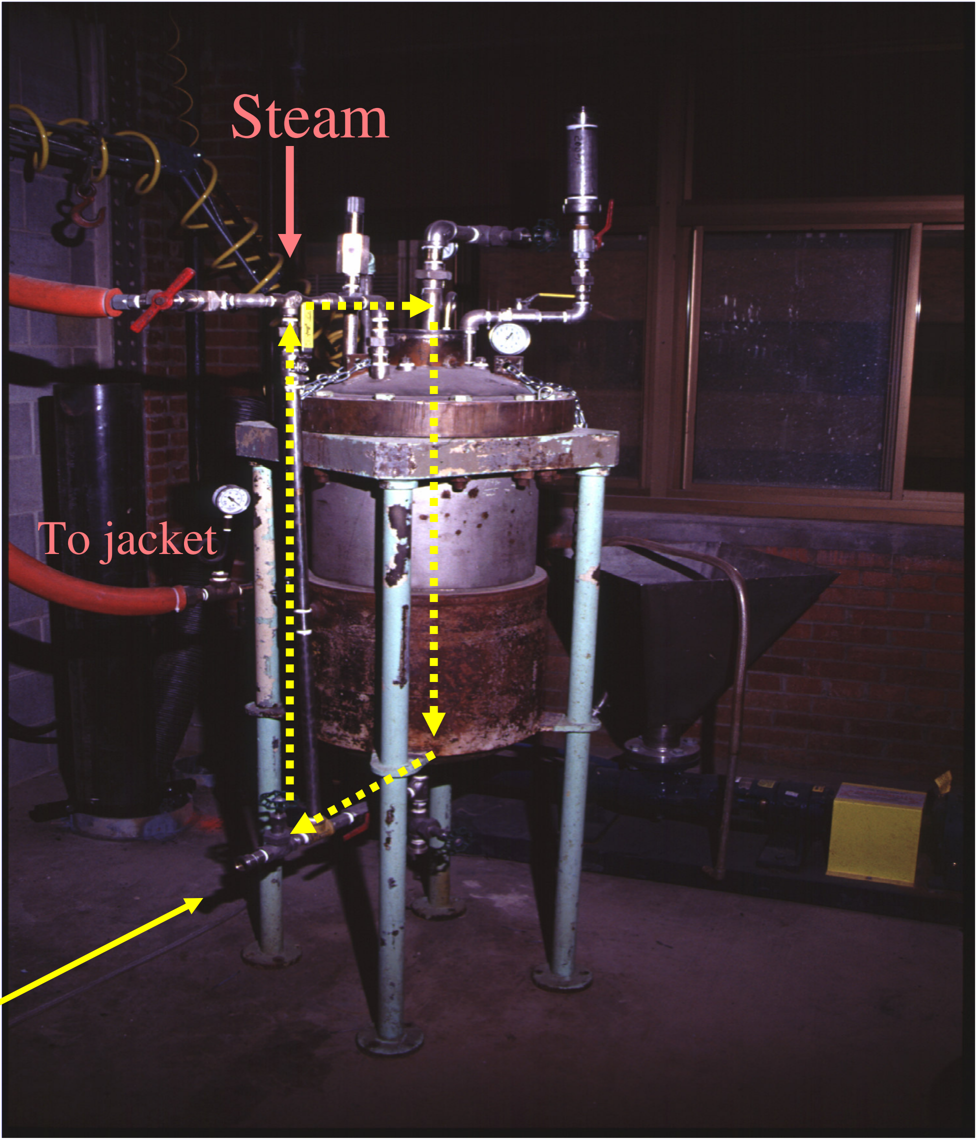
Solids
(cellulose)

Liq. Residues

Dry

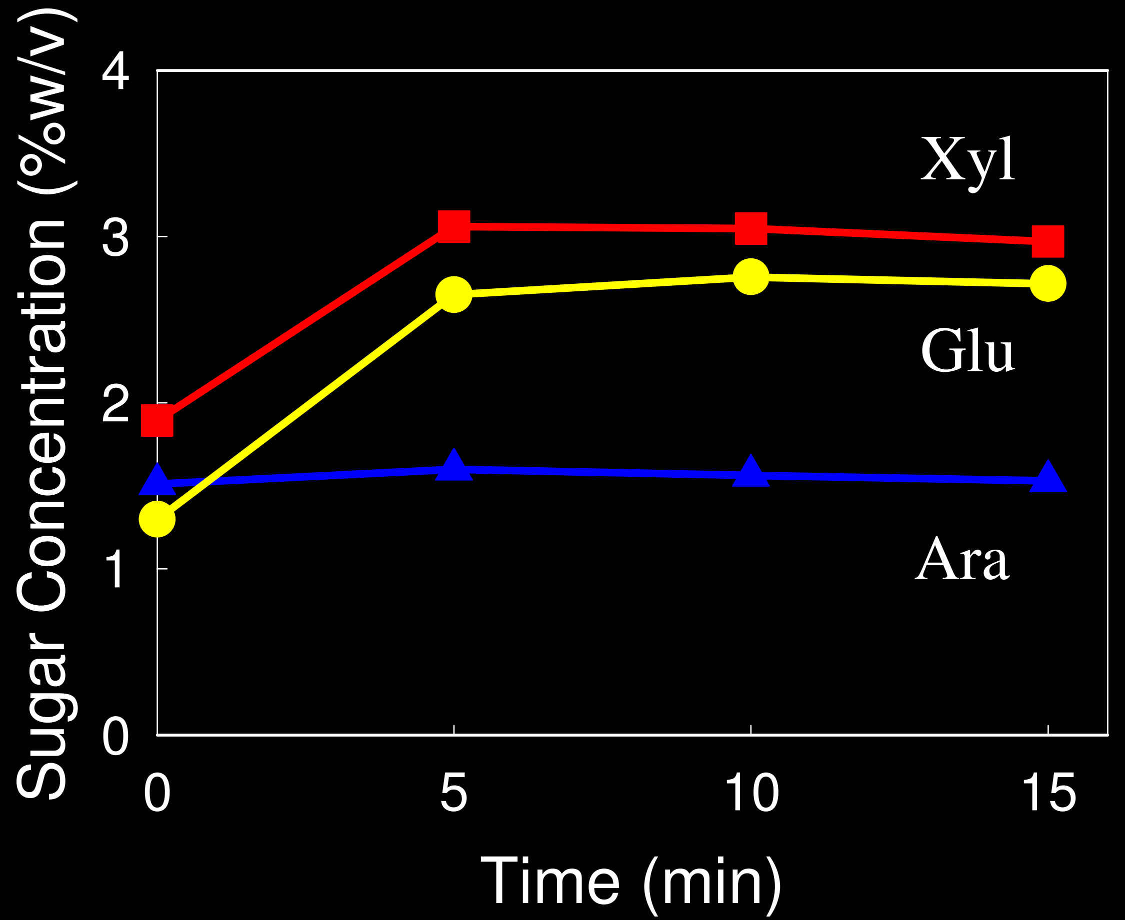
Animal Feed

Corn Fiber Reactor



Corn Fiber Slurry Enters Reactor

Rate of Corn Fiber Hydrolysis



Ethanol Producing Strains Capable of Fermenting Pentoses

Engineered to use pentoses
- *Zymomonas mobilis*
- *Saccharomyces*

Engineered to make ethanol
- *Escherichia coli*
- *Klebsiella oxytoca*

Natural microorganisms
- Pentose fermenting yeast
- Thermophiles
- *Saccharomyces* + xylose isomerase

Metabolic Engineering an ethanologenic bacterium

I. K12 converts glucose to mixed acids

Glucose → *wild-type K12* → Lactic Acid
Ethanol
Acetic Acid
Formic

II. Mutant does not ferment glucose (Dr. Clark, SIU)

Glucose → *pfl-, ldh-* → No Growth

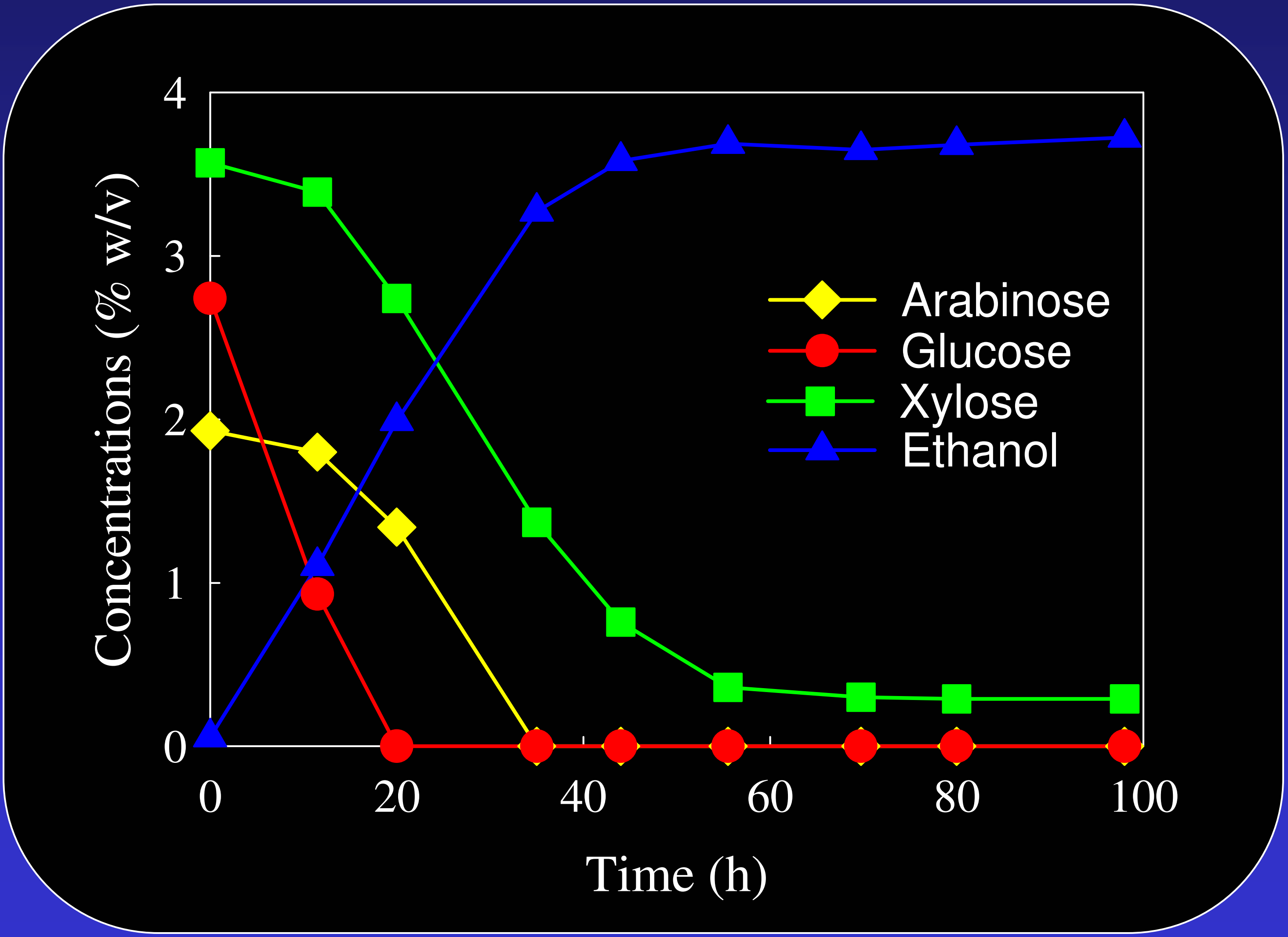
III. FBR5 ferments glucose selectively to ethanol (Dr. Ingram, U.Fl.)

Glucose → PET Operon → Ethanol

Ethanol Production Restores Anaerobic Growth

pfl-, ldh- **+ *pet* genes**

Ethanol Fermentation Of Corn Fiber Hydrolysate by *E. coli* FBR5



Fermenting Fibrous Components produced by Corn Milling

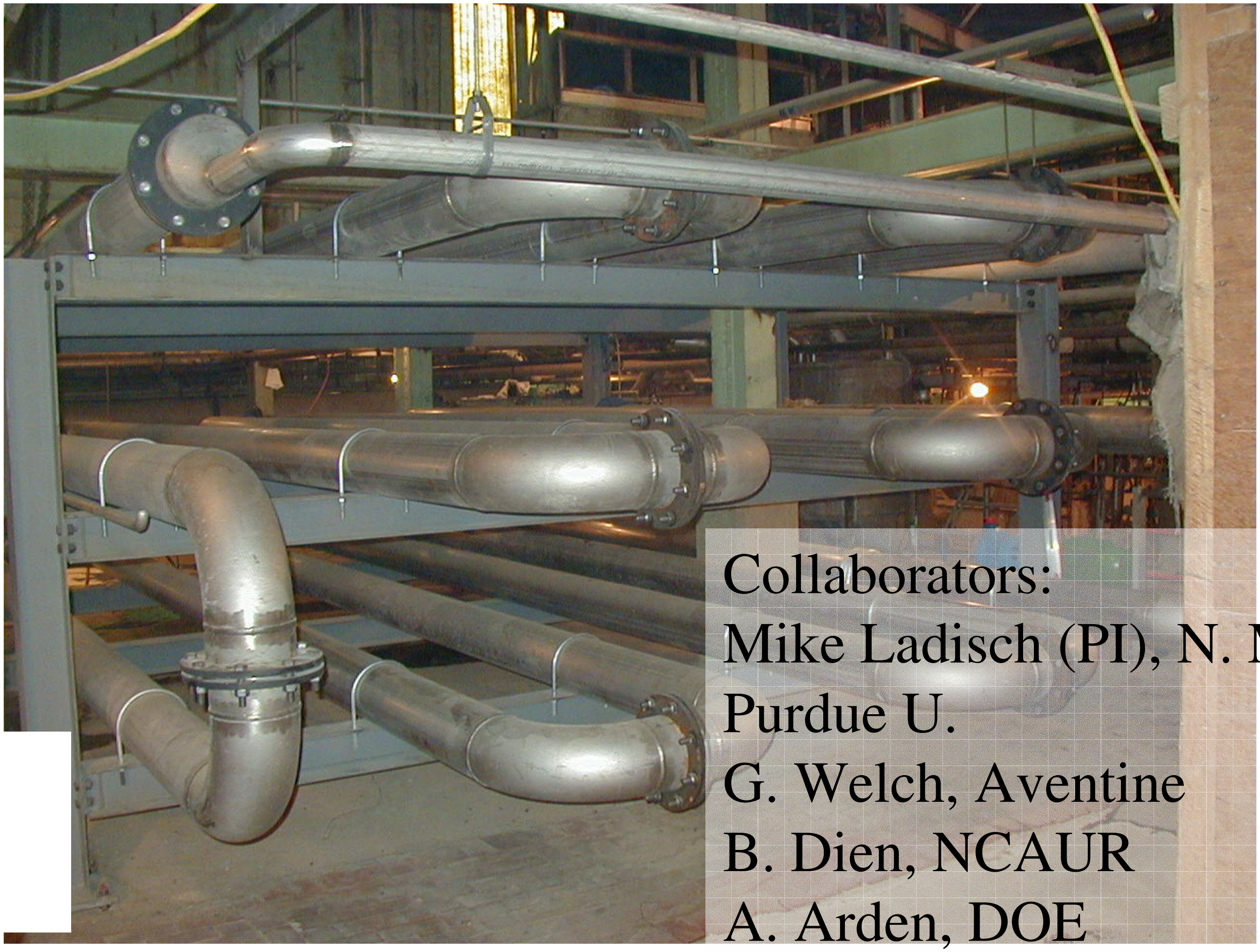
Feed stock	Sugars %w/v	Max. Ethanol %w/v	Ethanol Yield g/g	Ethanol Prod. g/l/h
DWG	3.98	2.12	0.49	0.71
Germ	4.15	2.19	0.50	0.56
Fiber	8.50	3.74	0.46	0.77

Comparison of Laboratory Microorganisms for Fermenting Biomass Hydrolysates

Pretreatment	Strain	Max ethanol (g/l)	Yield[1] (g/g)	Max. productivity (g/l/hr)
Dilute acid	*E. coli* K011	34.7	0.41	1.16
Dilute acid	*E. coli* SL40	31.7	0.42	1.12
Dilute acid	*E. coli* FBR3	28.0	0.46	0.97
Dilute acid	*Zymomonas* CP4 (pZB5)	22.6	0.47	1.05
AFEX	*Saccharomyces* 1400 (pLNH32)	21.0	0.50	1.60

- grams ethanol per grams sugar consumed; maximum possible is 0.51 g/g.

A review of more current work: Buffered hot-water pretreatment of corn fiber demonstration at Aventine Bioenergy



Aventine Bioenergy

Pekin, IL

Collaborators:

Mike Ladisch (PI), N. Mosier, Purdue U.

G. Welch, Aventine

B. Dien, NCAUR

A. Arden, DOE

Purdue's Buffered hot-water pretreatment

Advantages over dilute-acid:

- Lower capital costs
- Easy to integrate into process
- Maintain water-balance
- Does not generate gympsum
- Does not change color of corn gluten feed

Disadvantages compared to dilute acid:

- Does not completely hydrolyze hemicellulose

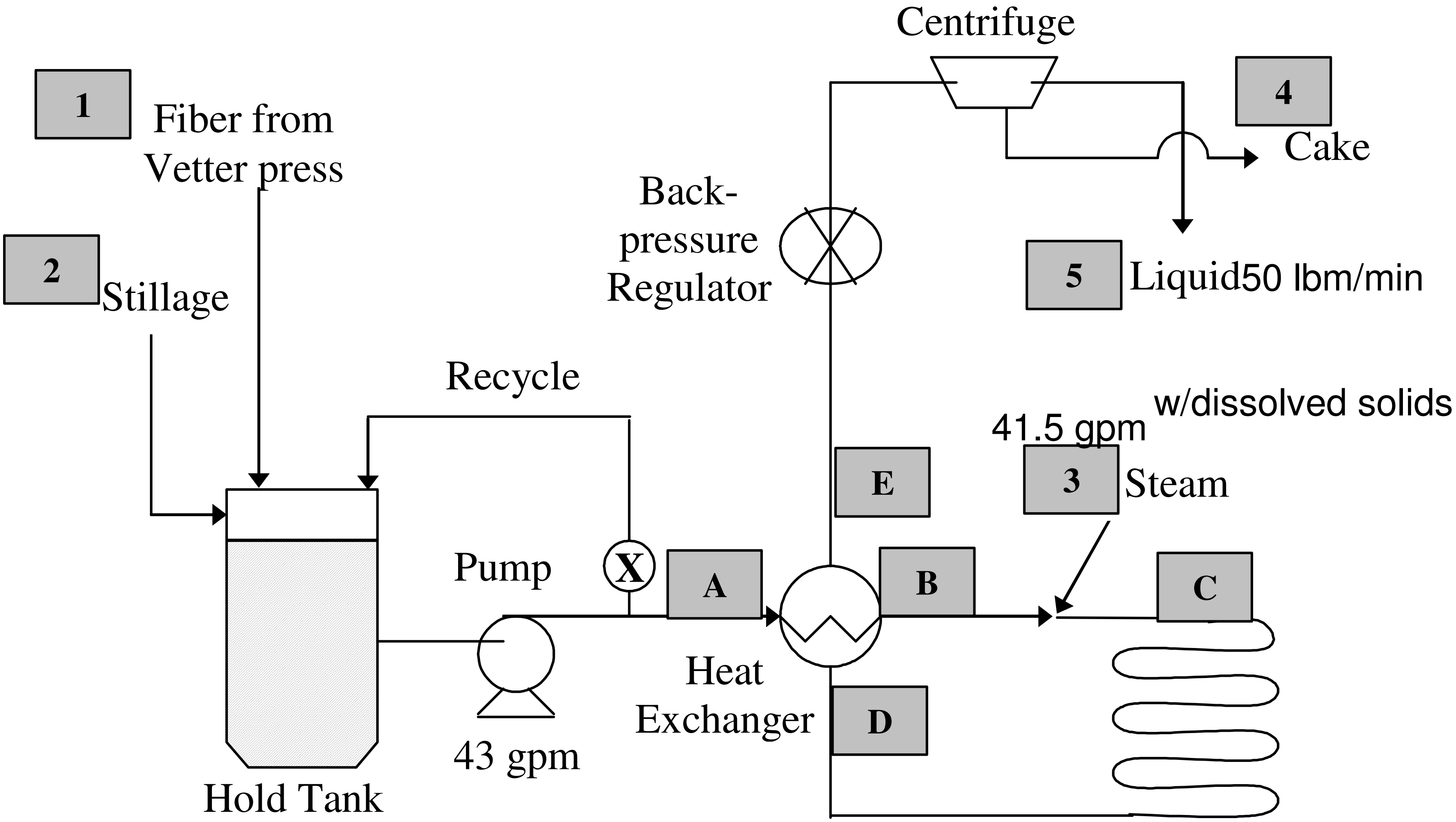
Conclusion: existing plant concerns trumped pretreatment concerns.

Principles of Liquid Water Pretreatment

a. Control (maintain) pH to prevent complete hydrolysis of the hemicellulose sugars – reduces formation of inhibitors
b. Use high temperature (160 – 180°C) to ensure disruption of cell wall and swelling of cellulose fibers

(Ladisch, et al.)

Pretreatment Flow Diagram



(Ladisch, et al.)

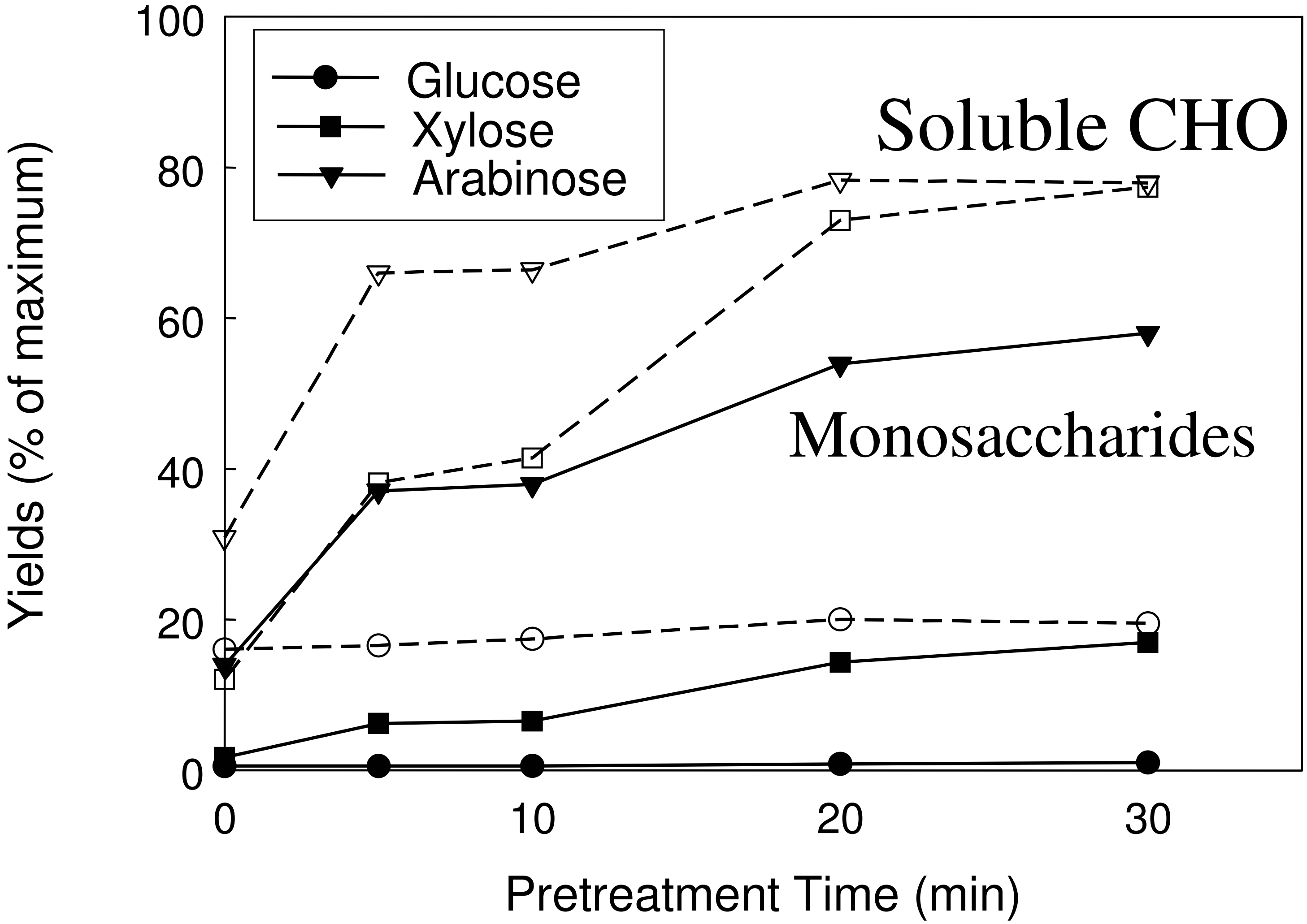
"Snake-coil" Plug Flow Pretreatment Coil

Aventine Bioenergy

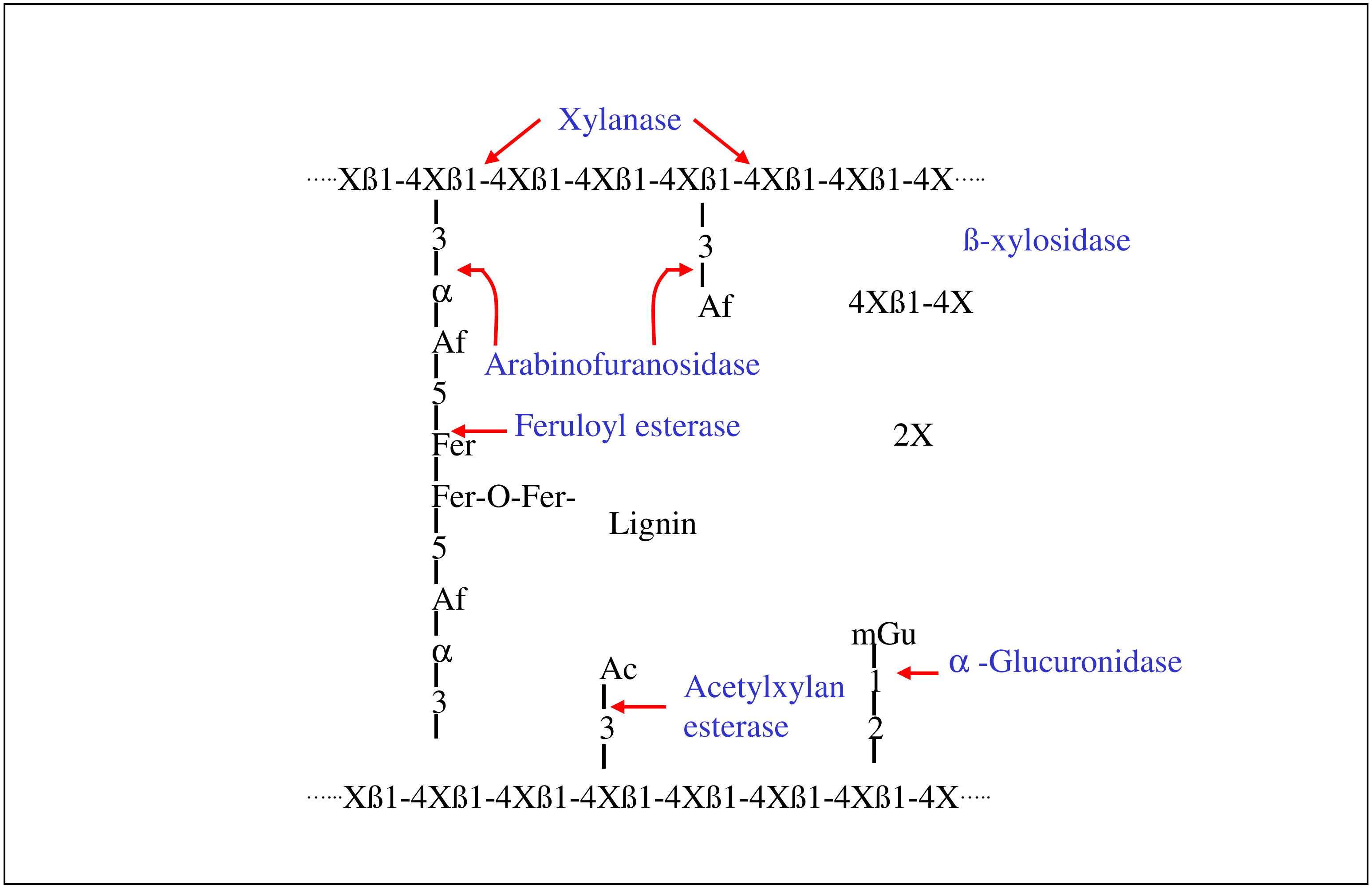
Pekin, IL

(Ladisch, et al.)

Release of sugars from corn fiber when treated with hot-water



Complex Mixture of Enzymes Needed to Degrade Arabinoxylan



Xylanase

·····Xß1-4Xß1-4Xß1-4Xß1-4Xß1-4Xß1-4Xß1-4X·····

ß-xylosidase

4Xß1-4X

2X

3

α

Af

Arabinofuranosidase

5

Feruloyl esterase

Fer

Fer-O-Fer-

Lignin

5

Af

α

3

3

Af

mGu

1

2

α -Glucuronidase

Ac

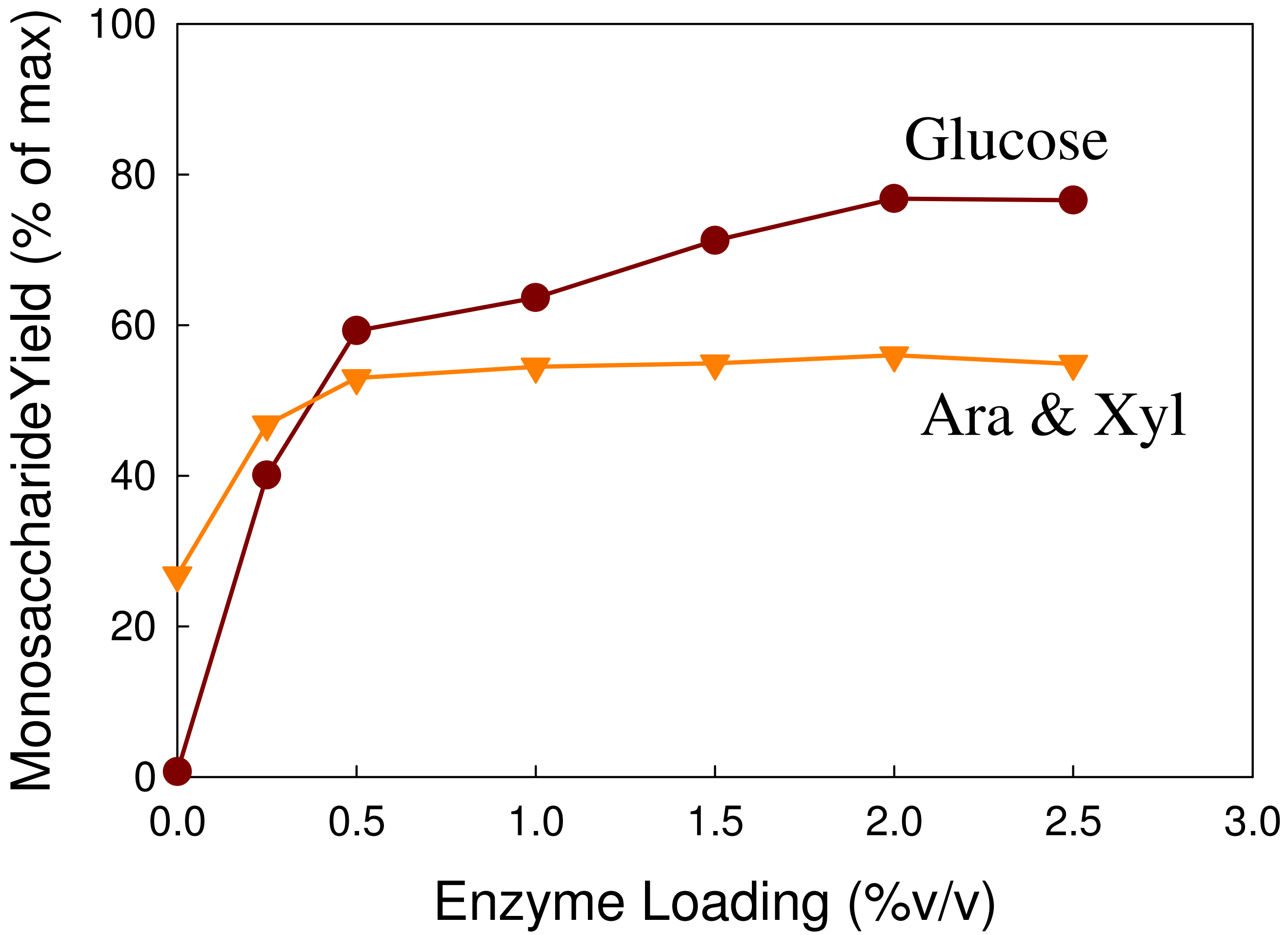
3

Acetylxylan esterase

······Xß1-4Xß1-4Xß1-4Xß1-4Xß1-4Xß1-4Xß1-4Xß1-4X·····

Selinger et al., 1996

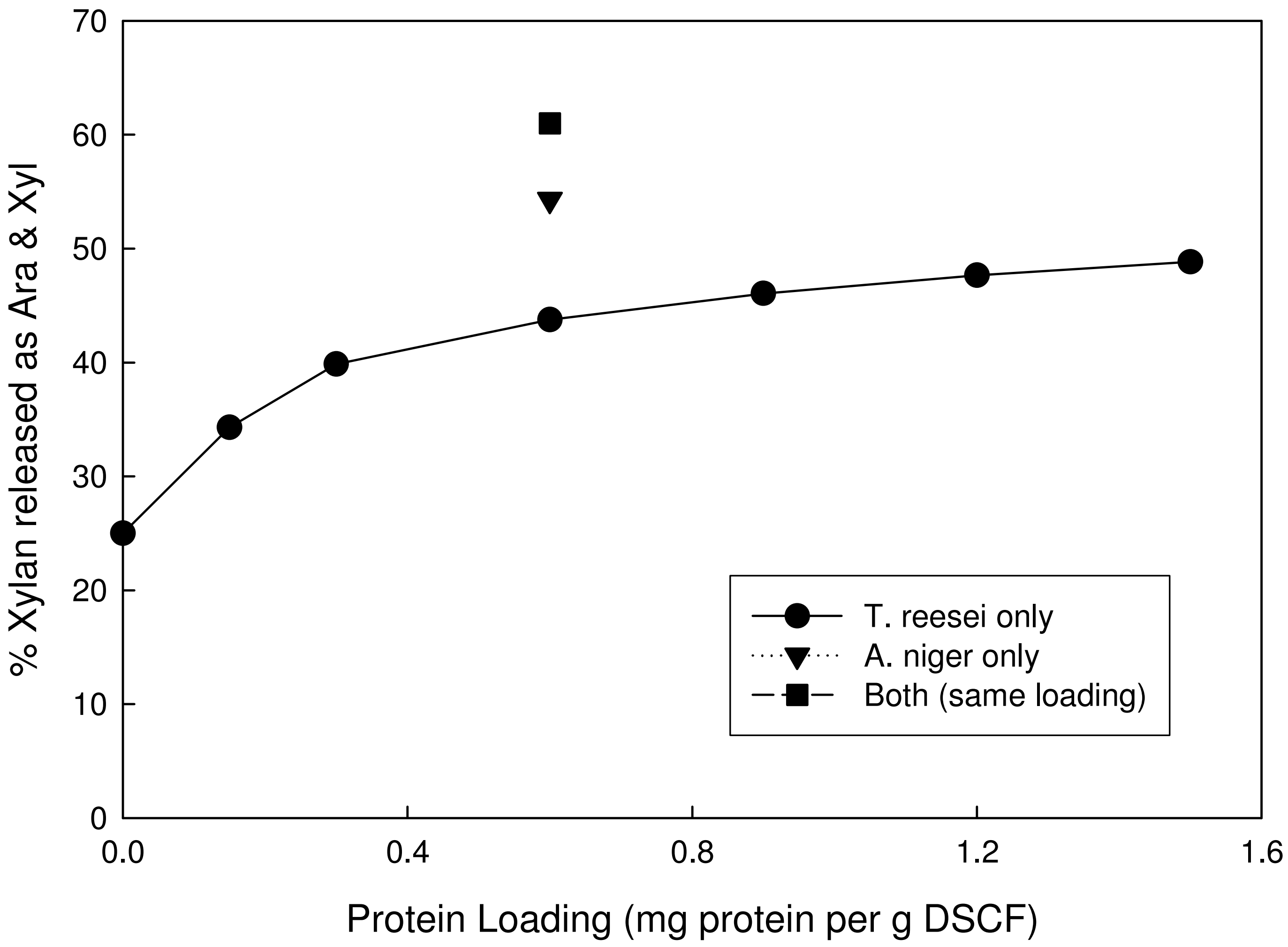
Digesting hot-water treated hot-water treated corn fiber w/ commercial enzyme



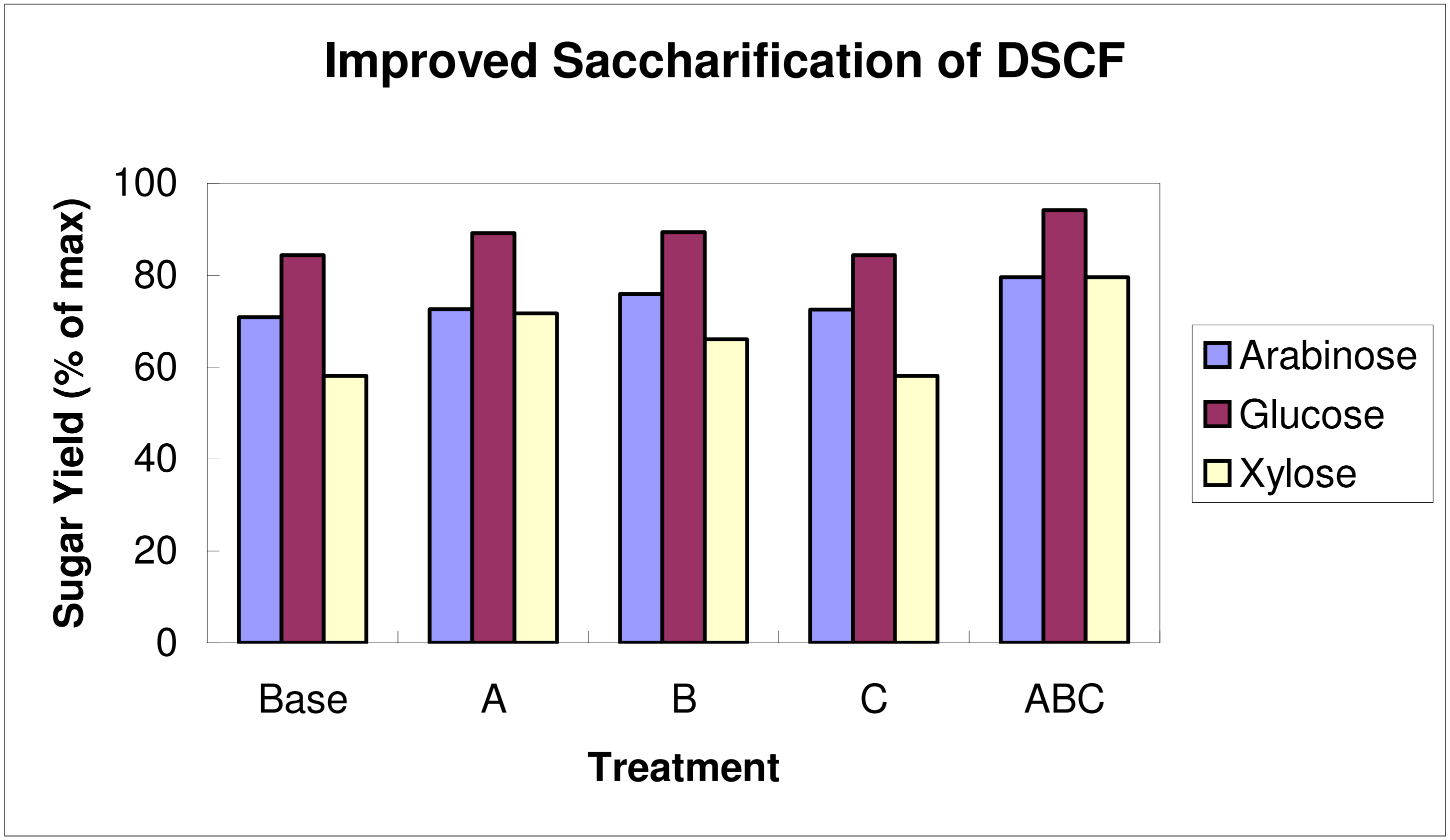
Preparing custom enzyme preparations by culturing fungi on corn fiber

Enzyme Preparation	Corn Fiber Pretreat.	Protein	Xylanase	Cellulase	FE Activity
		mg/ml	U/ml	U/ml	uM/m/ml
A. niger 2001	HW	1.24	113.5	5.0	2.25
A. niger 2001	Untreated	0.49	72.2	4.8	1.15
T. reesei RUT C30	HW	1.35	64.4	7.0	nd
T. reesei RUT C30	Untreated	1.42	46.3	8.3	nd

Enzymatic treatment of hot-water treated corn fiber

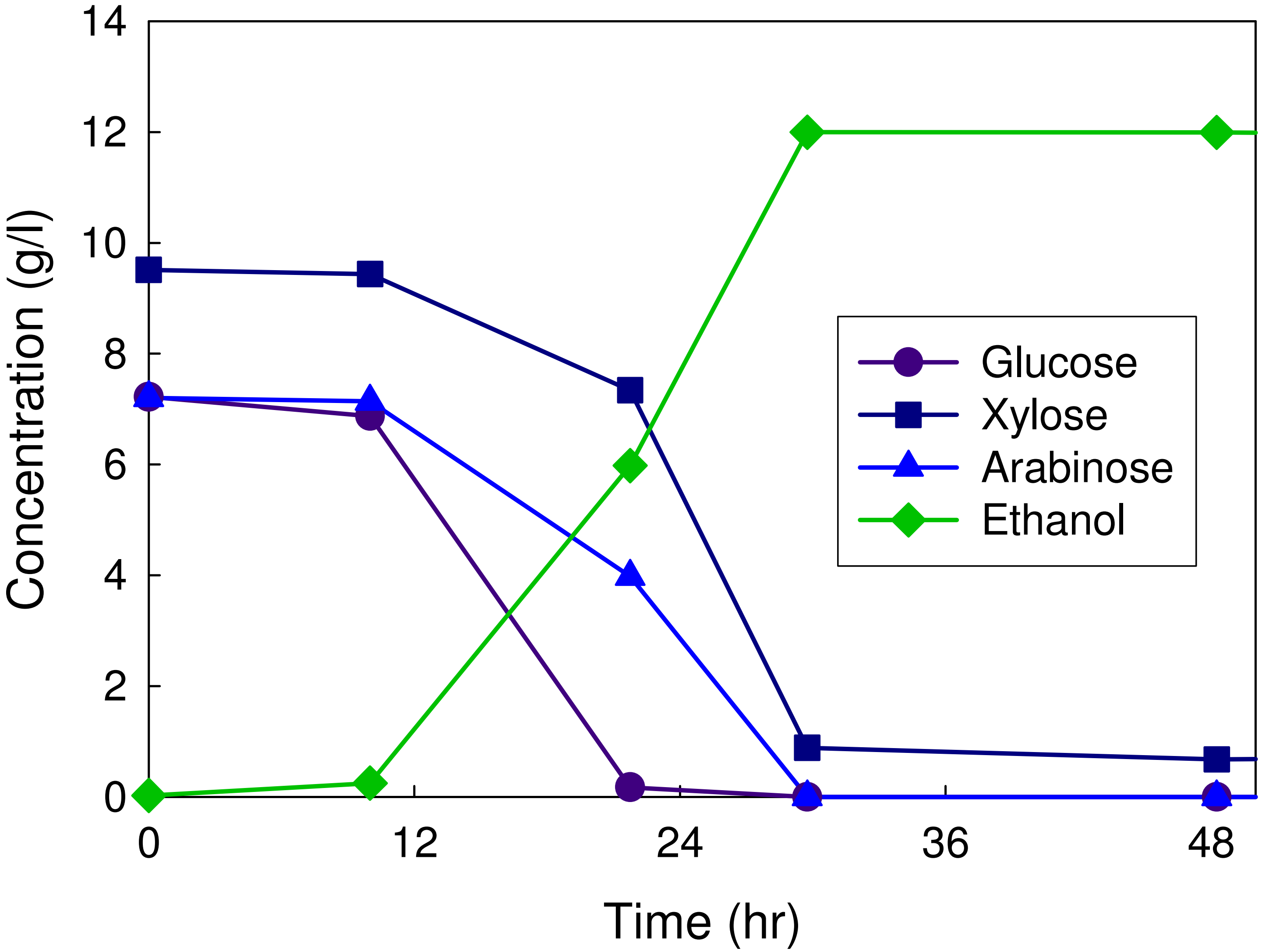


Factorial Design to Optimize Sugar Yields



A = increase from 20’ to 30’ pretreatment;
B = glucohydrolyases; C = feruolyl esterase

Fermentation of enzyme released sugars to ethanol



Summary

- Ethanol yield from corn can be increased 10% by converting fibers from the germ and pericarp into ethanol.
- Corn fiber can be converted to ethanol by treating with dilute sulfuric acid and fermenting with ethanologenic *E. coli*.
- Corn fiber can also be converted by pretreating with hot-water, but further work is needed to develop more efficient hemicellulases

Some Future Trends

- High-solids pretreatment of biomass
  - Lower chemical usage (e.g. acid or alkali)
  - Less energy required for heating
- High-solids saccharification or fed-batch SSF
  - More concentrated ethanol
  - Smaller unit operations
  - Possibility for using high-temperature enzymes

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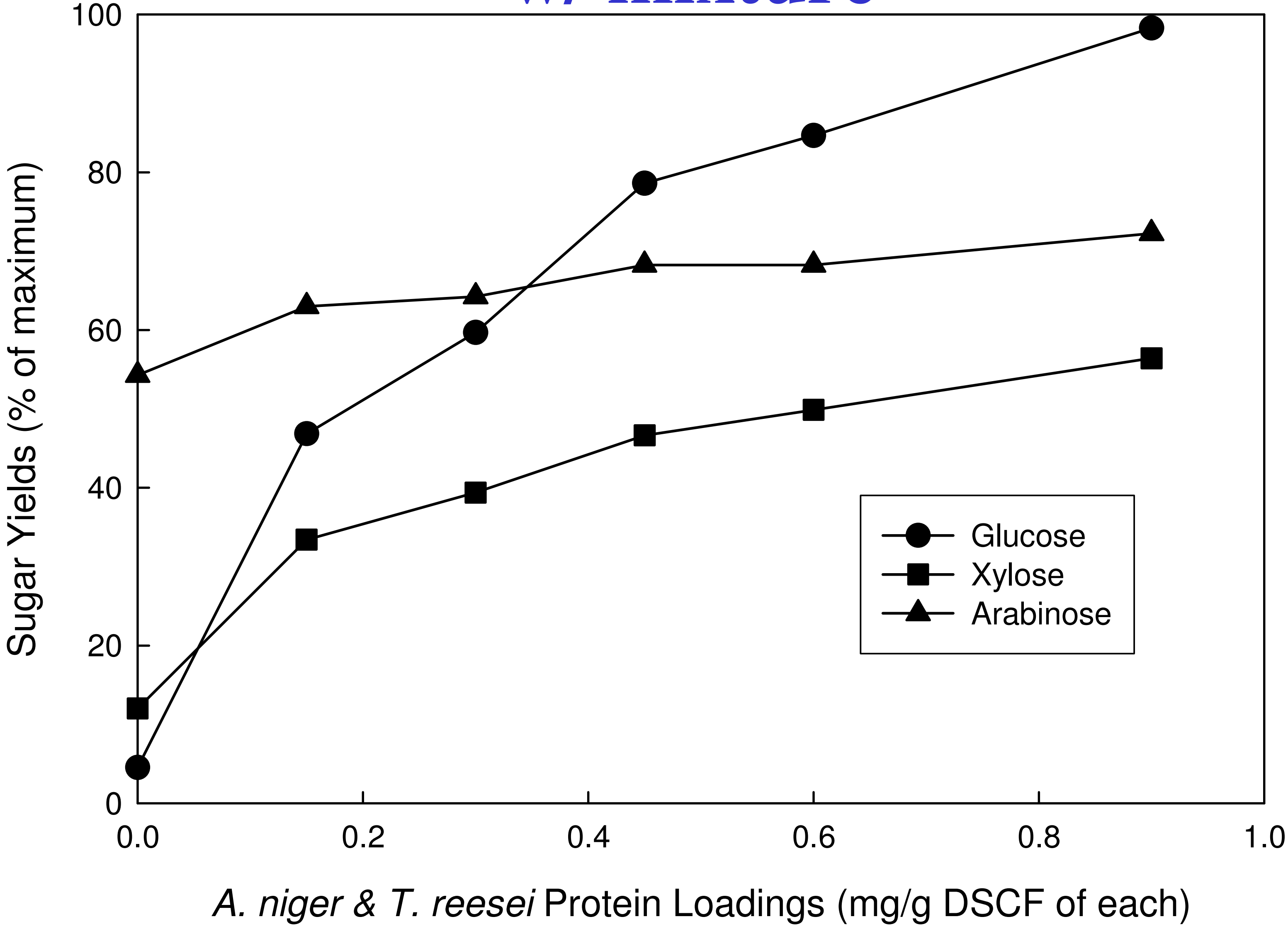
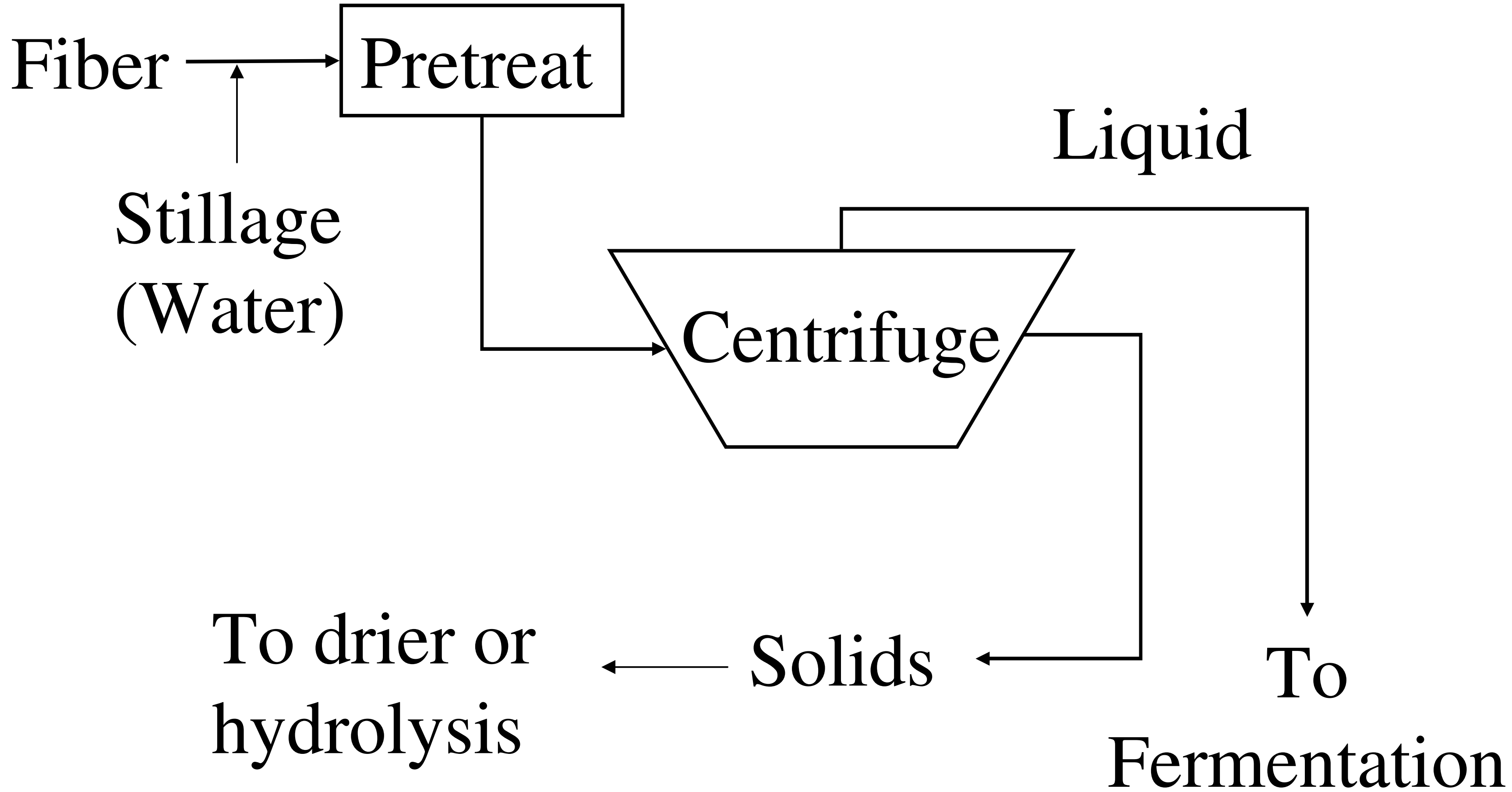
Enzymatic treatment of HW-CF w/ mixture



Process Description

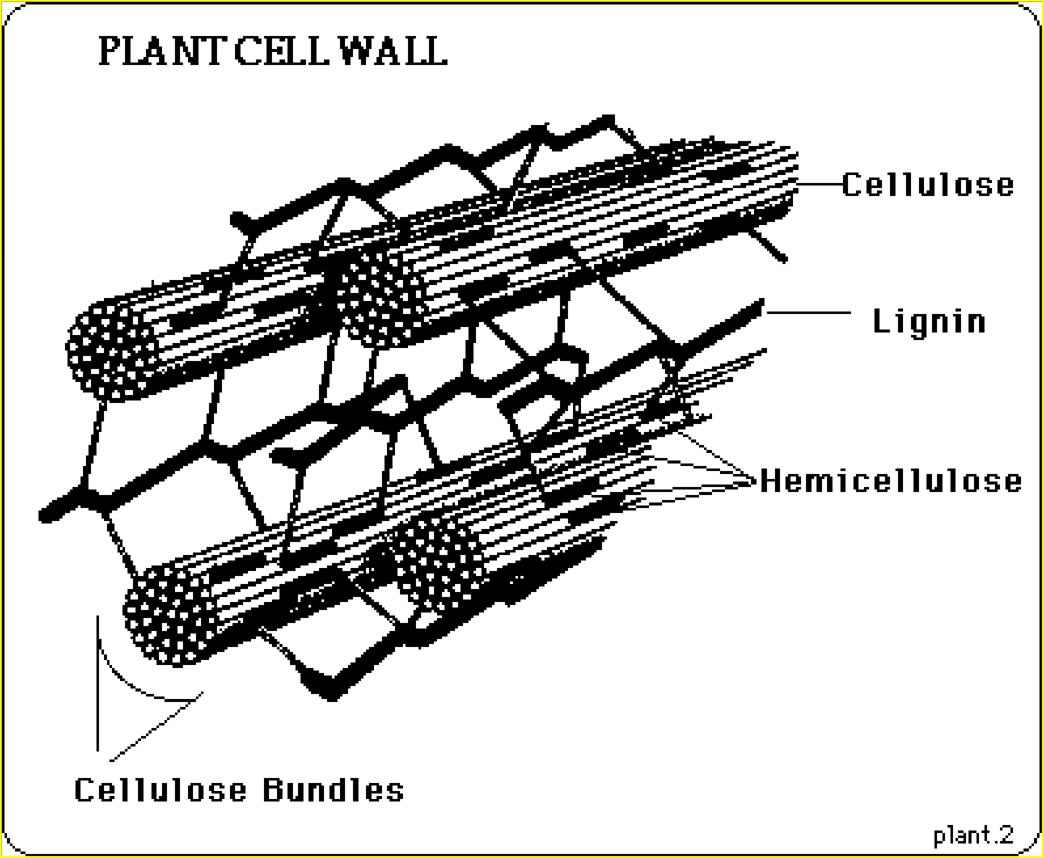
Pretreat Fiber and Liquid/Solid Separation



(Ladisch, et al.)

What is expected of a pretreatment?

Allow cellulase access to cellulose polymers by disrupting cell wall structure



- ✓ Dissolve Hemicellulose
- ✓ Displace Lignin
- ✓ Swell Cellulose Bundles

Chemical Mechanisms

Hemicellulose

Acid hydrolyzes, alkali dissolves, hot-water acts as week acid

Lignin

Molecular oxygen, ozone, peroxide break lignin ether bonds, alkali sponifies ferulic/arabinose ester bonds

Cellulose

Ammonia disrupts H bonds, solvents & conc. acid dissolves cellulose polymer