



Technology Brief

Ethanol from Biomass: The Five-Carbon Solution

NREL Breakthrough on Xylose Fermentation Greatly Improves Prospects for “Homegrown” Fuel

U.S. Department of Energy (DOE) scientists at the National Renewable Energy Laboratory (NREL) are making major progress on meeting the challenge presented by xylose—the five-carbon sugar prevalent in the hemicellulosic fraction of biomass. NREL scientists are developing a new approach to conversion of cellulose and hemicellulose to ethanol, a technology with potential for great economic and environmental benefit.

Unlike glucose, the familiar six-carbon sugar in cellulose, xylose is not readily fermentable by common industrial yeasts. One solution to this problem uses special microorganisms—a genetically modified bacterium or a yeast in carefully controlled conditions—that can ferment xylose directly to ethanol.

Another exciting solution uses a metabolically engineered bacterium specifically tailored to coferment both glucose and xylose. This new biocatalyst is a major breakthrough for the economics of biomass-to-ethanol technology because cofermentation greatly simplifies the biomass fermentation process—reducing both capital equipment requirements and operating costs.

Lignocellulosic biomass, such as wastepaper, agricultural and forestry residues, fast-growing grasses, and woody plant materials, consists primarily of cellulose, hemicellulose, and lignin. NREL is developing the technology for economical conversion of the carbohydrates cellulose and hemicellulose to ethanol—technology that industry will use to

produce ethanol as a clean-burning, “homegrown,” alternative transportation fuel. While microorganisms can efficiently ferment glucose, the six-carbon sugar in cellulose, to ethanol, conversion of the five-carbon sugars in hemicellulose—mostly xylose—is more difficult. Because feedstock costs are nearly 40% of total process costs, and because about one-third of the sugars in biomass feedstocks are in the hemicellulose, the rapid and efficient fermentation of xylose is essential for economical conversion of biomass to ethanol. Recent estimates suggest that fermenting both xylose and glucose in a combined process can produce ethanol for substantially less than the previously projected cost of \$0.32 per liter (\$1.22 per gallon).



Warren Greer, NREL

NREL's metabolically engineered *Zymomonas* biocatalyst will allow cofermentation of both major components of biomass in a single set of tanks, greatly reducing capital equipment and operating costs. These pilot-plant-size tanks are part of NREL's new Process Development Unit.

Expanding the Ethanol Potential

Ethanol can be used as a "neat" fuel substitute for gasoline; today, ethanol made from cane sugar provides about half of the fuel for passenger cars and light-duty vehicles in Brazil. It can also be used as an oxygenated fuel extender; ethanol made primarily from the starch in corn is now in about 9% of the gasoline sold in the United States. Using ethanol either as a neat fuel or as an oxygenated fuel extender provides energy security and diversity, improves global competitiveness, reduces oil imports, creates jobs, improves the economy, reduces the trade deficit, revitalizes agriculture and industry, and promotes energy competition. It also improves the environment by improving urban air quality, and by reducing the threat of global warming. DOE and NREL see great potential for use of ethanol as a fuel, and are eager to expand the range of useful feedstocks to include troublesome waste materials and non-food

"energy crops" grown specifically for ethanol production.

Cellulose and hemicellulose are polymers comprised of carbohydrates; they make up about one-half and one-quarter of most biomass, respectively. The trick to converting cellulose to ethanol is first breaking it down into its component sugar, glucose. Once that is done, the six-carbon glucose is readily fermented to ethanol by common yeasts. On the other hand, chemicals will break down hemicellulose into its component sugars relatively easily, but the five-carbon sugars produced—usually principally xylose—are much more difficult to ferment to ethanol. The common yeasts that ferment glucose and other six-carbon sugars are unable to ferment xylose.

Early work on xylose fermentation focused on using a bacterial enzyme, called xylose isomerase, to convert xylose to another five-carbon sugar, xylulose—one that several common yeasts can ferment. Scientists developed an innovative process that

effectively combined the conversion of xylose to xylulose with fermentation of xylulose to ethanol. NREL has patented a simultaneous fermentation and isomerization of xylose (SFIX) process based on this approach.

Direct Fermentation Major Improvement

Because converting xylose directly to ethanol would be efficient and cost effective, NREL scientists evaluated microorganisms that ferment xylose directly to ethanol and recently completed a comparative study of yeasts, fungi, and a recombinant strain of bacteria—all promising candidates for direct xylose fermentation on an industrial scale. They found the wild-type yeast, *Pichia stipitis*, and a recombinant *Escherichia coli* (*E. coli*) bacterium to have the best performance characteristics.

With a long history of use for industrial fermentation of six-carbon sugars and the potential of producing an animal feed coproduct from their cell growth—a very important economic factor in corn ethanol

Fermentation Process Development at NREL

Process Development Using the Data Acquisition and Control System:

The computer-controlled data acquisition and control system (DACCS) is one of the first systems designed to track anaerobic, microaerophilic (very low oxygen level), and aerobic fermentation processes. It monitors, records, and analyzes process data, including gas emissions. The ability of the system to measure carbon dioxide generation enables researchers to precisely determine how much sugar has been converted to ethanol, confirm process performance data, and better understand the biochemical processes involved. The system is valuable to other NREL bioprocess engineers and is available to outside researchers under cooperative arrangements.

Alternative Fuels User Facility and Process Development Unit :

NREL's new Alternative Fuels User Facility (AFUF) includes a process development unit (PDU) that occupies 743 square meters (8000 square feet) of space in the new building. The PDU's feedstock capacity of 900 kg (1 dry ton) per day will allow assessment of the biomass-to-ethanol process at the pilot-plant scale and testing of commercially available equipment. The PDU has four 9000-liter (2378-gallon) fermentation tanks, a continuous-process pretreatment reactor, a 12-meter (39-foot) distillation column, inoculation tanks, centrifuges, and utilities. NREL plans to add three additional fermentation tanks in 1995, bringing the PDU's capacity to 63,000 liters (16,643 gallons).

Process Integration Unit:

In addition to a number of other laboratories, the AFUF also contains two integrated process development laboratories in which researchers can test operations and processes linked as they would be in actual commercial production. Process integration studies identify key chemical and biological interactions between the various steps and will identify candidate processes for pilot-plant trials in the PDU.

production—yeasts have many advantages. But when it comes to xylose fermentation, they have one major problem: natural yeasts that ferment xylose do not perform well under either fully anaerobic or aerobic conditions. These yeasts require low levels of oxygen that are difficult to maintain at industrial scales. To better characterize the performance of one such yeast, *P. stipitis*, NREL researchers studied it in bench-scale fermentors with a powerful analytical tool developed at NREL—a bench-scale fermentation data acquisition and control system (DACS). With the DACS, NREL researchers fully analyzed the trade-off between the increase in the rate of ethanol production and the decrease in the yield per amount of xylose as aeration increases. This analysis confirmed the need for low aeration levels to achieve optimal performance with *P. stipitis*. NREL researchers also used the DACS system to evaluate and understand the recombinant *E. coli*'s production of ethanol from lignocellulosic biomass.

Metabolic Engineering Opens the Door to Cofermentation

At the same time researchers have been working to identify the best available microorganisms to ferment xylose directly, they also have been pursuing a totally different approach with tremendous potential for improving biomass-to-ethanol technology—cofermentation. NREL is now reporting an important breakthrough.

NREL's current biomass-to-ethanol technology uses dilute-acid pretreatment to break down the hemicellulose to xylose. The soluble xylose can then be separated from the solid cellulose and lignin fractions and fermented in separate fermentation tanks. The separate fermentation tanks and associated equipment are a sizable portion of the capital cost of the process. What if a single organism



Warren Gretz, NREL

NREL researcher works with an electroporator to open pores in cell membranes to facilitate transfer of genes for desired metabolic capability into *Zymomonas mobilis*.

could effectively ferment both five- and six-carbon sugars? With a major achievement in metabolic engineering, NREL researchers have developed such an organism, and they are now working to incorporate it into their biomass-to-ethanol process.

NREL researchers started by conducting a survey of several microorganisms used in industrial processes and compared their known metabolic characteristics. This allowed them to select one with the best characteristics for an economical biomass-to-ethanol cofermentation process. *Zymomonas mobilis*, a bacterium used in Mexico to make the alcoholic beverage pulque from the juice of certain agave plants, proved very attractive. *Z. mobilis*, the only species of the *Zymomonas* genus, displays many traits sought in an ideal

biocatalyst for ethanol production. It converts less of its food to its own body mass than other microorganisms and produces mostly ethanol rather than other products, so its yield of ethanol is very high. In comparative glucose fermentation performance trials, *Z. mobilis* achieved 5% to 10% higher yields and up to fivefold higher productivities than traditional yeasts. *Z. mobilis* also has: high ethanol tolerance, allowing high final ethanol concentration; the ability to ferment sugars at low pH; and considerable tolerance to the inhibitors found in pretreated biomass. In addition, the stillage from *Z. mobilis* fermentation is generally recognized as safe for use as an animal feed—a potentially important factor in ethanol production economics.

Metabolic Engineering at NREL

Metabolic engineering is the purposeful modification of a microorganism's metabolism through the use of recombinant DNA techniques. These techniques can:

- Amplify the concentration of rate-limiting enzymes to improve productivity
- Inactivate competing biochemical pathways to increase product yield
- Increase the substrate utilization range to permit the use of low-cost renewable feedstocks
- Introduce biochemical pathways for novel products.

Collectively, this approach provides a rational way to maximize fermentation yields and productivity. NREL research teams are using their metabolic engineering expertise to develop effective ways to produce ethanol and other chemicals from biomass.

But *Z. mobilis* naturally ferments only glucose, sucrose, and fructose—not xylose. Turning *Z. mobilis* into a universal biocatalyst for making ethanol from biomass required some highly sophisticated metabolic engineering. NREL's molecular biologists simultaneously introduced four genes, representing two new biochemical pathways—one for xylose assimilation and one for pentose metabolism—into the bacterium. These genes were isolated from another bacterium and precisely fused to strong promoters (control signals) from *Z. mobilis* that allow the genes to be turned on simultaneously, even in the presence of glucose.

The engineered *Z. mobilis* now produces ethanol from xylose and continues to produce ethanol efficiently from glucose. Because it uses less energy for cell formation, the NREL strain produces more ethanol per unit of available xylose than other microorganisms. And, what is most important, the presence of glucose does not shut down the xylose fermentation capability, nor does xylose affect the glucose fermentation. The NREL strain of *Z. mobilis* is an effective cofermenter and the result of a major metabolic-engineering breakthrough.

Industrial Interest Welcome

The new biocatalyst improves productivity and reduces fermentation

time. Efficient conversion of feedstocks that contain xylose increases ethanol yield and revenue. In addition, it provides the foundation for advanced process designs that significantly reduce capital and operating costs. NREL scientists are currently working to further improve their new *Z. mobilis* strain and develop cofermentation process designs that take full advantage of it. But they also are already looking for appropriate cooperative partners or licensees to make it available for industrial use. NREL welcomes interest in using the SFIX process or the Laboratory's direct xylose fermentation expertise as well as the DACS, process integration unit, pilot plant, and other ethanol research facilities.

With the cofermentation biocatalyst, NREL fully expects to eliminate xylose fermentation as a separate process and dramatically reduce the cost of converting biomass to ethanol—and thereby take a key step toward generating a major new domestic industry that creates jobs and helps to alleviate U.S. petroleum dependence, trade deficit, pollution, and climate change problems.

Publications

- Boynton, B.L.; McMillan, J.D. (1994). "High-Yield Shake Flask Fermentation of Xylose to Ethanol." *Applied Biochemistry and Biotechnology*. (45-46); pp. 509-514.
- Boynton, B.L.; Newman, M.M.; McMillan, J.D. (July 1993). "Comparative Evaluation of Candidate Xylose-Fermenting Microorganisms on Hybrid Poplar DN34 Hemicellulose Hydrolyzate." (Draft).
- Hinman, N.D.; Wright, J.D.; Hoagland, W.; Wyman, C.E. (1989). "Xylose Fermentation: an Economic Analysis." *Applied Biochemistry and Biotechnology*. (20-21); pp. 391-401.
- McMillan, J.D. (July 1993). "Construction and Validation of a Bench-Scale Fermentation Data Acquisition and Control System." (Draft).
- McMillan, J.D. (January 1993). *Xylose Fermentation to Ethanol: A Review*. NREL/TP-421-4944. Golden, CO: National Renewable Energy Laboratory.
- McMillan, J.D. (1994) "Conversion of Hemicellulose Hydrolyzates." *Enzymatic Conversion of Biomass for Fuels Production*. Himmel, M.E.; Baker, J.O.; Overend, R.P., eds. American Chemical Society Symposium Series, No. 566, pp. 411-437.
- Picataggio, S.K.; Zhang, M.; Finkelstein, M. (1994). "Development of Genetically Engineered Microorganisms for Ethanol Production." *Enzymatic Conversion of Biomass for Fuels Production*. Himmel, M.E.; Baker, J.O.; Overend, R.P., eds. American Chemical Society Symposium Series, No. 566, pp. 342-362.
- Zhang, M.; Eddy, C.; Deanda, K.; Finkelstein, M.; Picataggio, S. (1995). "Metabolic Engineering of a Pentose Metabolism Pathway in Ethanologenic *Zymomonas mobilis*." *Science*. (267); pp. 240-243.
- Zhang, M.; Franden, M.A.; Newman, M.; McMillan, J.D.; Finkelstein, M.; Picataggio, S. (1994). "Promising Ethanologens for Xylose Fermentation." *Applied Biochemistry and Biotechnology*. (In press).

For More Information

- General Information and other NREL Technology Briefs:
Technical Inquiry Service
(303) 275-4099
- Technical Information on this NREL Project:
Stephen Picataggio
(303) 384-6107
Jim McMillan
(303) 384-6861
- NREL Business Information:
Technology Transfer Office
(303) 275-3008

