# Banking on Biobutanol

## New Method Revisits Fermenting This Fuel From Crops Instead of Petroleum

A modified method of producing biobutanol is in the works to make the fuel more competitive with ethanol as a clean-burning alternative to gasoline and other fossil fuels.

Biobutanol offers several advantages over ethanol. It can be transported in existing pipelines, is less corrosive and less prone to water contamination, can be mixed with gasoline or used alone in internal combustion engines, and packs more energy per gallon than ethanol.

Up until the mid-20th century, biobutanol was produced from fermented sugars, such as glucose in corn or molasses. But low yields, coupled with high recovery costs and the increased availability of petroleum feedstocks after World War II, sidelined fermentation-based systems for biobutanol production.

Today, petrochemicals still reign supreme as the feedstock of choice for making butanol, a four-carbon alcohol that's mainly used as an industrial solvent. But jumps in the price of oil have rekindled interest in tapping butanol as biobased fuel, notes Nasib Qureshi. He's a chemical engineer with the ARS National Center for Agricultural Utilization Research at Peoria, Illinois. Indeed, in June 2006, DuPont, Inc., of Wilmington, Delaware, and the British energy company BP announced joint plans to operate a United Kingdom-based production plant dedicated to producing biobutanol from sugar beets.

### Turning Straw Into Biofuel Gold

Up until late 2003, Qureshi's own biobutanol studies dealt with new ways of fermenting glucose and other sugars from corn. But then he switched to wheat straw, drawn by its abundance and promise as a lower-cost alternative to glucose-based feedstocks.

"There's only so much you can do with sugar because of the competing uses for it," says Qureshi, who's in the Peoria center's Fermentation Biotechnology Research Unit. Wheat straw, by comparison, is typically left on crop fields after harvest to forestall soil erosion or used as feed or bedding for livestock—though new, value-added uses are being explored. The team is also working with barley straw, corn stover, and switchgrass.

Like other biobutanol processes, Qureshi's approach calls on species of *Clostridium* bacteria to carry out the critical task of fermentation. But before the bacteria can perform such work, the straw must be pretreated and hydrolyzed. The hydrolysis step uses enzymes to break apart the straw's cellulose and hemicellulose components. This liberates the simple sugars within so that the bacteria can ferment them into three products: acetone, butanol, and ethanol. Butanol is produced in the greatest concentration, but all three are valuable chemicals.

PEGGY GREB (D1240-2)

**Chemical engineer Nasib Qureshi adds enzymes into a bioreactor to simultaneously hydrolyze wheat straw into simple sugars and produce butanol.**

PEGGY GREB (D1241-1)

**Technician John Michael Henderson combines dilute sulfuric acid with ground wheat straw as a pretreatment. The wheat straw is then autoclaved and transferred to a bioreactor for butanol production.**

### Three Steps in One

Normally, four preparatory steps (pretreatment, hydrolysis, fermentation, and recovery) are carried out separately and sequentially. But in his studies, Qureshi—together with ARS Peoria colleagues Michael A. Cotta and Badal C. Saha—devised a way to combine three of the four steps.

After the wheat straw has been pretreated with dilute sulfuric acid or other chemicals, the material is fermented in a bioreactor containing three different types of commercial enzymes and a culture of *C. beijerinckii* P260, a strain Qureshi obtained from Professor David Jones of the University of Otago in Dunedin, New Zealand.

Qureshi's approach allows the enzymes and bacteria to do their jobs simultaneously. As soon as the enzymes hydrolyze the straw and release its simple sugars, the bacteria set to work fermenting them. Throughout, a procedure known as "gas stripping" (the fourth step) is used to remove the acetone, biobutanol, and ethanol as they're produced. Gas stripping also protects the bacteria by keeping biobutanol levels from reaching levels harmful to them.

In early trial runs, the method increased biobutanol productivity by twofold above traditional glucose-based fermentation—but the bacteria fermented the sugars faster than the sugars became available. So, to ensure optimum performance, Qureshi found it necessary to feed small batches of additional sugar to the bioreactor.

Later studies showed that the adjustment, called "fed-batch feeding," significantly increased biobutanol production. During a 22-day fed-batch operating period, a culture of *C. beijerinckii* P260 converted nearly 430 grams of sugar (glucose, xylose, arabinose, galactose, and mannose) into 192 combined grams of acetone, biobutanol, and ethanol.

If scaled up further, the process could yield 307 combined kilograms, or 99 gallons, of acetone, biobutanol, and ethanol from 1 ton of wheat straw. The P260 strain produces a specific ratio of the three chemicals, but efforts are now under way at Peoria to develop genetically modified bacteria that will make only biobutanol.

Qureshi says he is planning to scale up production levels in 2009. "Then, we'll look at the economics of using hydrolyzed wheat straw to see how we're doing and move this process forward."—By **Jan Suszkiw,** ARS.

*This research is part of Bioenergy and Energy Alternatives, an ARS national program (#307) described on the World Wide Web at www.nps.ars.usda.gov.*

*Nasib Qureshi is in the Fermentation Biotechnology Research Unit, USDA-ARS National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, IL 61604; phone (309) 681-6318, fax (309) 681-6427, e-mail nasib.qureshi@ars.usda.gov.* ✷

## Making Microbes Do More Work

ARS Fermentation Biotechnology Research Unit scientists Nasib Qureshi, Bruce Dien, and Loren Iten have another trick up their sleeves for making biobutanol. Together with Lars Angenent, a Cornell University professor in Ithaca, New York, and graduate student Matthew Agler, the team is developing a two-stage fermentation process for producing biobutanol from corn fiber.

The fibers are byproducts of corn wet-milling, but any plant material containing cellulose and hemicellulose will do. The first step involves pretreating the fiber with chemicals or hot water. Dien and Iten then ship the softened fibers to Angenent, who feeds the pretreated fibers to mixed cultures of different microbial species. By adjusting pH levels and other environmental factors, he coaxes the microbes to convert the fiber's sugars into butyrate, a butanol precursor. Angenent then ships the butyrate back to Peoria, where Qureshi uses his own special strain to make biobutanol.

"The enzymes usually used to convert pretreated biomass to fermentable sugars are very expensive. But the mixture of microbes used in this case to make butyrate produce their own enzymes. This means a huge potential cost savings," says Dien.

Unwelcome microbes pose a contaminant problem in traditional fermentation systems, he adds, but "we expect that the mixed microbial culture applied here will be more resistant to contaminants."

Dien and Qureshi's butanol research at Peoria falls under a competitive USDA grant.—By **Jan Suszkiw,** ARS.

PEGGY GREB (D1238-1)

**Biochemical engineer Bruce Dien (left) loads reactor vessels with corn fiber while microbiologist Loren Iten lowers samples into a sand bath heater to pretreat for fermentation.**