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Biobutanol: The Next Big Biofuel?

It's touted as a superior renewable fuel but challenges have stymied the industrial-scale production of biobutanol. Now, however, Dupont and BP have teamed to develop and commercialize the fuel. This comes as scientists announce advancements in the design of process technologies and the engineering of microbes aimed at improving the economics of mass-producing biobutanol.

by Jessica Ebert

It's certainly not new to the renewable fuel scene. In fact, some experts would say that, historically, the fermentation of sugar-based feedstocks into butanol takes a back seat in importance to ethanol. Biobutanol plants operated in numerous countries, including the United States, UK, China, Russia, South Africa and India, during the first two World Wars.

These plants were designed to harness the fermenting talents of microbes to produce acetone from feedstocks such as molasses and corn starch. The acetone was used to make a smokeless gun powder and a propellant for rockets. Interestingly, acetone was not the only product of this fermentation. Ethanol was produced in small amounts but the major product of the fermentation was butanol.

Starting in the 1960s, the growth of the petroleum industry and the cheaper cost of producing butanol from petroleum products rather than renewable feedstocks made the biobased butanol plant obsolete. The last significant vestige of the industry—a facility in South Africa—ceased its operations in the early 1980s. But rising oil prices and concerns surrounding climate change and national security have rejuvenated interest, research and development into biobutanol. Although the primary use for the alcohol is as an industrial solvent, it offers several advantages over ethanol as a transportation fuel. Since the molecule contains four carbons compared with the two of ethanol, those extra chemical bonds release more energy when burned. In addition, butanol is less volatile than ethanol, it can be used at a 100 percent blend in internal combustion engines without any modifications, it doesn't attract water like ethanol so it can be transported in existing pipelines and it is less sensitive to colder temperatures. "Butanol is an excellent fuel," says Nasib Qureshi, a chemical engineer with the USDA Agricultural Research Service in Peoria, Ill. "As a result of gas prices going up it is looking more effective than ethanol and more effective than gasoline."

Some big names in the energy business seem to agree. In 2006, BP and DuPont announced a joint venture to deliver advanced biofuels, initially targeting biobutanol. This past spring, the companies announced results from fuel testing including: that a 16 percent biobutanol blend performs similarly to a 10 percent ethanol blend and higher biobutanol blends also produce favorable results; that the energy density of biobutanol is closer to unleaded gasoline; and that biobutanol does not phase

separate in the presence of water. "Biobutanol addresses market demand for fuels that can be produced from domestic renewable resources in high volume and at a reasonable cost; fuels that can be used in existing vehicles and existing infrastructure; fuels that offer good value to consumers; and fuels that meet the evolving demands of vehicles," says Frank Gerry, BP Biofuels program manager.

Earlier this year, the companies announced that the partnership was developing biocatalysts for the production of 1-butanol as well as 2-butanol. (The latter is called an isomer of butanol because although it contains four carbons, the atoms of the alcohol are arranged differently). The goal of the partnership is to deliver a biobutanol production process with economics equal to ethanol production by 2010. Currently, the two companies have applied for more than 60 patents in the areas of biology, fermentation processing, chemistry and end uses for biobutanol.

The challenge to improve the process technology and the microbes that carry out the fermentation drives academic and governmental researchers as well. Qureshi, for instance, has been studying biobutanol production for more than 20 years. He came to the United States from New Zealand to develop a membrane process for more effectively recovering butanol from fermentation broth. He's also worked to develop efficient butanol bioreactors. In the past few years, however, his research has taken a different direction, one that focuses on optimizing the process for more economical substrates such as wheat straw, barley straw, switchgrass and corn stover. "We need to move toward more economical substrates," Qureshi says. "But it's not as simple as it looks."

First of all, there's an inherent paradox in the microbial fermentation of butanol: although butanol-producing bacteria produce the enzymes that convert simple sugars into the alcohol, butanol itself is toxic to those same bugs. This butanol inhibition results in a lower alcohol concentration in the fermentation broth, which leads to lower yields of butanol and higher recovery costs. These are the challenges that surface when highly pure feedstocks are used. When a cheaper, biomass substrate is used, additional microbial inhibitors are generated during the pretreatment process.

Strategies for reducing butanol toxicity and improving yield, including integrating several steps in the process and manipulating the microbial cultures, are advancing. "We've made good progress with raw materials, removing inhibitors and product separation," Qureshi says. The overall process that Qureshi's team has developed for the production of butanol from agricultural residues involves four steps: pretreatment, which opens the cell wall structure and removes lignin; hydrolysis of hemicellulose and cellulose into simple hexose and pentose sugars using enzymes; fermentation of simple sugars into butanol using a pure culture of *Clostridium beijerinckii* P206, an anaerobic bacterium; and recovery of the butanol. The unique aspect of the process is that the last three steps are combined and performed in a single reactor. "We've integrated the process and it appears to be very effective economically," Qureshi says. His team is currently in the process of filing a patent on the process.

In addition, Qureshi has teamed with Lars Angenent, an environmental engineer at Washington University, as well as other USDA-ARS researchers to improve the economics of the hydrolysis step. The idea is to replace the need for enzymes, which are often expensive, with a mixed culture of bacteria. "The real tenets of my lab involve studying nondefined mixed cultures and seeing what they can do," Angenent

explains. In the collaboration with Qureshi, Angenent will use microbes collected from the sludge of an anaerobic digester as well as microbes from sheep rumen to ferment pretreated corn fiber to butyric acid, a chemical found in rancid butter, parmesan cheese and vomit. The solution containing the acid will be sent to Qureshi's lab where it will be fermented into butanol by his pure cultures of *Clostridium*.

The collaboration is in its infancy, financed by a \$425,000 grant from the USDA. Currently, Angenent's team is working to optimize the butyric acid production by tweaking conditions like pH and temperature. "We try to steer the community to produce one product over another," he explains. Once conditions are right for the production of significant levels of butyric acid, Qureshi will take over.

Engineering Butanol-Fermenting Bugs

Whereas the approach spearheaded by Qureshi and Angenent involves optimizing butanol production by microbes that naturally produce it, a team of chemical and biomolecular engineers from the University of California, Los Angeles, recently reported a different course. In a recent issue of the journal *Nature*, the team led by James Liao, described how they genetically modified a well-known bacterium, *Escherichia coli*, to efficiently synthesize butanol, a molecule it doesn't normally produce.

To do this, the team reasoned that they could divert some of the metabolites that *E. coli* uses to make amino acids, the building blocks of proteins, to a metabolic pathway that would result in the production of butanol. "Amino acid biosynthesis is very well studied in *E. coli*," Liao explains. Using that knowledge, Liao's team inserted two genes into the *E. coli* genome: one from a microbe involved in the production of cheese and one from a yeast. These genes express proteins that convert keto acids, components of the amino acid biosynthesis pathway, into butanol. In addition, by inhibiting the expression of other genes and making changes in certain proteins in the pathway, Liao was able to increase the efficiency of the process to a level high enough for industrial use. "By using these two tricks we could force the flux to the desired direction," he says. "We were able to produce isobutanol very quickly and improved the titer in a few months."

The technology is so promising that Gevo Inc., a biofuels startup based in Pasadena, Calif., recently announced that it acquired an exclusive license to commercialize Liao's process. The company is currently scaling up the technology and deciding whether to go ahead with its own plans to build a butanol plant.

Liao, meanwhile, is working on converting cellulose waste materials into isobutanol as well as trying to implement the approach in other microbes. "We're very excited about the promise of the project," he says.

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