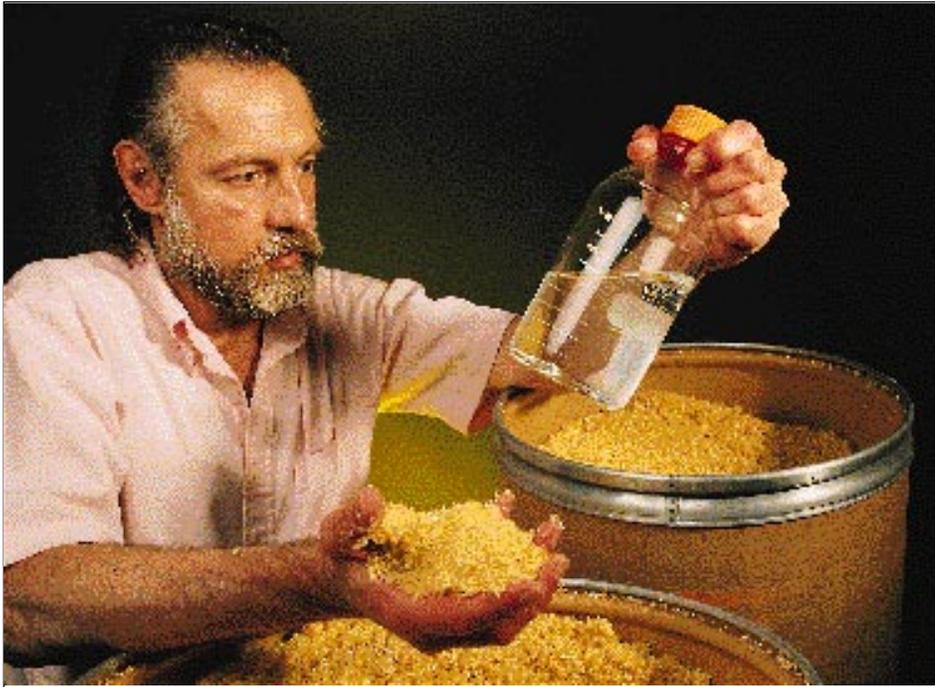


Improving Ethanol Yield From Corn

KEITH WELLER



The goal of microbiologist Robert Hespell and a team of Peoria, Illinois, scientists is to devise a way to ferment corn fiber into ethyl alcohol, potentially increasing yield by 0.3 gallon per bushel. (K7413-14)

Ethanol to fuel cars early in the 21st century may come from fiber-laden crop residues—instead of feed grains—if a vision of agricultural researchers at Peoria, Illinois, pans out.

But before that vision is fully realized, science and technology will most likely enable ethanol producers to squeeze a bit more ethanol from corn—the main source at present.

Through fermentation of starches and sugars found inside the grain, modern ethanol plants now produce 2.5 gallons of ethyl alcohol—ethanol—from each bushel of corn, says Rodney J. Bothast. He's an ARS microbiologist at the National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois.

Bothast and his colleagues have their sights set on making fiber in the grain's outer layer yield nearly 0.3 additional gallon per bushel.

In 1994, about 1.3 billion gallons of fuel ethanol were produced in the United States from corn, with more than 60 percent obtained through wet milling. The wet-milling process involves soaking, or steeping, the corn in water, grinding it, and separating high-protein germ, oil, and fiber from the starchy endosperm that is fermented to produce ethanol.

The current practice is to mix the fiber fraction with fermentation solubles before drying it and forming animal feeds.

Agricultural engineer Michael R. Ladisch and colleagues at Purdue University in West Lafayette, Indiana, teamed up with the NCAUR scientists to assess ways to increase ethanol production from corn.

"We estimate that if the fiber were also processed into ethanol, a corn wet-milling facility that produces 100 million gallons of ethanol per year

could generate an additional \$4 to \$8 million of annual income," says microbiologist Robert B. Hespell. He is project leader for ethanol research in Bothast's Fermentation Biochemistry Research Unit.

Stilling a Criticism

The increased efficiency of corn and ethanol production that has evolved over the last 10 years may also help to subdue criticisms that petroleum used to produce corn and process it into ethanol requires more energy than is released when the ethanol is burned.

According to "Estimating the Net Energy Balance of Corn Ethanol," a report published last year by USDA's Economic Research Service (which now includes the Office of Energy and New Uses), the ethanol energy now produced from each bushel of corn is 25 percent greater than the amount of energy used to grow and harvest the corn and distill it into ethanol. This is thanks to today's higher corn yields, more energy-efficient fertilizer production, and improved distillation technology.

Unlocking Fiber's Energy Potential

Hespell says his team's research strategy for economically converting fiber to ethanol is three-pronged. They hope to:

- find better ways to physically and chemically treat the fiber to expedite its conversion to sugars,
- find enzymes that better convert the fiber to sugars, and
- custom engineer suitable microbes to ferment the sugars D-glucose, D-xylose, and L-arabinose.

To achieve these goals, the researchers are combining their efforts with those of other ARS scientists and university cooperators.

At College Station, Texas A&M University researcher Mohammed

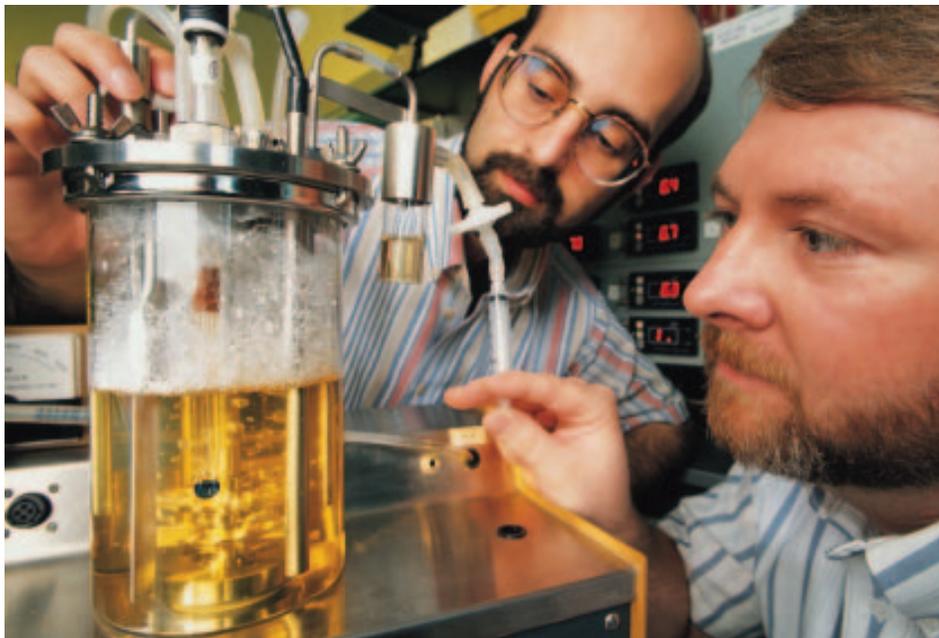
KEITH WELLER



Fungal physiologist Shelby Freer examines yeasts under high magnification, projected on monitor, for their ability to make enzymes needed for ethanol production. (K7416-8)

Moniruzzaman and chemical engineer Bruce E. Dale, who is now with Michigan State University, applied a pretreatment called ammonia fiber explosion (AFEX) to corn fiber to unlock its potential for fermentation.

KEITH WELLER



A small-scale bioreactor enables chemical engineer Bruce Dien (left) and microbial geneticist Herbert Wyckoff to see how well new genetically engineered microorganisms will produce ethanol in fermentation. (K7415-1)

while converting an additional 20 to 30 percent of the fiber into short sugar polymers.

In the AFEX process, a slurry of water and corn fiber is mixed with highly pressurized liquid ammonia. Quickly releasing the pressure splits the fiber's bundles of carbohydrate components that are normally rather inaccessible to chemicals or microbes because they are so tightly glued together by lignin.

Researchers treated the lignin-free polymers with mixtures of commercial enzymes. Some enzymes called amylases and cellulases thoroughly hydrolyzed, or split apart, chains of starch and cellulose into links of simple fermentable sugars such as glucose, each with a backbone of six carbon atoms. Today's ethanol plants typically use bakers' yeast—*Saccharomyces cerevisiae*—to produce ethanol only from these 6-carbon sugars called hexoses.

From the corn hemicellulose, or arabinoxylan, xylanase enzymes clipped off at least 25 percent of the component pentoses or 5-carbon sugars—monosaccharides such as arabinose and xylose. That success was enough to spur a search for xylanases that might be recruited to enhance ethanol production.

"If we can find bacteria that produce more active xylanases, this ethanol research might also lead to improving the efficiency with which ruminant livestock such as cattle and sheep digest hemicellulosic crop residues," says Hespell. He is also involved in research on ruminant microbiology.

Drawing On Archival Research

Another impetus for the current focus on freeing up pentoses for ethanol production is recent success by biotechnologists in transforming single microbial species to subsist on

both hexoses and pentoses.

Seeking an alternative pretreatment of the fiber to free up more simple sugars, the researchers took note of work done by John W. Dunning and Elbert C. Lathrop at NCAUR in one of the earlier ethanol research programs dating back more than 50 years.

Recognizing the large amount of sugars in corn cobs and other agricul-

tural residues, Dunning and Lathrop hydrolyzed hemicellulose with mild sulfuric acid treatments, forming a solution of mostly pentoses.

But further costly processing, such as deacidifying and removing the toxic byproduct furfural, was required before microorganisms could use the sugars.

ARS chemist Karel Grohman of Winter Haven, Florida, and Bothast

reasoned that formation of furfural and other chemicals that inhibit fermentation could be reduced by a two-stage process. First, they quickly hydrolyzed the fiber with hot, mild acid; then they quickly cooled it and added a mixture of cellulase and amyloglucosidase enzymes before further hydrolysis.

In the laboratory, Grohman found that the sequential treatment on

KEITH WELLER



Microbiologist Rodney Bothast (left) and technician Loren Iten add starter microorganisms to pilot-plant-size bioreactors used for brewing ethanol from sugar mixtures derived from corn fiber. (K7408-13)

batches of low-starch corn fiber resulted in about 85 percent of all polysaccharide becoming sugars in monomer forms.

Within 2 days, genetically engineered *Escherichia coli* bacteria fermented these sugars into solutions of more than 2 percent alcohol.

The hydrolysis is now being scaled up as a continuous process at NCAUR. A goal is to complete the acid hydrolysis phase within 2 minutes.

After successfully finding ways to break down corn fiber into hexoses and pentoses, the researchers' next challenge is to identify or develop strains of microorganisms that will convert both of these sugar types to ethanol as efficiently as bakers' yeast makes it commercially from hexoses.

The genetically engineered *E. coli* strain K011 that Grohman and Bothast used to produce ethanol from multiple sugars of acid-hydrolyzed fiber was developed by microbiologist Lonnie O. Ingram at the University of Florida in Gainesville. The evaluation of K011 was conducted by ARS under a cooperative agreement.

Starting with K011, microbiologist Herbert Wyckoff and chemical engineer Bruce S. Dien at NCAUR worked with Hespell and Bothast to further transform the microbe. Unlike the older version, the new one does not need antibiotics to survive in anaerobic fermentation environments like those of commercial ethanol plants. The scientists are scaling up their laboratory research to the pilot fermentors.

Another recombinant microbe that can ferment both glucose and xylose is a strain of *S. cerevisiae* yeast developed by geneticist Nancy Ho at Purdue. It was also evaluated under an ARS cooperative agreement.

Because this microbial species has long been used to make ethanol, a

modified version too might someday work well for the industry, says Bothast. Further research, however, is needed to develop Ho's strain into one that can survive better under industrial conditions, produce ethanol from other sugars such as arabinose, and quickly produce larger volumes of ethanol.

Fungi, Too, Might Join the Effort

In addition to bacteria and yeasts, genetically transformed filamentous fungi could become ethanol plant workhorses.

At NCAUR, microbiologists Christopher D. Skory and Shelby N. Freer envision harnessing industrial and food processing fungi for a "one-pot" method of producing ethanol. The microbes prodigiously spew out enzymes that efficiently break down the corn fiber's cellulose and hemicellulose while producing tiny amounts of ethanol from the resulting sugars. "Through both mutagenesis and genetic engineering, we hope to increase their ethanol production," says Skory.

Similar genetic research could lead to one-pot production of lactic acid that is valuable in food processing and for industrial applications, such as making biodegradable plastics. Developing such a wet-milling coproduct would help offset ethanol production costs, since ethanol is fairly low in economic value.

Considering alternative fermentations of glucose, Freer is screening part of the ARS Culture Collection located at the NCAUR for *Brettanomyces* yeasts that efficiently produce acetic acid from glucose. In earlier screening of the collection, Freer identified a microbe with a gene responsible for producing a beta-glucosidase enzyme that breaks down small cellulose polymers into

fermentable sugars. Skory has cloned the gene and inserted it into several other microbes, including ethanol-producing ones.

In still another screening, microbiologist Badal C. Saha has identified a yeast that produces a heat-stable beta-glucosidase that works in environments high in glucose. He is mutating the yeast to try to increase its production of the enzyme so that it can be used to efficiently convert cellulose to sugars.

In addition to trying to get more ethanol from a bushel of corn, the NCAUR researchers hope to increase the usefulness of other ethanol fermentation coproducts.

One abundant low-value product of fermentation is carbon dioxide. NCAUR plant physiologist Brent Tisserat is evaluating the ability of different CO₂ concentrations to speed the growth of plant tissue cultures. He envisions using such cultures one day to produce food flavorings and high-value pharmaceuticals.

At NCAUR, other scientists are researching potential value-added products that can be made from wet-milling coproducts.—By **Ben Hardin**, ARS.

Rodney J. Bothast heads the USDA-ARS Fermentation Biochemistry Research Unit, National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, IL 61604; phone (309) 681-6566, fax (309) 681-6686, e-mail rbothast@ncaur1.ncaur.gov ♦