SunEthanol

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Testimony

Before the

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Hearing on

*The Gas is Greener: the Future of Biofuels*

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**Testimony of Susan Leschine**

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I thank you for giving me this opportunity to testify today on the subject of biofuels and the impacts of biofuels development on energy independence and global warming. My name is Susan Leschine. I am a Professor of Microbiology at the University of Massachusetts, Amherst, and Founder and Chief Scientist at SunEthanol, a new biofuels technology company headquartered in Amherst. I also serve as Co-director of The Institute for Massachusetts Biofuels Research (TIMBR) at UMass Amherst, established by an interdisciplinary team of scientists and engineers to develop cost-effective technologies for producing biofuels and other value-added materials from biomass. Our goal is to establish the scientific and technological basis to enable the U.S. to meet the Department of Energy "30-30" goal, 30% gasoline reduction by 2030.

The link between fossil fuel combustion and global warming is compelling. We urgently must begin to limit greenhouse gas emissions. The need to limit greenhouse gas emissions has become even more critical with research results reported this week in the Proceedings of the National Academy of Sciences that carbon dioxide emissions are growing at a much faster rate than anticipated, and the ability of the land and the oceans to absorb carbon dioxide from the atmosphere has diminished.

Clearly, as we look to the future in meeting our transportation fuel needs, we must limit the use of fossil fuels. The only form of energy that can contribute substantially to fulfilling transportation fuel requirements at costs competitive with fossil fuel is solar energy captured by photosynthesis in plants and stored as biomass. At present, plant biomass is the only significant source of liquid transportation fuels that may replace the world's finite supply of oil.

Ethanol derived from biomass is one of the most promising biofuels. In addition to reducing our dependence on imported oil, thereby improving domestic energy security and lowering the U.S. trade deficit, biomass ethanol production will also yield environmental benefits in the form of reduced greenhouse gas emissions. In addition, the increased value of agricultural crops, crop residues, and new energy-specific crops will benefit rural economies through higher incomes and increased employment opportunities. Economic modeling studies suggest that simply integrating cellulosic biomass crops into the agricultural rotation of existing cultivated acreage could increase the net income of U.S. farmers by 32%, or $23 billion. In Massachusetts, where forest growth exceeds wood harvest, biomass from wood is a sustainable resource. The total woody biomass supply in Massachusetts has been estimated to be 4.4 million tons per year, which could theoretically be used to produce more than 400 million gallons of fuel ethanol.

The relative benefits of biomass ethanol compared with fossil fuels have been passionately debated. Important questions arise concerning the "energy return on investment" (ROI): the ratio of ethanol energy output compared to the nonrenewable energy input required to produce ethanol fuel. It is very important to note that several peer-reviewed studies have concluded that the energy return on investment for fuel ethanol production is favorable. Corn ethanol energy yields are favorable, and cellulosic ethanol energy yields have the potential to be even more favorable. Clearly, the production of ethanol from cellulosic biomass, such as wood chips, switch grass, corn stover or other agricultural waste has a clear advantage over gasoline. In large part, this energy advantage arises from the fact that biomass ethanol production makes use of the whole plant. The fermentable components of plants – cellulose and other polysaccharides – are separated from the non-fermentable lignin component, which can be burned and used to power ethanol production facilities.

It is very important to point out that the corn ethanol industry will play a central role in the future development of biofuels in this country. New technologies for cellulosic fuels are being built upon the pioneering expertise developed by the corn ethanol industry. Also, the industry has demonstrated that the agricultural sector of our country can play a key role on our path to energy independence.

Cellulosic ethanol is a reality. Demonstration plants are in operation and full-scale commercial plants are in construction. At the same time, new technologies are being developed – and must be developed - for more efficient and more cost-effective conversion of biomass to ethanol biofuel, specifically to overcome the resilience of cellulosic biomass. Plants are tough! Plant biomass is composed of highly ordered sugar polymers such as cellulose. These plant components are shielded by a matrix of other complex polymers. The recalcitrance of cellulosic biomass to bioprocessing (e.g., by enzymes) poses a significant obstacle to developing cost-competitive cellulosic ethanol technologies.

To overcome this biological hurdle, at SunEthanol we are developing a microbial bioprocessing technology. This technology arose from research in my laboratory at the University of Massachusetts Amherst.

For many years I have been investigating bacteria that decompose plant material or biomass. I am interested in these microbes because they play a very important role in the environment, in the global carbon cycle, and also because they have the potential capacity to convert plant biomass into useful products such as ethanol.

One such microbe was first isolated from forest soil near Massachusetts' Quabbin Reservoir. This microbe turned out to be particularly interesting because it decomposes nearly all of the components of biomass, and it produces ethanol as its primary product. We determined that this isolate from forest soil is a novel microbe, and we gave it a name, *Clostridium phytofermentans*. We continue to study the unique biological properties of this microbe in my lab at UMass.

We also described a new technology for producing cellulosic ethanol, which makes use of a strain of this bacterium, the Q microbe. This new technology involves the direct conversion of biomass to ethanol, consolidating several steps into one, a technology known as **C**onsolidated **B**io**P**rocessing (CBP). This technology has the potential to greatly improve the economics of ethanol production from biomass. For example, the separate production of costly enzymes may be completely eliminated in the Q microbe cellulosic ethanol process. The Department of Energy Biofuels Roadmap (June 2006), "Breaking the Biological Barriers to Cellulosic Ethanol," recognizes CBP as "*the ultimate low-cost configuration*" for cellulosic ethanol production.

Currently at SunEthanol we are taking this technology from the lab to the marketplace, developing an economically viable process using the Q microbe to produce ethanol from biomass as a renewable and sustainable transportation fuel.

In conclusion, cost-effective cellulosic ethanol production is achievable in the near term. This will be a monumental task. It is essential that there be significant resources invested for research and development at both the applied and basic science levels. Such investments will have enormous positive impacts on the environment and the economy, especially benefiting rural economies. Given that biomass is a regional resource, the impacts will be broad and widespread across the country. Perhaps most importantly, it is essential that we begin to limit greenhouse gas emissions. Renewable and sustainable biofuel production must form a key component of our energy future.

**Appendices**

Appendix 1. "*Biomass to Biofuel Technology: A Novel Bacterial Catalyst for Consolidated Bioprocessing of Biomass to Ethanol*," a white paper by Susan Leschine. January 2007

Appendix 2. "*In Microbe, Vast Power for Biofuel*," by Steven Mufson. Washington Post, October 18, 2007.

# Biomass to Biofuel Technology:

## A Novel Bacterial Catalyst for Consolidated Bioprocessing of Biomass to Ethanol

Susan Leschine, Ph.D.

The Institute for Massachusetts Biofuels Research (*TIMBR*)
University of Massachusetts Amherst

January 2007

Plant biomass, produced in nature using solar energy captured by photosynthesis, is generally regarded as the only source of liquid transportation fuels that may replace the world's finite supply of oil. Ethanol derived from biomass is one of the most promising such fuels. In addition to reducing our dependence on imported oil, thereby improving domestic energy security and lowering the U.S. trade deficit, biomass ethanol production would also yield environmental benefits in the form of reduced greenhouse gas emissions and air pollution. Moreover, the increased value of agricultural crops, crop residues, and new energy-specific crops would benefit rural economies through higher incomes and increased employment opportunities. Economic modeling studies suggest that simply integrating cellulosic biomass crops into the agricultural rotation of existing cultivated acreage could increase the net income of U.S. farmers by 32%, or $23 billion (3). In Massachusetts, where forest growth exceeds wood harvest, biomass from wood is a sustainable resource. The total woody biomass supply in Massachusetts has been estimated to be 4.4 million tons per year (1), which could theoretically be used to produce more than 400 million gallons of fuel ethanol.

Over the past twenty-five years, the relative benefits of biomass ethanol compared with fossil fuels have been passionately debated. Important questions arise concerning the "energy return on investment" (ROI). Because traditional ethanol production involves a series of steps, nonrenewable energy must be expended during processing. Therefore, the ratio of ethanol energy output compared to the nonrenewable energy input required to produce ethanol fuel is a critical metric. Two recent, peer-reviewed analyses concluded that the energy ROI of fuel ethanol production is favorable (2, 4). However, not all ethanol is created equal: existing methods for production of ethanol from corn yield only a marginally favorable energy return. On the other hand, production of ethanol from cellulosic biomass, such as wood chips, switch grass, corn stover or other agricultural waste has a clear advantage over gasoline. In large part, this energy advantage arises from the fact that biomass ethanol production makes use of the whole plant. The fermentable components of plants – cellulose and other polysaccharides – are separated from the non-fermentable lignin component, which can be burned and used to power ethanol production facilities.

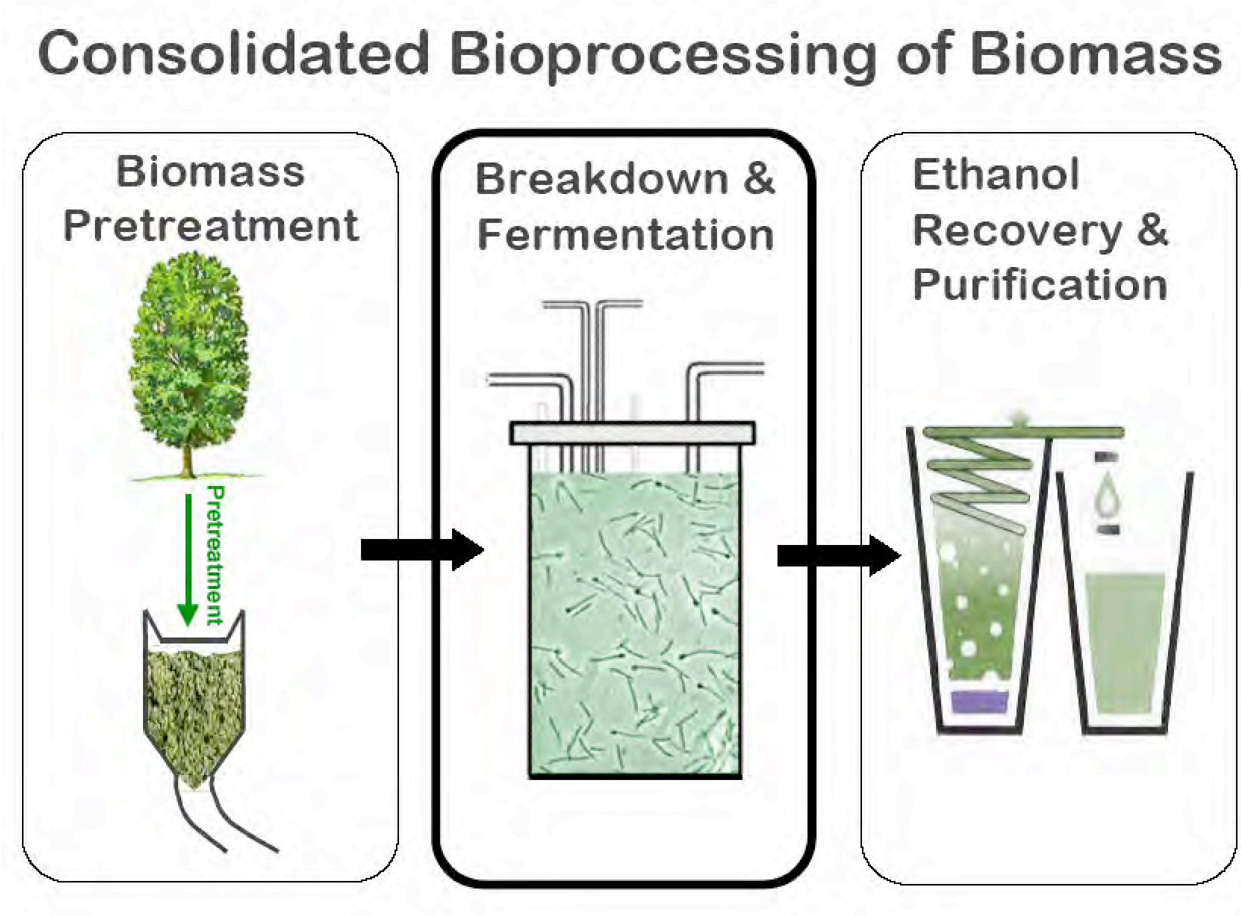


**Fig. 1.** Consolidated bioprocessing (CBP). Production of cellulase enzymes, cellulose breakdown, and fermentation are consolidated in a single step in a bioreactor ("Breakdown & Fermentation"). Shown in the reactor are cells of the bacterium *Clostridium phytofermentans*, which serve as biocatalysts in the CBP process. Bacterial cells were imaged using phase-contrast light microscopy.

### New Technology for Biofuel Production

Large-scale use of ethanol for fuel will require new technologies for the efficient conversion of biomass to ethanol biofuel. The recalcitrance of cellulosic biomass to enzymatic bioprocessing poses a significant obstacle to the development of these technologies. Cellulosic biomass is composed of highly ordered sugar polymers, which are shielded from enzyme attack by a matrix of other complex polymers. A microbial bioprocessing strategy can be employed to overcome this biological hurdle. In this regard, technology under development at the University of Massachusetts Amherst involves the use of a novel bacterium, which was first isolated from forest soil near Massachusetts' Quabbin Reservoir.

The bacterium *Clostridium phytofermentans* actively and efficiently decomposes cellulose and produces ethanol. Cellulose-fermenting cultures of *C. phytofermentans* produce prodigious amounts of ethanol and they also form $H_2$. In addition, *C. phytofermentans* possesses exceptional nutritional versatility and is capable of decomposing more components of biomass than most other known microbes. Furthermore, we have recently discovered that *C. phytofermentans* ferments unusually high concentrations of cellulose with increased ethanol production.

Our recent investigations have revealed several unusual and unexpected properties of *C. phytofermentans* that indicate it would be an ideal organism for use in the commercial development of large-scale direct conversion of cellulosic biomass to ethanol. This direct conversion biomass-processing scheme is referred to as consolidated bioprocessing (CBP) because production of the cellulase enzymes, cellulose decomposition, and fermentation are all consolidated in a single step (Fig. 1). Patent applications have been filed, including a US utility application and an international (PCT) application. These applications relate to the compositions, systems, and methods for producing biofuels such as ethanol, and other chemicals from cellulosic biomass.

**How does the *C. phytofermentans* CBP technology compare with other technologies for biomass ethanol production?**

The overall conversion of biomass to ethanol can be viewed as a multi-step process involving the enzymatic decomposition of complex cellulosic materials (polysaccharides) into simple sugars, and the fermentation of these sugars to ethanol. To date, most research has focused on biomass processing schemes that separate the process of cellulase enzyme production from the hydrolysis (breakdown) of complex cellulosic materials, and subsequent fermentation. An advantage of the *C. phytofermentans* CBP technology is that enzyme production, hydrolysis of biomass polysaccharides, and fermentation of the resulting simple sugars occur simultaneously in a single bioreactor.

The inherent simplicity of the *C. phytofermentans* CBP technology presents an obvious advantage, but in addition, the technology obviates the need for separate and costly enzyme manufacture. A recently published study estimates that production of ethanol by CBP would reduce costs by 25% or more as compared with processes involving separate enzyme production (6). Additionally, microbial activity may facilitate cellulose breakdown in ways that are not yet fully understood. For example, it has been suggested that the presence of insoluble cellulose might trigger an increase in cellulase enzyme production by a cellulose-fermenting microbe (9).

At present, a major limitation to the development of biomass refineries is the lack of appropriate microbial catalysts that are capable of fermenting the wide range carbohydrates found in biomass, particularly five-carbon sugars and five-carbon sugar polymers, such as xylose and xylan, which make up the hemicellulosic portion of biomass (5, 6). This is a critical limitation, given that the hemicellulosic portion of biomass may constitute 20 to 35% of plant dry weight (7). Most cellulolytic bacterial strains are incapable of fermenting five-carbon sugars and five-carbon sugar polymers due to the narrow growth substrate range of these strains. Researchers pursuing potential solutions to this problem are investigating recombinant DNA techniques to genetically modify strains in order to expand their substrate range, or coculturing cellulolytic bacteria with other microbes that are capable of fermenting five-carbon sugars and polymers (7).

The alternative approach we have followed taps the natural diversity that exists in anaerobic biomass-decomposing microbial communities. *C. phytofermentans* was isolated from such a microbial community in forest soil near an intermittent stream. An advantage of the CBP technology we are developing is that it employs the properties of naturally-occurring *C. phytofermentans*, which has an uncommonly broad range of growth substrates including such five-carbon sugar polymers as xylose and xylan (8). We have recently discovered that *C. phytofermentans* is able to simultaneously ferment different polymeric components of biomass (e.g., cellulose and xylan), a property that would be particularly useful for the efficient production of ethanol from biomass.

The natural fermentation characteristics *C. phytofermentans* are uniquely well suited to cellulosic ethanol CBP technology currently under development at UMass Amherst. Future advancements in this technology might involve genetic modifications of the microbe to further improve its fermentation properties. As part of ongoing research, we are exploring genetic engineering strategies to modify the metabolic properties of *C. phytofermentans* in order to maximize the cellulosic ethanol yield and increase the microbe's tolerance to ethanol. These strategies are anticipated to enhance cellulase activity while at the same time eliminating unwanted byproduct formation. In support of this research, the genome sequence of *C. phytofermentans* has been determined in collaboration with the U.S. Department of Energy Joint Genome Institute. The availability of genome sequence data for *C. phytofermentans* is a significant advantage that will greatly facilitate future development of this ethanol-from-biomass consolidated bioprocessing technology.

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Thomas Warnick, a University of Massachusetts lab technician, near the spot in the Quabbin Reservoir where he filled a jar with dirt that turned out to have a cellulose-chomping, ethanol-producing microbe.

**Photo Credit:** By Steven Mufson -- The Washington Post Photo

# In Microbe, Vast Power For Biofuel

Organism's Ability To Turn Plant Fibers To Ethanol Captures Investors' Attention

By Steven Mufson
Washington Post Staff Writer
Thursday, October 18, 2007; D01

QUABBIN RESERVOIR, Mass. Ten years ago, an assistant from a microbiology laboratory took a hike near the shore of the vast Quabbin Reservoir, which supplies water to Boston. At one point, he crouched alongside a brook in the shade of towering hemlock trees, dug up some moist dirt, put it in a jar and took it back to the lab.

Today, some investors are betting that the jar of dirt could help change the biofuels industry.

Inside the jar, microbiology professor Susan B. Leschine found curious lollipop-shaped microbes with an uncommon ability to break down leaves and plant fibers into ethanol. For 30 years, Leschine has been researching this sort of thing and writing about it for publications such as the International Journal of Systematic and Evolutionary Microbiology.

Some venture capitalists in the area have convinced Leschine that her tiny microbe could be very big business. Now Leschine, who teaches at the University of Massachusetts at Amherst, is also chief scientist at SunEthanol, a start-up firm with about a dozen employees.

The firm has attracted an equity investment from VeraSun Energy, one of the nation's biggest producers of ethanol derived from corn and used as motor fuel. It is VeraSun's first investment in the next generation of ethanol, known as cellulosic ethanol, made from switch grass, wood chips and other plant fibers. Now SunEthanol is racing to gear up for commercial production of the microbe so it can move from the cloudy test tubes in Leschine's cluttered lab into the giant vats at VeraSun's refineries.

SunEthanol is just one of countless firms searching for ways to make cellulosic ethanol a commercially viable business. At the moment, they have a way to go. Unlike ethanol made from corn, not a drop of cellulosic ethanol is being commercially produced. Half a dozen pilot projects are being built -- with the help of $385 million in Energy Department grants -- but no one claims to have a sure thing.

"We're optimistic, but we're also realistic that this is an early-stage company and it still has many hurdles to cross," said Bill Honnef, VeraSun's vice president for strategic initiatives. "We will look at the program over the next year and figure out how we're doing. At that point, we will decide whether to make further investments."

Congress is working to prime the cellulosic ethanol pump. The Senate version of the energy bill being considered would require the oil industry to use 21 billion gallons annually of "advanced biofuels," including cellulosic ethanol, by 2022. A tax break would allow companies to deduct half the cost of a new plant in the first year of operation. And cellulosic ethanol would draw generous subsidies for oil refiners who mix it into their gasoline.

Still, many technological hurdles remain, and much of the venture capital poured into the cellulosic ethanol industry is going into companies like SunEthanol that are searching for ways to make the manufacturing process more efficient and profitable.

A key part of the challenge is figuring out how to better break down cellulosic material -- such as cornstalks or wood chips -- into ethanol. Many firms are trying to do that in two steps, first breaking down cellulose into sugars and then fermenting sugars to produce ethanol for use in motor fuel.

Many companies are genetically engineering enzymes to do the first task. Those enzymes tend to be expensive. On Monday, Genencor, a division of Danisco, announced that it had developed a new product, Accellerase 1000, that it said contains a combination of enzymes that reduces cellulosic biomass into fermentable sugars.

"Lots and lots and lots of groups and companies are looking for new cellulases," or enzymes that process cellulose, said J. Craig Venter, who raced the federal government in mapping the human genome. Venter's company, Rockville-based Synthetic Genomics, is searching for naturally occurring chemicals that can turn sugar into diesel fuel. "A key part of nature is breaking down plant debris," he said. "So we find all kinds of environments with unique cellulases in them."

Leschine says her microbe has the advantage of performing both the breakdown of plant fibers and the production of ethanol. "Creating one microbe that does what enzymes and fermentation do is regarded as the Holy Grail because of the savings in costs," she said.

For the microbe, the plant fibers are food while ethanol and carbon dioxide are waste products. "These are tiny little cells we can't even see. They don't have mouths. How do they do it?" she said with a sense of wonder.

Whether SunEthanol will succeed remains unclear, but it is a good example of the hopes and hurdles for companies in the cellulosic biofuel business. Many of those firms rely on the research, and serendipity, of scientists like Leschine.

For years, Leschine scoured soil samples from around the world in search of the perfect microbe, one that would excel at breaking down what nature casts on the ground in damp places like the reservoir. When friends or colleagues traveled, she would ask them to bring back soil in jars or old plastic film canisters.

Yet the best microbe may have been here all along, lurking near some ferns and an old stone dam just 20 minutes from her university lab. She has dubbed it the Q microbe for the Quabbin Reservoir.

Apart from its lollipop appearance, it didn't stand out at first. But the more she tested it, the more unusual it seemed. Most microbes have about 20 machine-like proteins for absorbing sugars; the Q microbe has more than 100 for various nutrients, half of them for sugars, she says. The Q microbe also works in moderate temperatures, making it useful for manufacturing.

Leschine's interest predates the current fervor over biofuels. Since her graduate school days, she has been interested in the role of microbes in the carbon cycle. On the shelves of her office sit beanbags in the shapes of microbes. In a corner stands a bag of dirty cornstalks.

"The last thing in the world I wanted to do was start a company," Leschine said as she stood next door to her lab, where an assistant heated mixtures in test tubes. "It seemed like more of a distraction."

Then she talked to someone teaching business at the university, and he and a group of venture capitalists persuaded her to commercialize it. She came to feel that it was her obligation because she had received Energy Department research grants.

Next came lengthy negotiations with the university, which owned the intellectual property rights for work done in its labs. SunEthanol negotiated an exclusive licensing agreement with the university, which owns the patent.

The Q microbe's future hinges on the SunEthanol founders' skill at navigating talks like this to raise more money and persuade ethanol makers to use the Q microbe instead of a competing bug or enzyme. A small business is like Leschine's test tube samples, and scaling it up is no science. SunEthanol's chief executive Jef Sharp knows that from experience. He started a successful clothing firm. Later, he started X-S Capacity, which he called a sort of eBay for excess manufacturing capacity. It flopped. More recently, he launched Tech Cavalry, which provides computer support for individuals and businesses. This time, he says, "the wind is at our backs."

SunEthanol has received help from the Energy Department in mapping the genetic code of the Q microbe. The firm also hopes to receive an Energy Department grant through a program to help small businesses. "It could be lifeblood for us," said Jonathan Gorham, SunEthanol's marketing director.

SunEthanol's founders fear that the regulations are written in a way that will restrict federal funding to enzyme development, not microbe development. They have lobbied the Energy Department and members of Congress to make sure their work will be considered.

Next SunEthanol must figure out how to go from working with a liter or two of Q microbes in the lab to churning out millions of gallons of the microbe. VeraSun, for example, has fermentation vats that are more than 100,000 gallons each. SunEthanol must also test temperature and acidity conditions for the Q microbe, which will be expected to break down plant material very different from what it consumes near Quabbin's shore.

VeraSun's Honnef hopes that cellulosic ethanol plants can be built next door to the company's corn ethanol distilleries. Those distilleries use kernels and leave the rest, known as corn stover. The corn stover is largely waste material now but shows cellulosic possibilities.

"The conversion of cellulosic material to ethanol has been proven out in a lab," Honnef said. "The challenge is that it can't be done today on a commercial basis on a large scale."

Leschine is hopeful. "What we've demonstrated is that if you go out and look in nature, you can find a microbe that does what you want to do," she said. "The advantage of working with a natural microbe is that . . . you don't have to do all the complicated genetic engineering."

But she knows that she doesn't know much about engineering or big industry. She notes the dirty cornstalks in her office. They are there, she said, "to remind me that this is a daunting task."