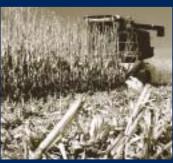
Bioethanol-Moving Into the Marketplace





Advanced Biotechnology Becoming Reality



Warren Gretz NRFI/PIX 0226



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What is biomass? Biomass includes the full range of plant and plant-derived materials. Starches and sugars from which ethanol are currently made are just a very small portion of available biomass. The great bulk of biomass consists of cellulose, hemicellulose, and lignin. Advanced bioethanol technology allows fuel ethanol production from the cellulose and hemicellulose, greatly expanding the renewable and sustainable resource base available for fuel ethanol production.

Technology for producing transportation fuel from biomass is moving out of the laboratory and into the marketplace. In the past decade, advances in biotechnology have allowed us to reduce the projected cost of producing bioethanol from biomass materials other than starches and sugars now used by nearly 25%. In the 1990s, the U.S. Department of Energy (DOE) National Bioethanol Program:

- Developed new, more versatile, microorganisms capable of squeezing more ethanol from biomass
- Gained a greater understanding of how the individual technology components work together in an integrated process
- Supported the private sector's initiatives to commercialize bioethanol technology

As we enter the 21st century, we are seeing federal investment in research beginning to pay dividends in the market-place. Meanwhile, the DOE Bioethanol Program is building on these successes. Our research program targets process improvements that will ultimately allow bioethanol to compete head to head with gasoline as a fuel supply for the U.S. transportation sector. Our strategy is simple. We will ride the growing wave of biotechnology advances to build efficient bioprocesses for ethanol production.

THE BIOFUELS PROGRAM MISSION:

To develop cost-effective, environmentally friendly technologies for production of alternative transportation fuels and fuel additives from plant biomass

The Biofuels Program is managed by the Office of Fuels Development (OFD) at the U.S. Department of Energy (DOE). Biofuels research and development includes work on a range of renewable liquid fuels, and covers the whole spectrum of technology development from basic science to commercial deployment. DOE's Bioethanol Program is by far the largest of several fuel development efforts managed by OFD under the auspices of the Biofuels Program.

The National Bioethanol Program

Today, fuel ethanol in the United States is made from corn starch, a biopolymer of glucose that is readily broken down to sugars. The goal of the National Bioethanol Program is to develop technology which can produce ethanol from the sugars in cellulose and hemicellulose, two of the main components of the fibrous material that makes up the bulk of most plants. This opens up a wide range of feedstock materials for bioethanol production to supplement current production from starch.

Biomass Feedstock Development

DOE researchers are developing new sources of biomass for bioethanol production. These include the residues left over after harvesting of existing food crops and, further down the road, new energy crops like switchgrass. Today, farmers leave millions of tons of residue on the ground after harvesting corn. The responsible collection and use of this residue—known as corn stover—offers a huge opportunity for expanding the supply of ethanol from its current level of 1.6 billion gallons per year (2000) to more than 10 billion gallons per year. As demand expands beyond this level, newly developed energy crops will come into play.

Bioethanol Conversion Technology

Two key steps are at the heart of the DOE Bioethanol Program's research and development activities for bioethanol conversion technology:

1. Hydrolysis. This is a chemical reaction that releases sugars, which are normally linked together in complex chains. In early biomass conversion processes, acids were



Min Zhang, NREL/PIX 06812

Zymomonas recognized by scientific peers

1995—R&D 100 Award

1995—Science magazine publication, "Metabolic Engineering of a Pentose Metabolism Pathway in Ethanologenic Zymomonas mobilis"

1996—U.S. Patent #5,514,583 "Recombinant Zymomonas for pentose fermentation"

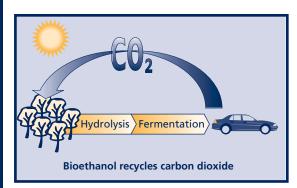
1998—U.S. Patent #5,712,133 "Pentose fermentation by recombinant Zymomonas"

1998—U.S. Patent #5,726,053 "Recombinant Zymomonas for pentose fermentation"

1998—U.S. Patent #5,843,760 "Single Zymomonas mobilis strain for xylose and arabinose fermentation"

used to accomplish this. Recent research has focused on enzyme catalysts called "cellulases" that can attack these chains more efficiently, leading to very high yields of fermentable sugars.

2. Fermentation. Microorganisms that ferment sugars to ethanol include yeasts and bacteria. Research has focused on expanding the range and efficiency of the organisms used to convert sugar to ethanol.



Breakthroughs in Fermentation Technology in the Past Decade Lead to Commercialization of Biomass Conversion Technology

Common sense suggests that we need to convert every bit of biomass into fuels and coproducts. For ethanol production, this means using all the available sugars. For most of this century, researchers assumed that many of the sugars contained in biomass were not fermentable—those contained in hemicellulose. This meant that as much as 25% of the sugars in biomass were out of bounds as far as ethanol production was concerned. In the 1970s and 80s, microbiologists discovered microbes that could ferment these sugars, albeit slowly and inefficiently. The race was now "on" to under-

stand how these organisms handled these sugars, and to create new organisms capable of efficient conversion of all the sugars found in biomass. With the advent of new tools in the emerging field of biotechnology, researchers at DOE labs and at universities across the country, have succeeded in producing several new strains of yeast and bacteria that exhibit varying degrees of ability to ferment the full spectrum of available sugars to ethanol. The advances made in the 1990s are now the starting point for entrepreneurs interested in realizing a new bioethanol industry. Existing ethanol producers are also looking to these new organisms as a pathway for improving their own bottom line as well.



Warren Gretz, NREL/PIX 0094

At the Bioethanol Program's one-ton-per-day Process Development Unit, bioethanol developers can test proposed processes under industrial conditions without having to build their own pilot plants.

ADVANCED BIOETHANOL TECHNOLOGY—PROVIDING MULTIPLE OPTIONS

The race to create new microbes capable of fermenting the full range of sugars found in biomass has followed several successful pathways. Dr. Lonnie Ingram at the University of Florida started with an *E. coli* bacterium capable of metabolizing multiple sugars and added the ability to make ethanol—a feat for which he received U.S. Patent #5,000,000 in 1990. His work was sponsored by the Biofuels Program and others.

Taking an approach that complements Dr. Ingram's *E. coli*, other DOE researchers started with the bacterium

Zymomonas, a naturally efficient ethanol-producing bacterium, and added the capability for utilizing multiple sugars (see "Zymomonas recognized by scientific peers," above left).

DOE also helped support Purdue's Dr. Nancy Ho, who started with the "industrial workhorse" for ethanol production—the yeast *Saccharomyces*—and added the capability for utilizing multiple sugars.

All three organisms are now being tested by industrial partners for use in bioethanol production.

A Decade of Generating **Engineering "Know-How"**

Along the continuum of technology development from basic science research to commercialization, process engineering data bridges the gap between scientific inventions in the lab and commercial production facilities. The Bioethanol Program, over the past ten years, has increased the engineering knowledge base by collecting rigorous material and energy balance data on integrated bioethanol processes. Today, we have greater confidence about projected process performance and cost, and a far more realistic understanding of the engineering issues remaining to be solved. This kind of information is critical to entrepreneurs and financiers looking at multimillion-dollar investments in bioethanol technology.

Support for Today's Industry and Tomorrow's Pioneers

The Department of Energy's Bioethanol Program supports a portfolio of activities that is balanced across the spectrum of technology development. To this end, we supplement

our core Research and Development program with activities focused on near-term deployment opportunities. Our goal is to plant the seeds today for the technology we are developing for tomorrow's renewable fuel industry.

Giving a boost to today's fuel ethanol industry Today's ethanol producers are looking for ways to push their yields as high as possible. They are turning their attention to corn fiber—the shell of the kernel—as a source of additional sugars for ethanol production. But, corn fiber, like other forms of biomass, contains sugars that are not fermentable by today's industrial fermentation organisms. The National Corn Growers Association and the Corn Refiners Association are working with DOE researchers to tailor new microbes that can ferment these specific sugars. This is work that builds directly off the Bioethanol Program's successes of the past decade. Customized organisms developed in this cooperative project will be available to the member companies of these two important industry trade groups.

Supporting industry pioneers for a new bioethanol industry Several companies are now pursuing niche opportunities



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Current U.S. ethanol producers use only the starch in the corn kernels. The fiber left from that processing, however, plus the cobs, husks, and stalks all contain sugars that can also be made into ethanol.

CONCENTRATED ACID TECHNOLOGY

Masada Resource Group. Masada is a waste management company that views bioethanol as one of several tools in its aresenal for managing waste and recycling operations. They are planning on constructing a bioethanol plant in New York State, where solid waste disposal costs are very high. The plant will convert the biomass portion of municipal solid waste into fuel grade ethanol. DOE researchers have worked with Masada to collect engineering data on one of the key process steps—the separation and recycling of sulfuric acid from the sugar stream prior to fermentation.







DILUTE ACID TECHNOLOGY

BC International. BCI is building a facility that will initially produce 20 million gallons per year of ethanol from bagasse—the residue left over after sugarcane production. BCI will utilize an existing ethanol plant located in Jennings, Louisiana. At the heart of BCI's process is the recombinant E. coli originally patented in 1990. DOE researchers have worked with BCI to collect critical process engineering data.

♦ Groundbreaking ceremony for BCI's bagasse-to-ethanol plant in Jennings, Louisiana.

ENZYME TECHNOLOGY

Fungal production of cellulase.

logen. logen is a Canadian enzyme producer who recently entered into a joint venture with PetroCanada to demonstrate the use of cellulase enzymes in the production of bioethanol. The Bioethanol Program is supporting research at the University of Wisconsin and the University of Toronto to evaluate the use of a yeast strain and DOE's recombinant Zymomonas in their process. logen is targeting agricultural residues such as wheat straw and corn stover for their initial commercial demonstration.





Mike Himmel, NRFI/PIX 05014

Genencor/Novozyme. Because enzyme technology offers such great promise for ethanol production if the cost of cellulase enzymes can be reduced—the Bioethanol program has contracted with two of the largest industrial enzyme producers to develop low-cost cellulases. Both Genencor International and Novozymes Biotech expect to be able to dramatically reduce cellulase cost over the next three years; this would greatly improve the economics of bioethanol technology.

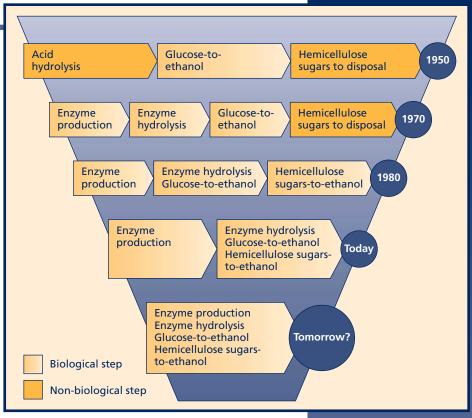
◆Computer animation of cellulase enzyme attached to cellulose.

for introducing bioethanol technology in the U.S. Each of these companies has identified one of several variations of the process for converting biomass into ethanol. Two of the processes involve the use of sulfuric acid, in either concentrated or dilute form, to hydrolyze the cellulose and hemicellulose, while the third introduces the use of enzymes called cellulases to hydrolyze the most challenging of the biopolymers—cellulose.

Bioprocessing—a strategy of adding, improving and combining biological process steps

A strategy that reflects past and future accomplishment Today we talk about our strategy for future technology improvements in terms of "bioprocessing," but, in fact, this is not a new approach. Bioprocessing captures the approach that we have taken over the past few decades, as suggested by the illustration above. Dilute acid hydrolysis reached the limits of its capabilities after several decades of research that led to the construction of a plant during World War II and further refinements in the 1950s. At that time, the biological steps were limited to the conversion of glucose to ethanol by conventional industrial yeast. Early in the 1970s, research began on the use of cellulase enzymes to hydrolyze cellulose—an approach that offered higher yields and elimination of unwanted side reactions. The 1980s saw dramatic improvements in enzyme performance, and the first efforts to consolidate biological hydrolysis and glucose fermentation. Today, we have taken bioprocessing of ethanol from biomass one step further by genetically engineering microbes capable of fermenting hemicellulosic sugars as well as cellulosic sugars. What will bioprocessing mean for tomorrow's technology? The exact form is not clear. We are attacking the technology on several fronts—by applying new biotech tools to improve cellulase enzymes and continuing to enhance the fermenting organisms.

The Marketplace So, what does all this technology improvement mean for the market-place? Before the introduction of microbes capable of handling multiple sugars, ethanol from biomass was projected to cost about \$1.58 per gallon for the enzyme process. A



1999 study projected that cost at \$1.16 per gallon. This cost assumes access to moderately priced feedstocks (at around \$25 per dry ton), and reflects a combination of the best results reported by various research groups in industry and in the private sector. The fuel ethanol market currently supports a price of anywhere from \$1.00 to \$1.40 per gallon. Though the high capital investment and higher risk of deploying new technology are still hurdles to be overcome, it is clear that the new bioethanol technology is poised for commercial introduction.

In 2000 the fuel ethanol market accounted for 1.6 billion gallons per year in sales (2.0 billion capacity). This market is now constrained—because of cost—to the use of ethanol as a blending agent in gasoline, with only limited sales of "E85"—85% ethanol fuel. As technology costs drop, bioethanol will add sales of anywhere from 6 to 9 billion gallons year. With the blend market saturated at this level of ethanol sales, we will then set our sites on the bulk fuel market—competing head-to-head with gasoline at ethanol costs of around \$0.60 per gallon.

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