

NEW TECHNOLOGIES IN BIOFUEL PRODUCTION

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INTRODUCTION

The National Corn-To-Ethanol Research Center (NCERC) on the campus of Southern Illinois University Edwardsville is the only facility in the world that fully emulates both a wet-mill and a dry-grind commercial fuel ethanol production plant. This flexible research facility was constructed to test new technologies on a pilot scale, a critical step in assisting in the determination of their commercial viability. The NCERC is ideally suited to validate new technologies for producing ethanol more cost effectively from corn. This capability will positively impact the economics and the speed of commercialization of technical advancements of this domestically produced, environmentally friendly renewable fuel. Expansion of fuel ethanol markets by using renewable feedstocks like corn is a national priority. Advantages of increased ethanol production include: increasing national energy independence, improving the environment, and promoting rural development. In 2004, 3.4 billion gallons of fuel ethanol were produced from over 10% of the corn crop. Ethanol demand is expected to more than double in the next ten years. For the supply to be available to meet this demand, new technologies must be moved from the laboratories to commercial reality.

CURRENT ETHANOL PRODUCTION PROCESSES

Today, most fuel ethanol is produced from corn by either the dry-grind (67%) or the wet-mill (33%) process. Current technologies allow for 2.5 (wet-mill) to 2.8 (dry-grind) gallons of ethanol per bushel. Recent growth in the industry has been predominantly with dry-grind plants because of (2-4x) less capital costs/gallon and incentives for farmer-owned coops. These incentives also promote markets and jobs in rural America. The wet-mill industry has relied on expanding existing facilities rather than building new plants. A schematic of both processes is shown in Figure 1.

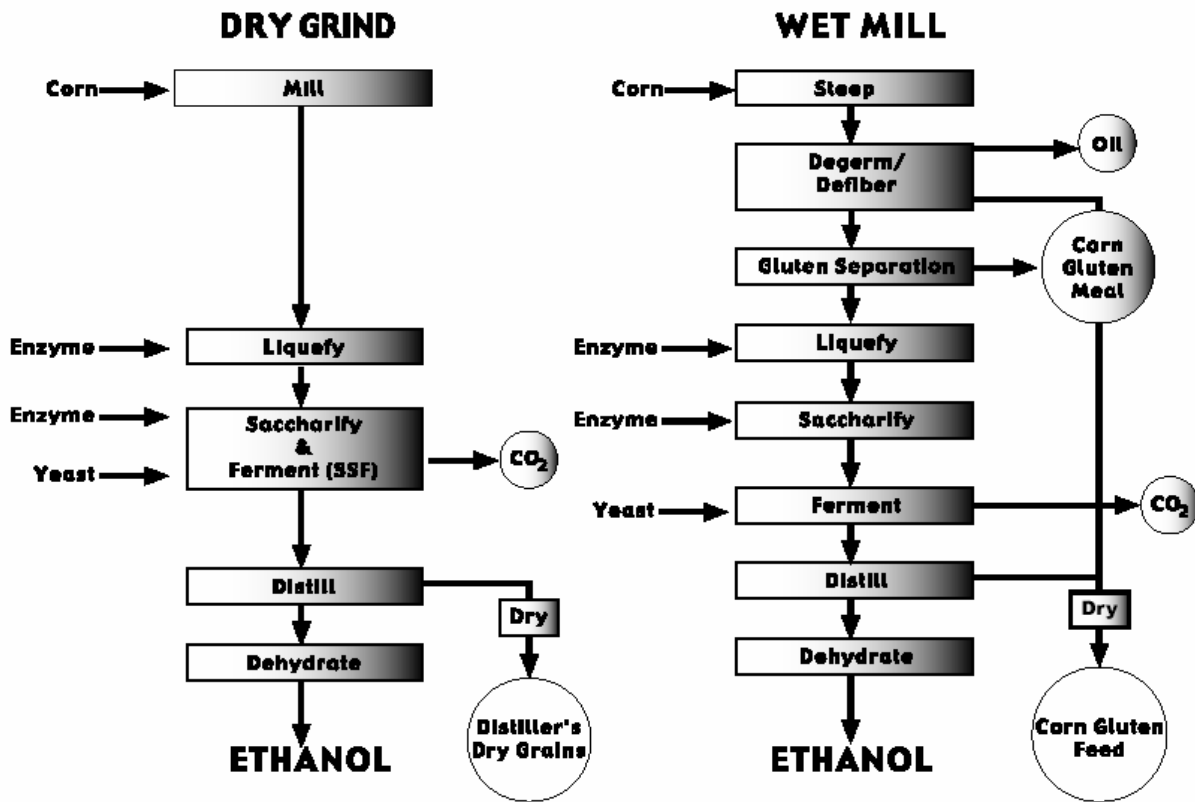


FIGURE 1: ETHANOL PRODUCTION PROCESSES

FUTURE DIRECTIONS

A number of surveys have been conducted by the Renewable Fuels Association, the Illinois Department of Commerce and Economic Opportunity, the Illinois Corn Growers Association and the Iowa Corn Growers Association to determine the research priorities of the industry. The national priorities in ranked order are: New Coproducts, Plant Emissions, Fermentation, Feedstocks, Fiber Recovery, DDG(S), Separation, Pretreatment, Saccharification, Germ Recovery, Distillation, Starch Hydrolysis, and Carbon Dioxide. For the sake of this presentation, we have highlighted four areas (high fermentable hybrids, recovery of new and high-value ethanol coproducts, new process and enzyme technology, and conversion of fiber (biomass) component of the kernel to ethanol with the best near term opportunities to produce ethanol more cost efficiently.

A. High Fermentable Hybrids

There is considerable interest today by seed companies to market specific hybrids bred for enhanced ethanol production. Corn hybrids are being developed with higher extractable starch or with higher fermentable starch content for wet mill and dry grind ethanol

production respectively. Early efforts are in selective breeding versus a transgenic approach. The two largest U.S. corn seed companies, Pioneer and Monsanto, both have ongoing research efforts to identify and develop new corn hybrids with these features and to understand the impact of agronomic practices and the environment on the hybrid characteristics. Additionally, both have commercial seed corn today specifically labeled for the ethanol industry. Not yet evaluated in either of these programs, but important research to be conducted, is the impact on the composition of coproducts from hybrids resulting in higher ethanol production.

Pioneer's research efforts include work to both generate hybrids more conducive to the ethanol production process and to understand how to optimize these characteristics under field conditions. Pioneer laboratory and commercial testing has demonstrated that corn hybrids differ in ethanol yield potential. When samples of corn grain, independent of hybrid and environment, were compared in a lab-scale fermentation assay, the highest ethanol-yielding samples produced about 15% more ethanol than the lowest ethanol-yielding samples. When the effects of growing environment are controlled and hybrid difference are compared, the highest ethanol-yielding hybrids produced 6% more ethanol than the lowest ethanol-yielding hybrids. Commercial testing of grain from these hybrids, now designated as "High Total Fermentables" (HTF) has shown that ethanol yields of up to 4% above mixed commercial grain are possible. This could mean an increase of \$1M-\$2M in profitability for a 40 million gallon per year ethanol production facility. Their research has shown that the HTF trait is a more accurate indicator of dry grind ethanol production than total starch or extractable starch.

Pioneer has analyzed data from over 18,000 of corn grain from North America including inbreds, hybrids, waxy hybrids, high/low moisture, various drying temperature, and samples containing elevated foreign matter to create a laboratory spectroscopic method using whole-grain Near Infrared (NIR) technology for predicting the ethanol yield potential of corn grain samples. In November 2004, Pioneer donated a royalty-free license of this technology to the National Corn Growers Association to allow broad market access to this predictive system. (Haefele, 2004)

Pioneer's efforts to understand the impact of agronomic practices and environmental conditions in optimizing hybrid production for the ethanol industry has also progressed significantly. Their research to-date suggests an optimum exists for grain yield and extractable starch or total fermentables based on plant population and applied nitrogen per acre. Pioneer recommends hybrid selection for yield and agronomics first, followed by the hybrid designation as either high total fermentables or high extractable starch. Additionally, their research suggests that managing the field for optimum yield will also maximize extractable starch or total fermentables (O'Bryan 2004, Haefele 2004).

Monsanto's efforts have focused on the dry grind industry. They have also developed a list of hybrids for ethanol production termed "Processor Preferred Fermentable Corn" (HFC) geared toward dry mills and High Extractable Starch (HES) geared towards wet milling. For the 2004 crop year, these hybrids were offered in nearly 60 independent seed brands in addition to the Monsanto DEKALB and Asgrow brands. Additionally, Monsanto also

provides NIR instrumentation to the 23 plants participating in their marketing program (Anderson 2003). Monsanto reports that in over two years of testing of over 5 million bushels they have demonstrated a 2.7% average ethanol yield increase with the HFC hybrids.

Syngenta, under the Northrup King brand, Agrigold and additional Regional Seed companies have also begun to market hybrids reported to enhance ethanol yields. Syngenta Seeds entered the market in 2004 with the NK Brand Extra Edge corn hybrid geared towards dry grind ethanol production. Currently 27 corn hybrids carry the NK Brand Extra Edge label. Another area of industrial interest is illustrated in the efforts of Syngenta Biotechnology to direct the accumulations of starch hydrolyzing enzymes in the endosperm of transgenic corn kernels (Craig et al. 2004). Stable accumulations of enzymes, without detriment to grain viability and composition, allows “processing capability” to be built into the grain itself. Self processing grains can be designed to meet specific, and novel, process constraints due to flexibilities in engineering enzymes with distinct biophysical properties and enzymatic specificities.

Longer term efforts to create modified starches or other complex carbohydrates from genetically modified corn are intended to provide new functionalities that will make possible additional markets and products for corn. These hybrids may result in starch with improved gelling properties, viscosity, and temperature stability, improved flavor or flavor stability, improved adhesion or film formation or properties that enhance the efficiency of processing.

B. New and High Value Coproducts

As new technologies are implemented, adding value to coproducts is essential to the profitability of the fuel ethanol business. (Anonymous NRC 1999) This will require a more holistic approach to ethanol production in the dry grind plant. Optimization of coproducts as well as ethanol yield must be considered. A number of new processes have been developed in the laboratory. Examples include the Quick Germ (Singh and Eckhoff 1996), Quick Fiber (Singh et al 1999), and Enzymatic Milling (Johnston et al 2003) as well as the COPE Process (Cheryan 2002). These process modifications may allow cost effective removal of coproducts such as corn oil, zein, germ, pericarp fiber and endosperm fiber at the beginning of the process, prior to fermentation. Potential benefits of these processes are: 1) recovery of high quality corn germ oil and fiber for corn fiber oil, 2) an increase in protein quality of residual DDGS after fermentation and 3) additional production of ethanol per batch. All await validation at the pilot scale. Within the concept of “biorefining,” a cadre of products can be listed (Leathers 1998; Wyman 2003). Examples include: corn fiber oil, sweeteners, polysaccharides, pharmaceuticals, nutraceuticals, fibers, biodegradable films, organic acids, solvents, amino acids, pigments, enzymes, polyols, vitamins, etc. Also in the coproduct arena, today’s DDGS feed customers are asking for more information than the traditional moisture, protein, fat and fiber analyses. Animal nutritionists want complete nutrient profiles of the ingredients and they want to know the variability of these nutrients as well as the ability to select which nutrients they need. In Minnesota, a certification program for DDGS has been developed that is resulting in market premiums for certified DDGS (Bryan 2003). Research projects are underway that would modify the amino acid composition, protein composition, or phosphorous content of DDGS. DDGS market expansion beyond cattle to

swine, poultry and aquaculture is dependent on improving the quality and consistency of the DDGS coproduct. In addition to feed uses, numerous other uses for DDGS are finding their way to the marketplace. Examples include: deicers, cat litter, “lick barrels”, worm food, etc.

C. New Process and Enzyme Technology

Alcohol fermentations from ground rice and maize without cooking was successfully carried out on a laboratory scale at Kyushu University, Japan, in 1963. Twenty years later, Suntory, Central Research Institute, Osaka, published the results of the first successful industrial scale alcohol fermentation from grains with a non-cooking system. This system allowed mashing at an extremely high concentration (1:2, maize:water) because starch in the uncooked mash did not gelatinize. Such high solid concentrations improved productivity and yielded high concentrations of alcohol which helped control bacterial contamination. This past fall, similar “raw starch hydrolysis” processes were unveiled by two of the leading process technology firms, each collaborating with a separate enzyme producer. ICM Inc. working with Genencor International is commercializing a technology that involves the direct conversion of starch to ethanol without a cook step. Likewise, Broin Companies announced that it had filed patents on a new process developed under the name Broin Project X (BPX) that utilizes a patent-pending enzyme system developed with Novozymes. Genencor claims that the benefits of a no-cook process include: a continuous supply of glucose; reduced capital, raw material, energy, water and waste costs; improved bioconversion efficiency resulting in increased yield; improved DDGS final product quality; improved product recovery; and lowered overall manufacturing costs. Through ICM laboratory and pilot-scale testing at the National Corn-to-Ethanol Research Center, ICM Inc. has developed a commercial system. Testing is continuing at a functioning Fagen/ICM ethanol plant. According to Broin, BPX will reduce energy cost, release additional starch for conversion to ethanol, increase protein content and quality of byproducts, increase byproduct flowability, potentially increase plant throughput, and significantly decrease plant emissions. Broin developed the BPX process in its laboratories and optimized it in its production scale research facility and the process has been implemented in three major U.S. ethanol plants. The impact of these developments on the dry grind ethanol industry remains under evaluation.

D. Fiber Conversion

Fermentation of the fiber fraction of the corn kernel can increase ethanol yield from a bushel of corn by 10% and subsequently yield a higher-value and higher-protein feed coproduct than is typically recovered in corn gluten feed and DDGS (Gulati et al. 1996). Expanding fuel ethanol production beyond 10% of our liquid transportation needs will require developing a lower cost feedstock and only feedstocks containing lignocellulosics are available in sufficient quantities to substitute for starch as an ethanol source.

Corn fiber is particularly attractive as a novel source of sugars for ethanol fermentation. Corn fiber has a high carbohydrate content that can be converted into fermentable sugars and is stockpiled at central locations – in many cases at existing fermentation facilities.

Currently, most corn fiber is incorporated into low-value animal feeds which may face a limited market in the future as ethanol production continues to grow. In addition to using corn fiber as a feedstock for ethanol, it may serve well as a feedstock for such value-added fermentation products as lactic acid, xylitol, lycopene, etc. (Leathers 1998). In addition to the fibrous component of the kernel, the rest of the corn plant (e.g., corn stover) could also serve as a feedstock for ethanol (Wyman 2003). Corn stover contains 58% carbohydrates and 1.0-1.5 lb of stover is produced per pound of harvested corn. Unlike DDG and corn fiber, collecting and storing corn stover represents a formidable challenge. However, fermenting available corn stover could significantly boost ethanol production. To date, no commercial process is in operation for conversion of corn fiber or corn stover into fuel ethanol.

Major technical constraints to commercialization exist in the conversion of lignocellulose to ethanol. These constraints are primarily in the areas of pretreatment of the substrate, enzymatic hydrolysis of the substrate to fermentable sugars and strain development for fermentation of multiple sugars. Development of efficient substrate pretreatments that increase the susceptibility of crystalline cellulose and hemicellulose to enzymatic hydrolysis will lower the cost of producing ethanol from lignocellulosic biomass. Pretreatment of lignocellulose, coupled to enzymatic hydrolysis, generates a stream of mixed sugars including arabinose, glucose, and xylose (Grohmann and Bothast 1997). However, enzymes for hydrolysis of biomass remain cost prohibitive, (~ \$.25/gal) and better enzymes are required. For example, commercial hemicellulase mixtures are ineffective for hydrolyzing corn fiber (Hespell et al 1997). Improved microbial strains to ferment mixed sugars are also required (Bothast et al 1999). Industrial yeast strains, used for fermenting corn starch, are unable to ferment arabinose and xylose; the few naturally occurring strains that do ferment pentoses, grow slowly, and produce low ethanol yields. Another challenge is to obtain strains that can tolerate the inhibitory compounds generated during pretreatment and hydrolysis. A fiber conversion process requires implementation of all unit operations used in biomass conversion, i.e. size reduction, pretreatment, enzymatic saccharification, fermentation, and product recovery.

Tiffany and Eidman, 2004 conducted a comprehensive assessment of the economic competitiveness of ethanol produced from corn grain by the dry grind process versus ethanol produced from corn stalks (lignocellulosic biomass). Their conclusions suggest that lignocellulosic processing (estimates from the National Renewable Energy Laboratory) may be competitive with the state of the art dry grind process when corn prices approach \$3.00 per bushel, which equates to an ethanol cost of about \$1.40 per gallon.



READY for RESEARCH

The National Corn-to-Ethanol Research Center, Edwardsville, IL has opened its doors to those who want to test the latest ethanol technology.

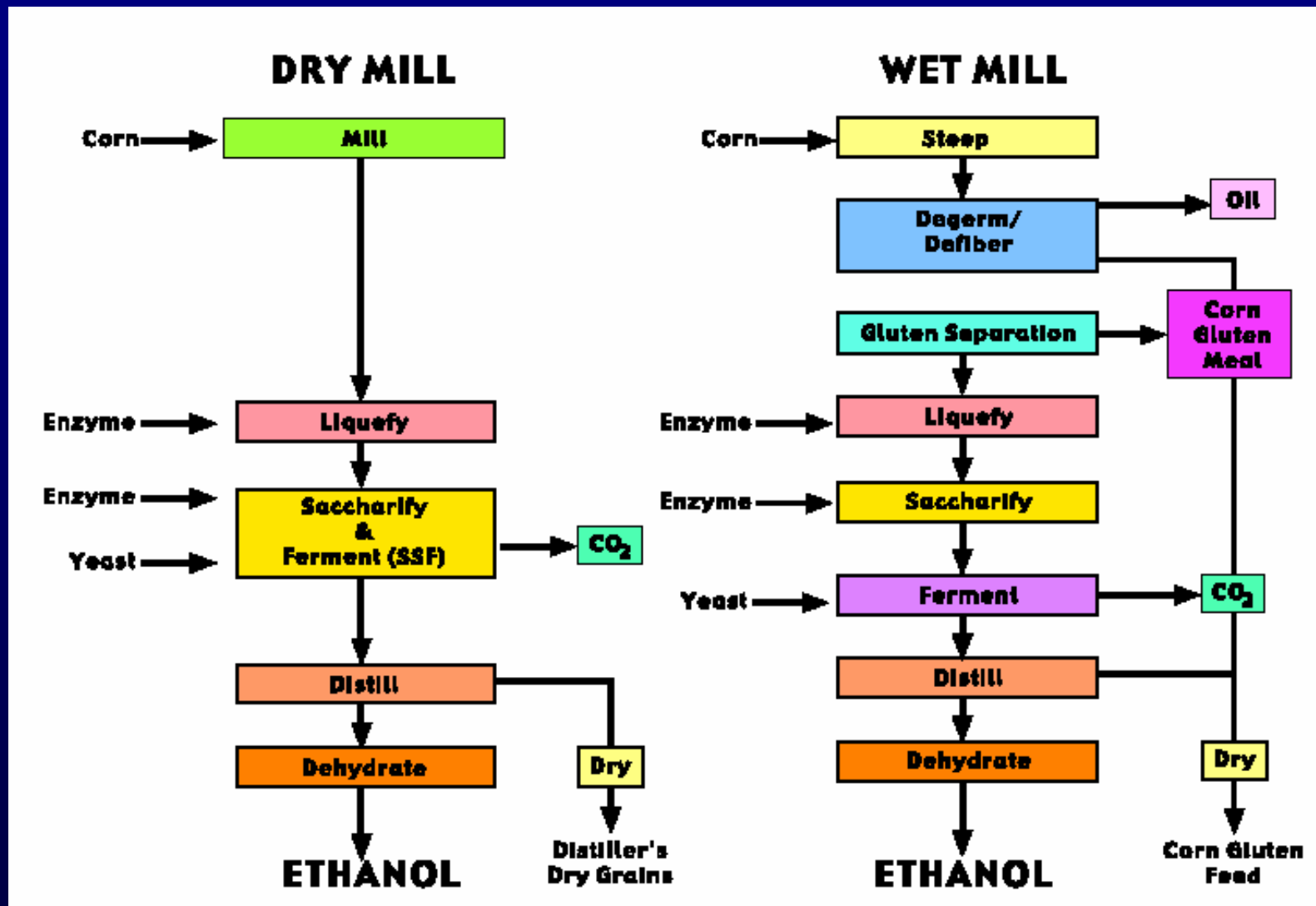


New Technologies in Biofuel Production

Rod Bothast
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February 24th, 2005



Current Ethanol Production Methods



Demonstrated Improvements

Corn Yields

Hybridization; annual yield improvements –
5 fold increase

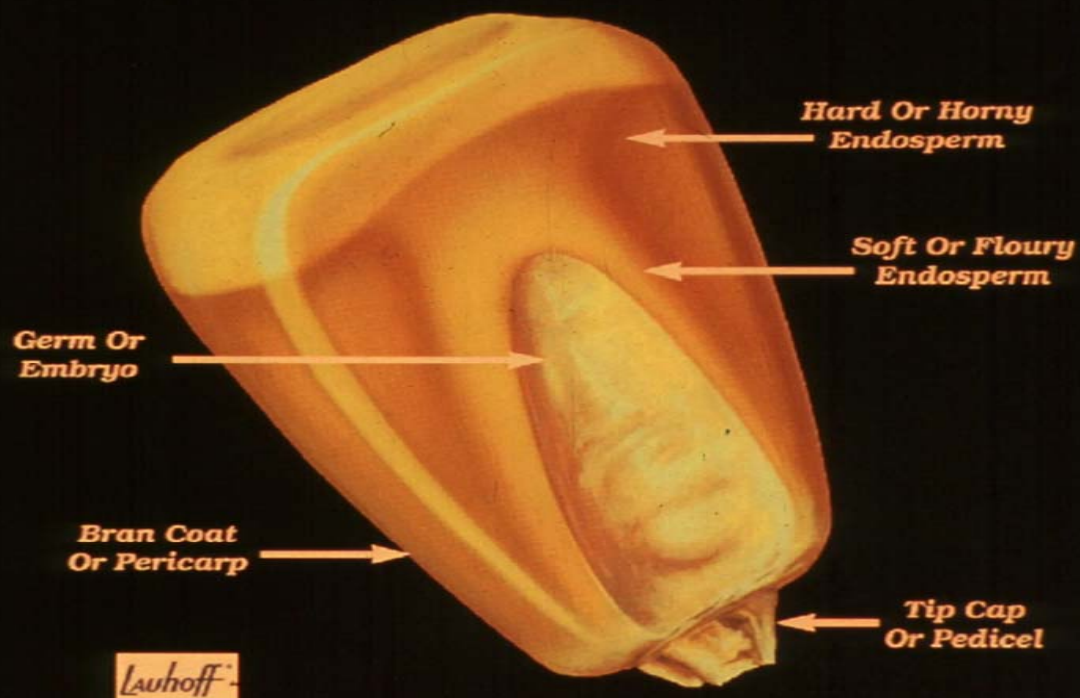
Construction Costs – 55.5% cost reduction
Currently \$1-\$1.5/gallon

Net Energy Value - raised
from 22 to 67% (1995 to 2001)

Process Efficiencies

2.7-2.8 gal/bu – dry grind process

Typical Composition Of Yellow Dent Corn



Component	Portion Of Kernel, % Dry Basis	Composition Of Component Part, % Dry Basis					
		Starch	Protein	Oil	Ash	Sugars	Fiber
Endosperm	82.9	88.4	8.0	0.8	0.3	0.6	1.9
Germ	11.0	11.9	18.4	29.6	10.5	10.8	18.8
Bran Coat	5.3	7.3	3.7	1.0	0.8	0.3	86.9
Tip Cap	0.8	5.3	9.1	3.8	1.6	1.6	78.6
Whole Kernel	100.0	75.0	8.9	4.0	1.5	1.7	8.9

Typical Composition Of Yellow Dent Corn



Component	Portion Of Harvest, % Dry Basis	Composition Of Component Parts, % Dry Basis				
		Shank	Hull	Germ	Tip Cap	Total
Endosperm	82.0	88.4	4.0	0.0	0.0	7.4
Germ	11.0	11.0	18.4	25.0	10.0	14.4
Straw Coat	2.0	7.2	0.7	1.0	0.0	9.2
Tip Cap	1.0	0.0	0.0	0.0	1.0	1.0
Whole Harvest	100.0	76.6	8.9	1.0	1.7	88.2

Improving Hybrids for Dry-Grind Ethanol Production

Numerous commercial hybrids now available

Pioneer(HTF), Monsanto(PPF), Syngenta(NKEE), Regional Seed Companies

Ethanol yields increase from hybrid selection up to 4%

\$1-2M annually for a 40mg ethanol plant

Extensive research has resulted in a better understanding of the complexity

Influenced by agronomic practices (plant population, applied nitrogen, etc), environment, hybrid selection and performance

Self-processing grains

Starch hydrolyzing enzymes in transgenic corn kernels
(Syngenta)

New and High Value Coproducts

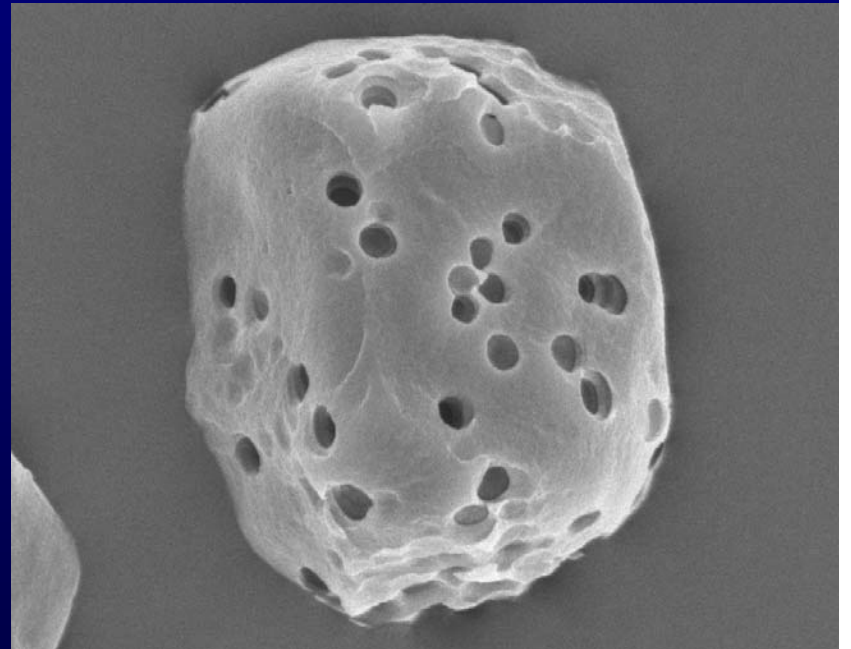
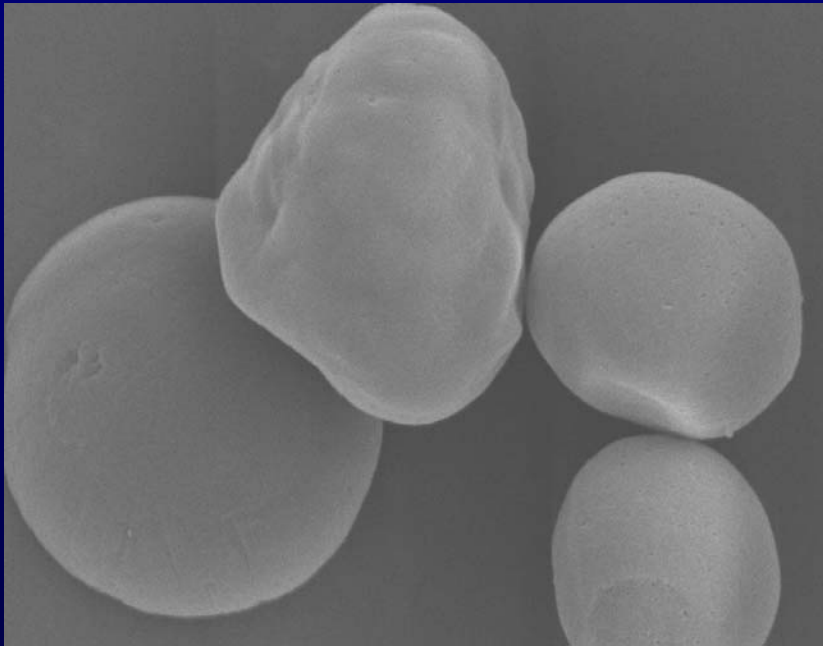
New processes

QG, QGQF, Enzymatic milling, COPE,
Modified DDGS with nutrient profile

Coproducts

Corn oil, zein, fiber, corn fiber oil, sweeteners,
polysaccharides, pharmaceuticals,
nutraceuticals, biodegradable films, organic
acids, solvents, amino acids, pigments,
enzymes, polyols, vitamins, etc

Raw Starch Hydrolysis



From David Johnston, *Cereal Chem.* 79:523-527

Commercial Developments

ICM/ Genencor

Broin/ Novozymes(BPX)

- Direct conversion without a cook step
- Reduced energy input
- Reduced capital
- Increased yields
- Lower overall costs

US Biomass Sources

ECONOMICAL



Sugar Cane



Corn Starch



Corn Fiber



Paper



Switch Grass



Wood Chips



Cottonwoods



Stover

ABUNDANT & AVAILABLE

Corn Kernel Cellulosics

Near Term Technology Validation



**No incremental supply chain
Costs**

Potential 14% Yield increase

**4.5 M gal Ethanol per plant
Annually**

Minimal incremental capital

DDGS weight reduced 44%

No increase in corn acres

Amounts of feedstocks to produce 10 ml ethanol

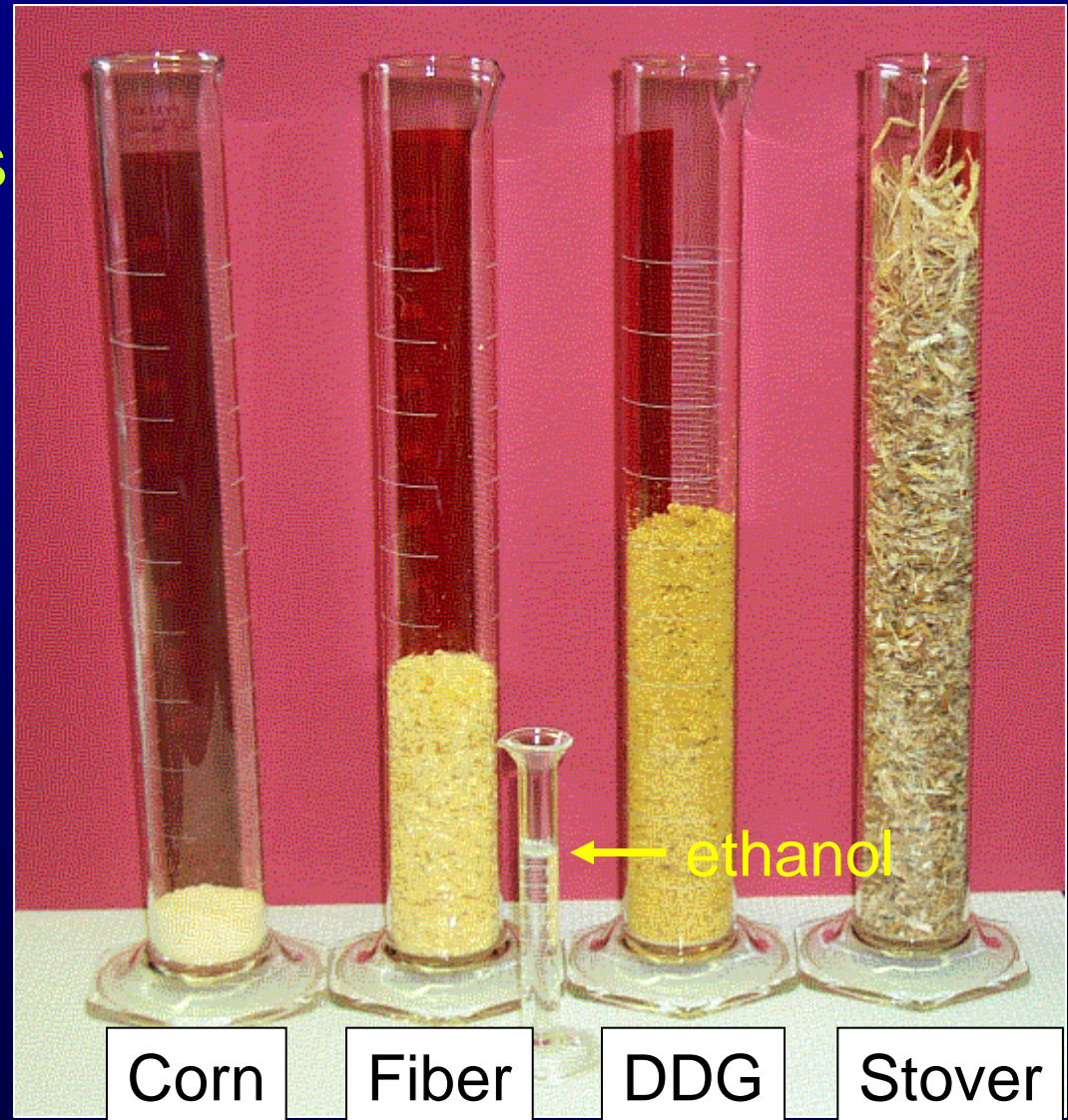
Moisture Content

Corn 15%

Fiber 46%

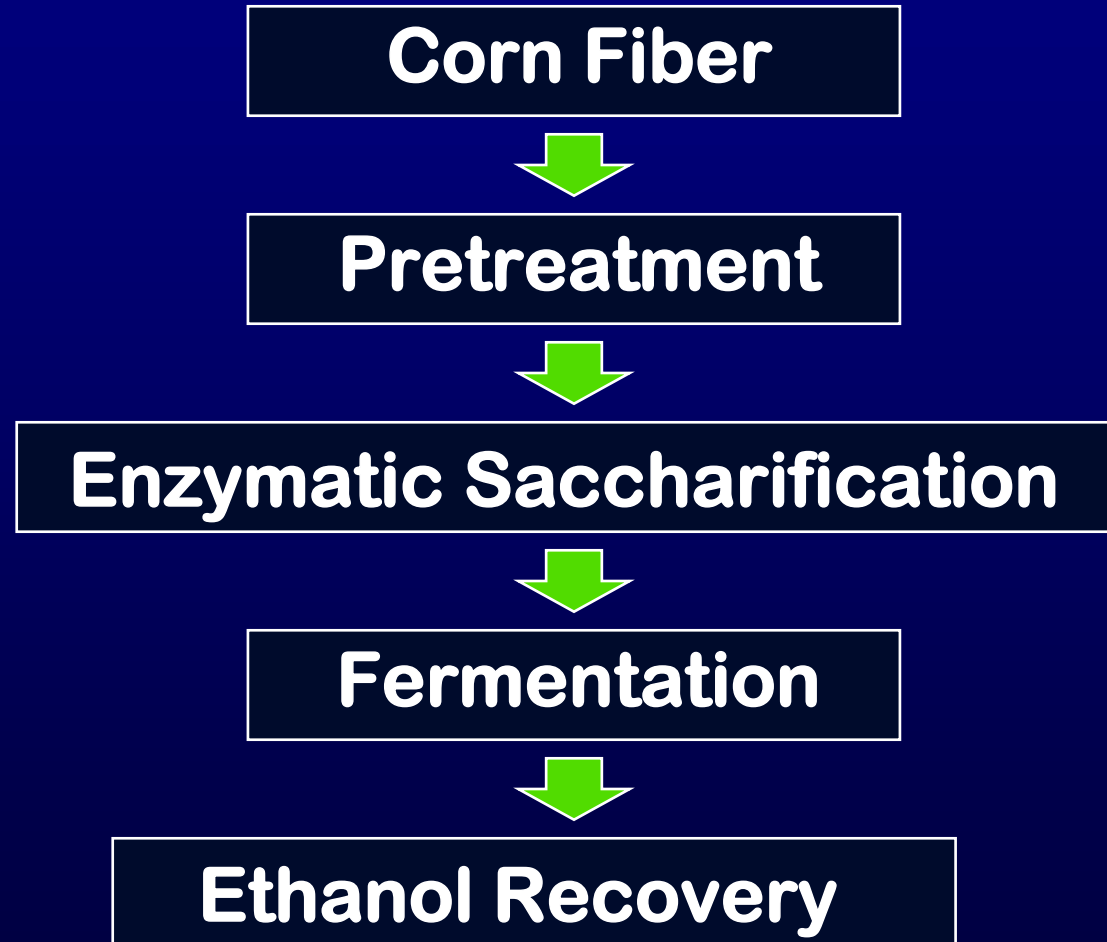
DDG 64%

Stover 5%



500 ml graduated cylinders used for comparisons

Utilization of Biomass for Production of Fuel Ethanol



Selected Pretreatment Strategies

Acid



Base

Pretreatment

Pentoses

Inhibitors

Strong Acid

+

++

Dilute Acid

+

++

Hot Water

-

+

AFEX

-

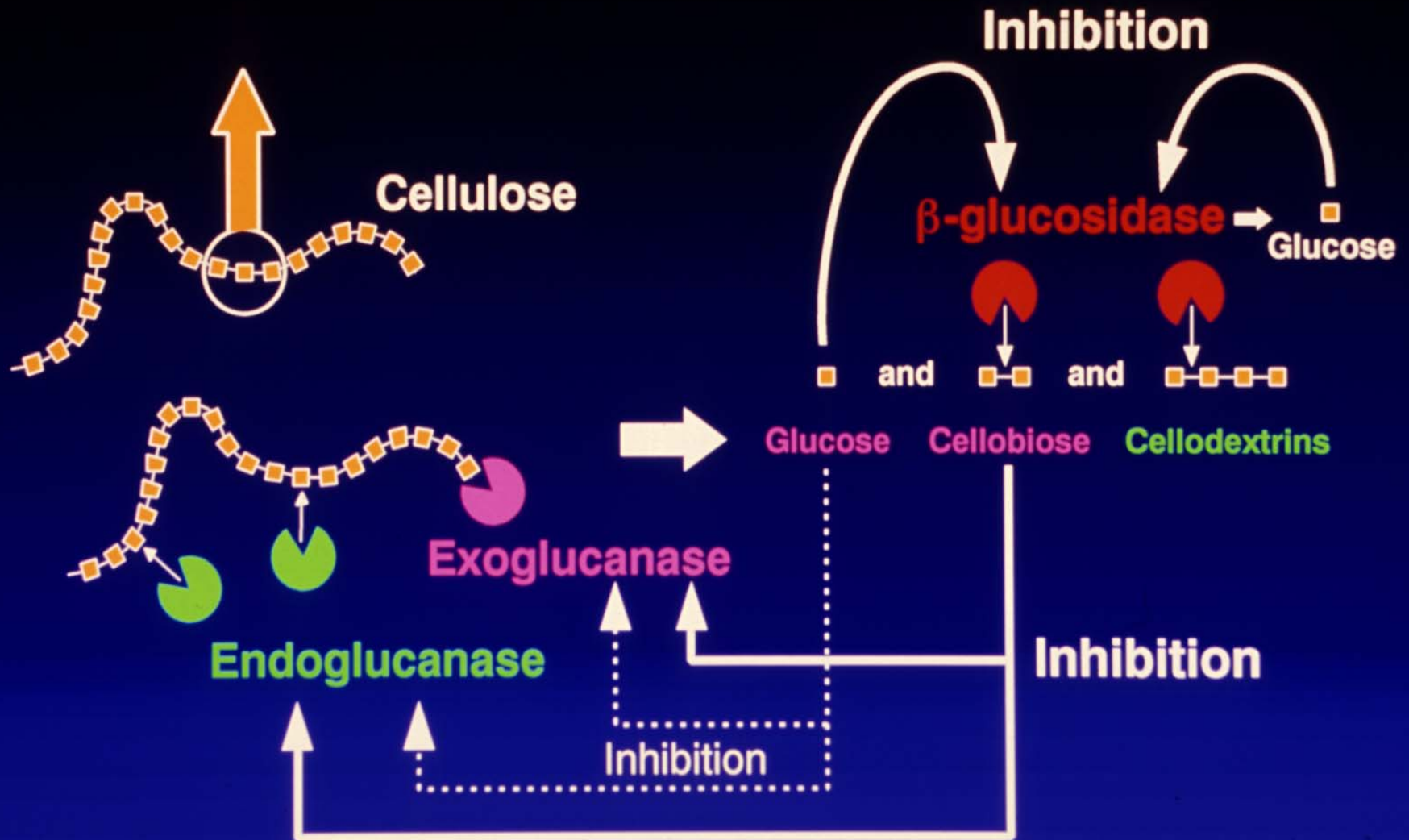
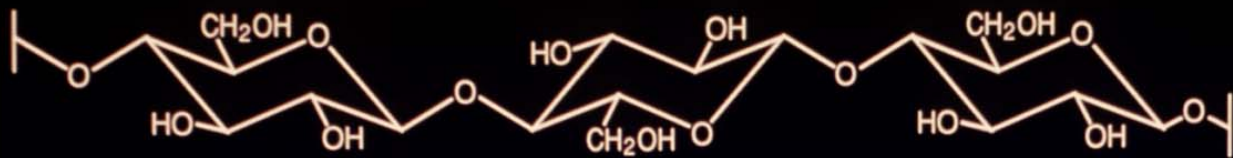
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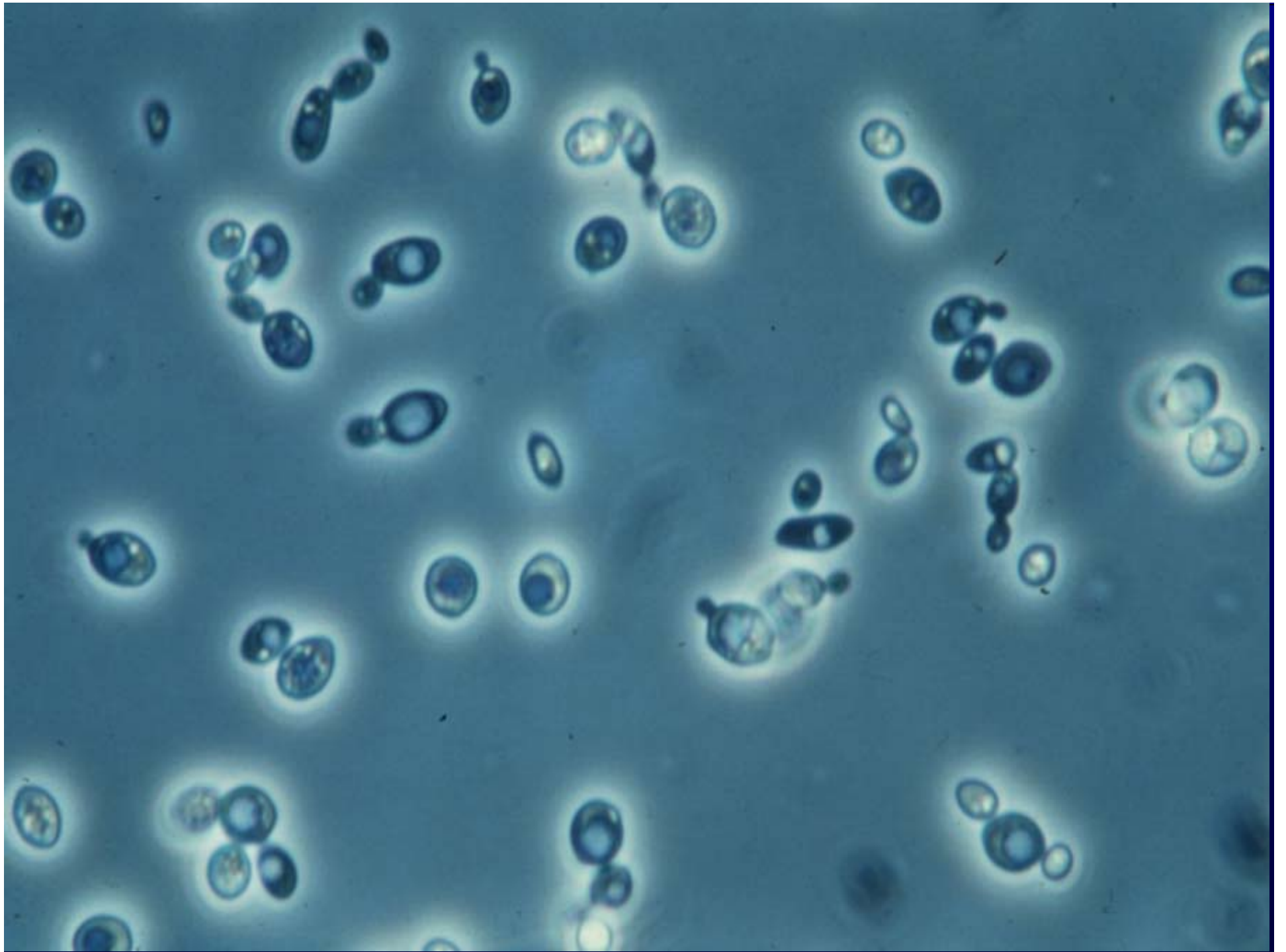
Alkaline
Peroxide

-

-

Enzymatic Hydrolysis of Cellulose





Recombinant Microorganisms for Fermentation of Mixed Sugars to Ethanol

- Recombinant organisms are now available
 - Recombinant *Escherichia coli*
 - Recombinant *Saccharomyces*
 - Recombinant *Zymomonas*
 - Recombinant *Klebsiella oxytoca*
- Commercialization prospects
 - BCI with recombinant *E. coli*
 - Iogen with recombinant *Saccharomyces*

Ethanol cost derived from \$50/ton corn stover versus equivalent corn prices in dry-grind processing

	Conversion Rate Gallons Per Ton	Enzyme Cost Per Gallon	Cost Per Denatured Gallon	Corn Equivalent Prices
Future	89.7	\$0.10	\$1.25	2.35
		\$0.25	\$1.40	2.98
Base	68.0 ^b	\$0.10	\$1.65	4.02
		\$0.25	\$1.79	4.62

From Tiffany and Eidman, 2004



Corn Stover



Acre Expansion Coproduct Utilization



Corn Kernel Cellulose



Agronomic Improvements



Tailored Hybrids



Process Improvements



Today

What is the Impact

Corn Acres

Billion Gallons