Fermentation of "Quick Fiber" Produced from a Modified Corn Milling Process into Ethanol and Recovery of Corn Fiber Oil

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Ethanol Dry Mills

- Over two billion gallons of ethanol were produced in 2002 in the U.S.
- Projected ethanol production is expected to more than double by 2010.
- Currently, 60% of all US ethanol is produced using dry mill technology.
- Traditional starch based operation using standard *S. cerevisiae* cultures.

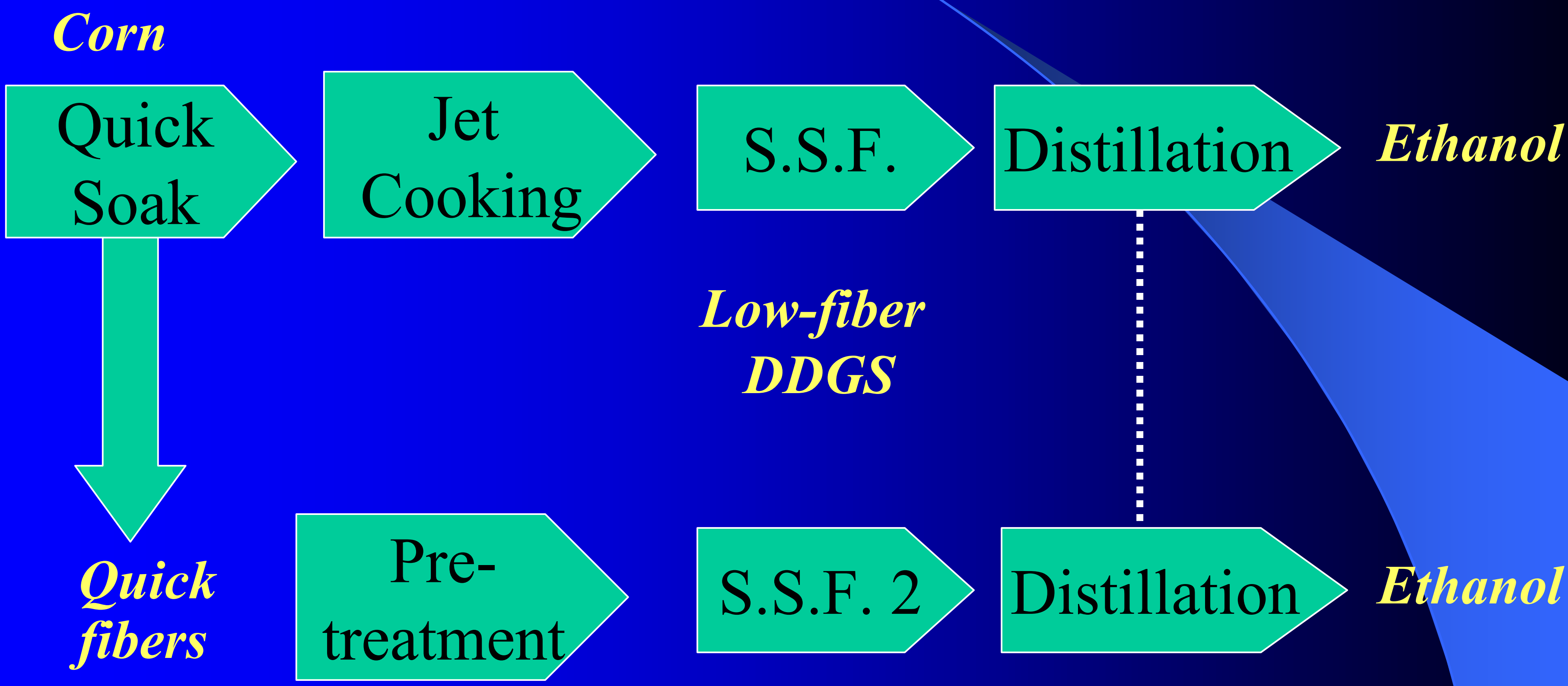
Ethanol Dry Mills cont.

- Two major co-products from the dry mill process:
  - Distillers grain (DG)
  - Carbon dioxide
- Over three million tons of DG are produced each year.
  - Used in feed formulations to replace soy bean meal for cattle.
  - Price and market concerns as DG production increases from increased ethanol production.

Quick Fiber Project Objectives

- Improve profitability of the dry mill ethanol industry.
  - Model the wet milling process with a diversity of co-products from corn processing.
  - Increase ethanol production.
  - Produce higher quality DDG and DDGS.
  - Increase efficiency for the dry mill process

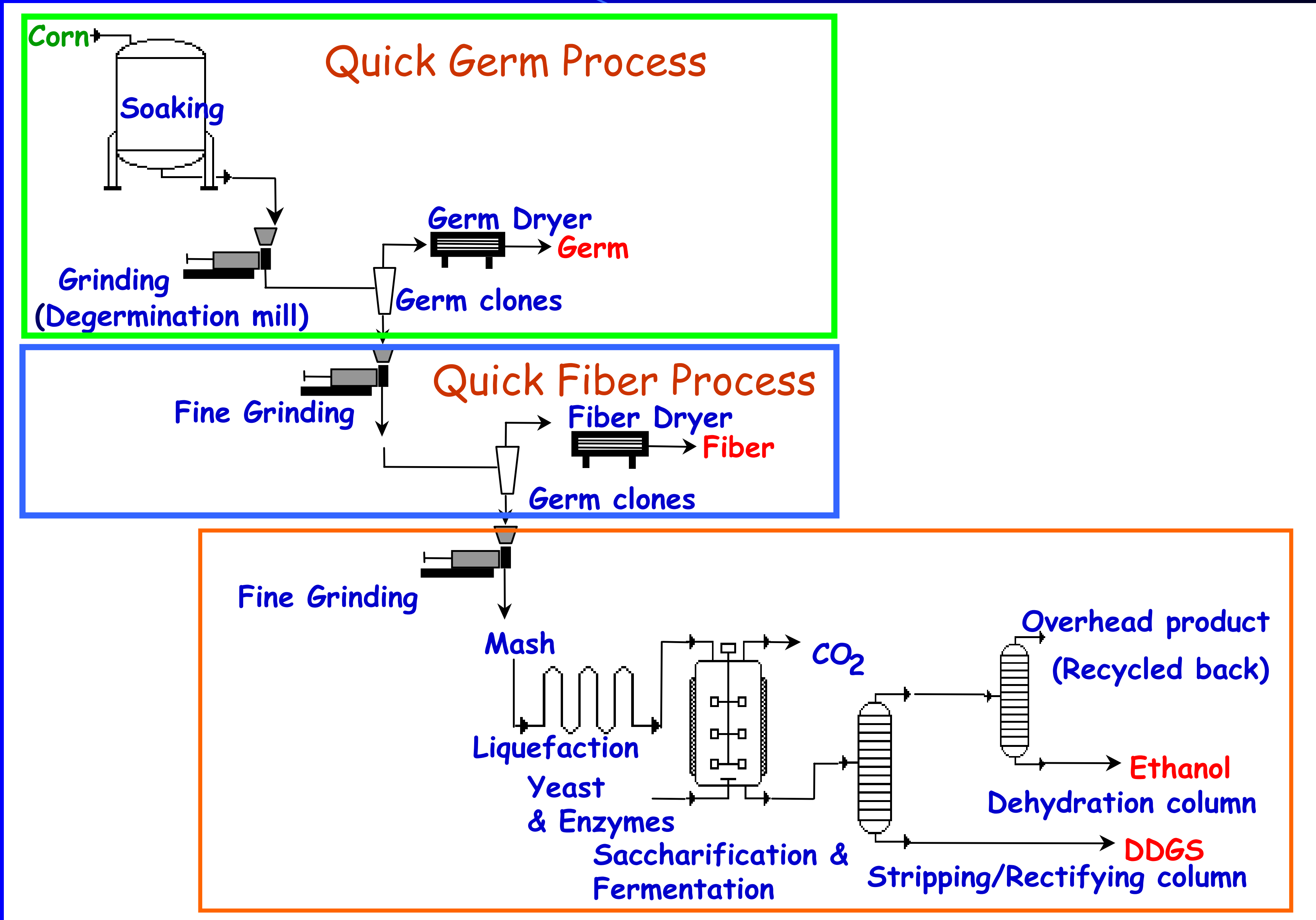
Quick Germ and Fiber Process* with Fiber to Ethanol Conversion



*Developed by V.J. Singh, U of IL US patent #6,254,914

*Cereal Chem. 1999 76(6):868-872

Germ & Fiber Recovery in Dry Mill Process



Fiber Composition

Component	Corn Fiber*	Quick Fiber*
Glucose **(Starch)	11-23	15
Glucose (Cellulose)	12-18	22
Xylose	18-29	17
Arabinose	11-19	11
Protein	11-12	11
Oil	3	1

- * % w/w db
- **anhydrous basis

Theoretical Ethanol Yield from a Bushel of Corn

Product (per bu)	Ethanol Yield (gallons)
Starch	2.5-2.7
Quick Fiber*	0.2

*Assumes 90% efficiency & 3.8 lb Q.F./bu

One bushel of corn weighs 56 lbs

One gallon of ethanol = 3.785 L = 6.58 lbs

Potential Co-Products from Quick Fiber Separation

- **Corn Fiber Oil**
  - Extracted from the pericarp, aleurone, and tip cap.
  - Contains phytosterols
  - Phytosterols have been recognized to lower serum cholesterol.
- **Corn Fiber Gums**
  - Comprised mainly of hemicellulose (arabinoxylan)
  - Can be used as a substitute for gum arabic (food emulsifier) or in industrial films and adhesives.

Experimental Protocol

- Pretreat Quick Fiber using dilute acid.
- Condition and neutralize resulting pretreatment residue and hydrolyzate.
- Determine bioconversion potential of pretreated residue using *S. cerevisiae* and the hydrolyzate using a recombinant *E. coli*.
- Extract oil from starting material, pretreatment residue and SSF residue.

Dilute Acid Pretreatment of Quick Fiber (Yeast Fermentation)

- **Solids loading of Quick fiber was 10-20% dry wt. basis.**
- **Acid loading at 3.2 % of H_2SO_4 per dry wt. of biomass.**
- **Temperature: 150°C.**
- **Hydrolysis Time: 10 minutes.**
- **After hydrolysis, hydrolysate was neutralized with $Ca(OH)_2$.**

Conditions based upon Grohmann and Bothast, 1993

Dilute Acid Pretreatment of Quick Fiber (Bacterial Fermentation)

- Quick fiber was ground using a small grinder.
- Solids were pretreated at 121^{o}C using 1% H_2SO_4 for one hour.
- Liquor was filtered from the solids and overlimed using $Ca(OH)_2$ for one hour.
- Liquor was neutralized to pH 7.0 and centrifuged to remove gypsum
- Final hydrolzate was filtered sterilized prior to fermentation

SSF Conditions

- Solids were loaded at 16.4% db.
- Cellulase (*15 FPU/g cellulose*), β-glucosidase and glucoamylase were added.
- Inoculated with S. cerevisiae and incubated at 32°C for 70 hr in a temperature controlled shaker at 150 rpm.
- Ethanol, sugars and organic acids were analyzed periodically.

Bacterial Fermentation

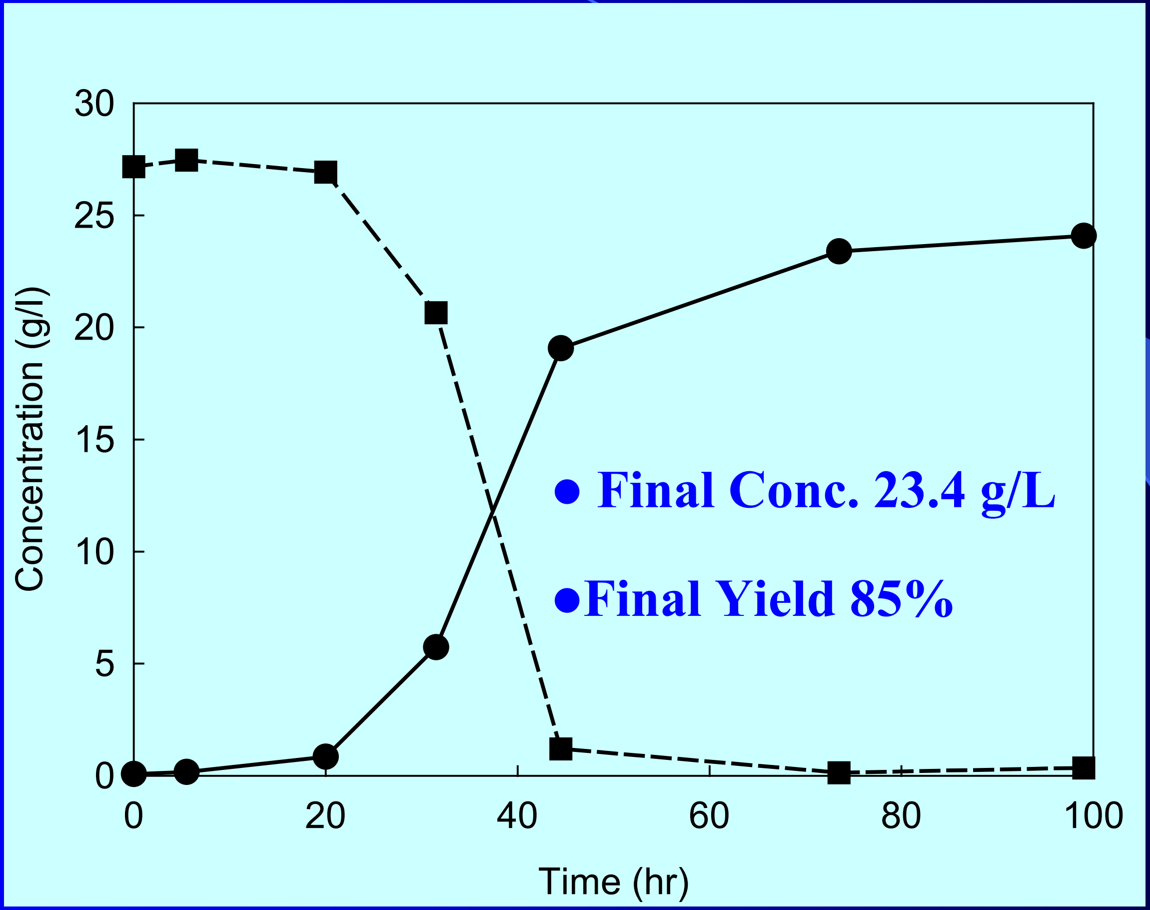
- Recombinant strain E.coli FBR5 was used to ferment all of the sugars in the hydrolyzate.
- Mini-bioreactors with pH control were used.
- No added cellulase – cellulose partitioned w/ solids.
- Inoculated with E. coli FBR5 at 5% v/v inoculum.
- Fermentation held at pH 6.5, 35^{o}C for 70 hr.
- Ethanol, sugars, and organic acids were analyzed periodically.

Pretreatment Results from the Hydrolysis of Quick Fiber Using Dilute Acid

% Acid Loading [a]	Glucose [b]	Xylose [b]	Arabinose [b]	pH[c]
0	75±0	16±0	39±0	4.41 ±0.07
0.8	92±2	50±6	77±2	2.78 ±0.00
1.6	90±1	74±9	86±3	2.12 ±0.07
3.2	92±3	98±6	98±4	1.89 ±0.07
4.8	87±4	98±8	87±0	1.56 ±0.09

[a] % g H_2SO_4 per g biomass
[b] % of theoretical yield
[c] measured following pretreatment

SSF of Pretreated Quick Fiber by S. cerevisiae

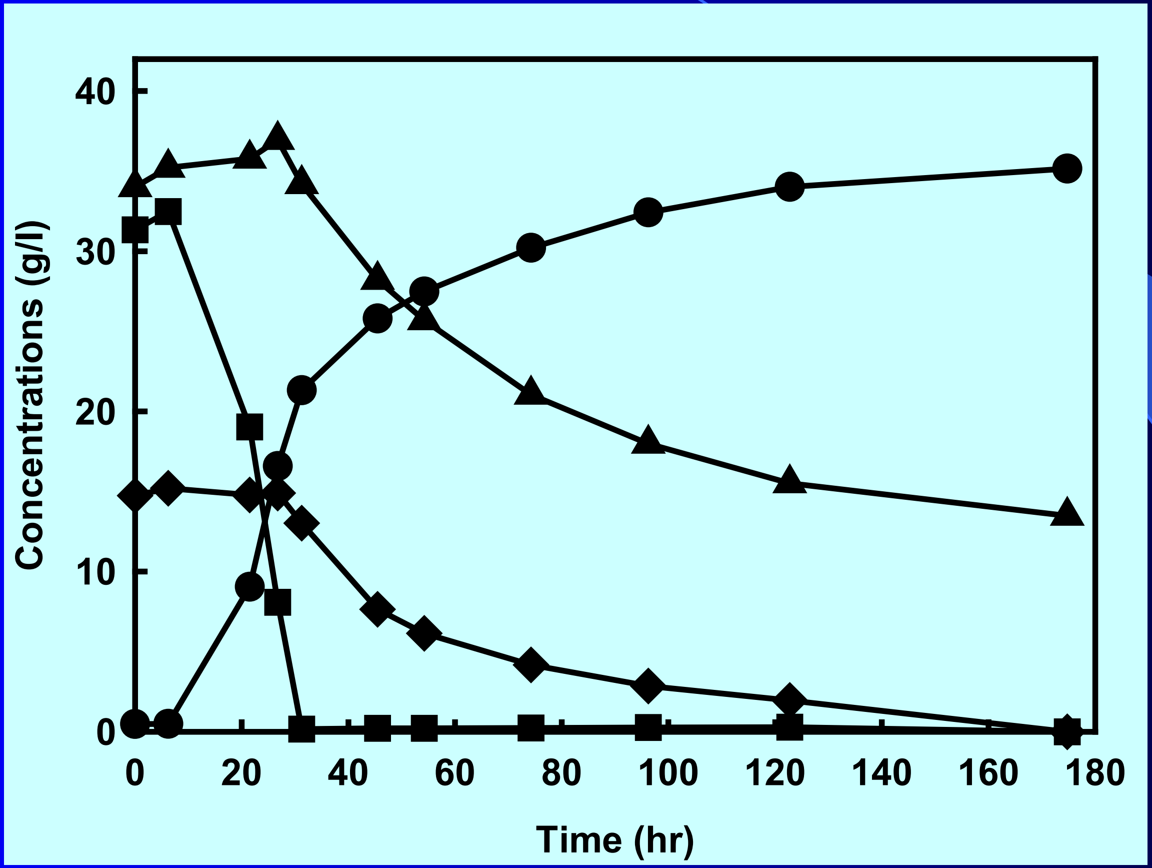


Legend: ■ Glucose ● Ethanol.

Fermentation Results For Various Fibrous Feedstock's Using Ethanologenic Strain FBR5

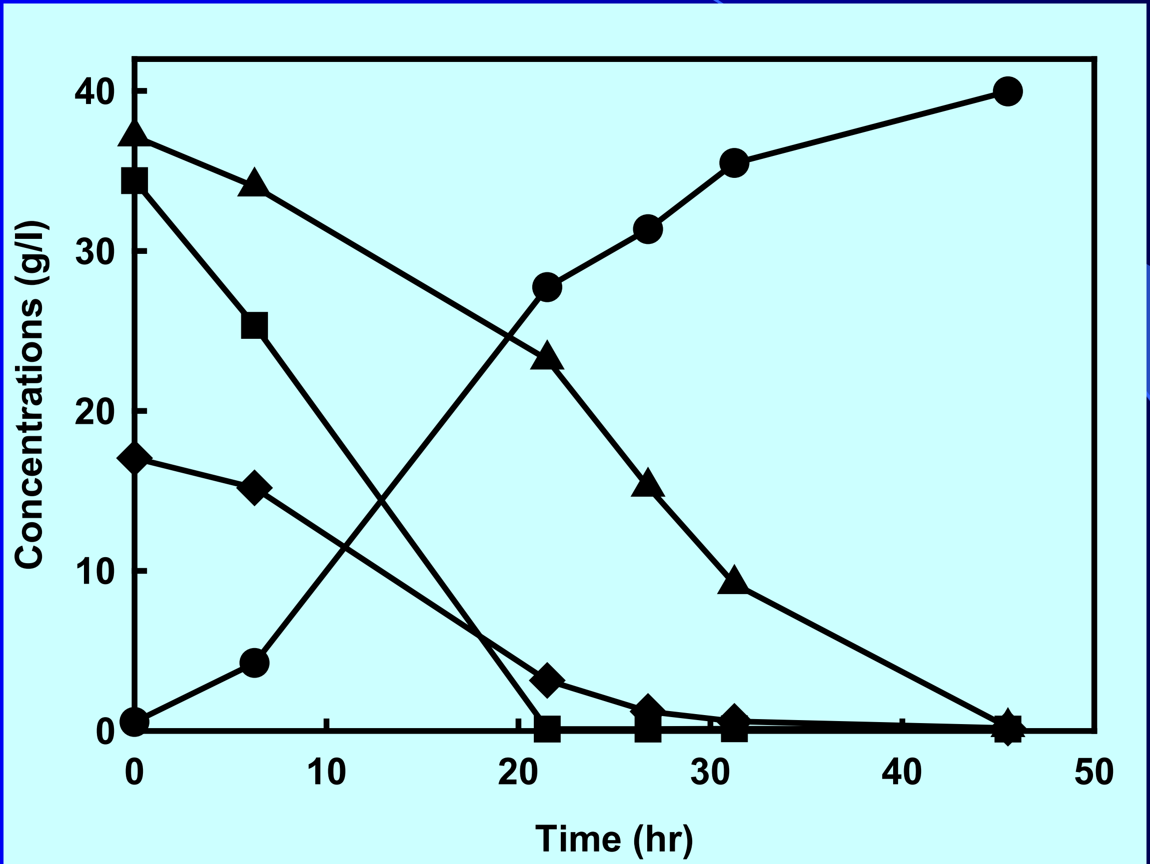
	Initial Hydrolyzate Sugar Concentrations % w/v			Maximum	Ethanol	Ethanol
Feedstock	Arabinose	Glucose	Xylose	Ethanol %w/v	Yield g/g	Productivity g/l/hr
Quick Fiber	1.47	3.13	3.40	3.52±0.03	0.44±0.00	0.43±0.04
DWG	0.79	1.96	1.23	2.12±0.05	0.49±0.01	0.71±0.01
Corn Fiber	2.00	2.80	3.70	3.74±0.01	0.46±0.00	0.77±0.05

Fermentation of Quick Fiber Hydrolyzate by Strain FBR5



Legend: ▲ Xylose ■ Glucose ◆ Arabinose, and •Ethanol

Fermentation of Control Sugar Mixture by Strain FBR5



Legend: ▲ Xylose ■ Glucose ◆ Arabinose, and •Ethanol

Recovery Of Corn Fiber Oil From Process Fiber Residues

Fiber Source	Total Oil % w/w	Free Sterol Yield w% oil	FPE[1] Yield w% oil	St:E[2] Yield %w oil
Pre SSF	7.9 ± 0.1	4.43 ± 0.19	3.27 ± 0.04	7.9 ± 0.1
Post SSF	1.8 ± 0.5	6.03 ± 3.74	5.82 ± 3.66	1.2 ± 0.57
Post FBR5 Ferm	12.2 ± 1.8	5.80 ± 0.79	4.29 ± 0.69	12.2 ± 1.8

[1] FPE= Ferulate Phytosterol Esters

[2] **St:E** = Phytosterol Fatty Acyl Esters

Conclusion

Quick Germ and Quick Fiber Process :

- Remove non-fermentable material from process stream.
- Increase fermentor capacity leading to increasing ethanol production.
- Achieve high yields of sugars resulting from pretreatment.
- Achieve high levels of bioconversion using C5 or C6 organisms.
- Lead to potential co-products from corn fiber oil and gum.
- Allow for the dry mill process to model the wet milling process using less capital investment.
- Increase profitability and efficiency of the dry mill process.

Future Work: Scale-Up Fiber Conversion
Acid Impregnation Study

30 qt. bread dough mixer

Fiber sprayed with red dye

Scale-Up of Fiber Conversion Using Several Types of Pretreatment Reactors

Zipperclave

4 L Steam Gun

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[3]Eastern Regional Research Center, Agricultural Research Service. USDA

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