FY 2008 Annual Report
NP307-- Bioenergy and Energy Alternatives

The ARS Bioenergy program develops technologies that can enable sustainable production of biofuels by the agricultural sector in ways that both enhance the natural resource base but do not disrupt existing markets for food, feed or fiber.

The growth and long-term viability of bioenergy production in the Nation is impeded by a number of technical barriers, and ARS research focuses on barriers which ARS is best suited to addressing. ARS has adequate and unique research capabilities to optimize both the production of bioenergy feedstocks as well as the biorefining of those feedstocks. Consequently, ARS will pursue the following major areas of bioenergy research:

- Enable new varieties and hybrids of bioenergy feedstocks with optimal traits;
- Enable new optimal practices and systems that maximize the sustainable yield of high-quality bioenergy feedstocks; and
- Enable new, commercially preferred biorefining technologies.

Accomplishments

Component I: Feedstock Development

Switchgrass genomics techniques help advance development of biomass crops. Rapid breeding of switchgrass varieties with desired traits will require sequence information for the switchgrass genome. ARS scientists led a national effort to produce 500,000 switchgrass sequence tags that have been released to the public through GenBank. Analysis of the sequences has identified approximately 76,000 unique genes. These sequences have enabled comparative genomics with other grass species, and gene families associated with both cell wall biosynthesis and photosynthesis are being investigated. In addition, the sequences have been screened for common marker classes, thereby enabling marker-assisted selection.

Regional gene pools for switchgrass identified to enhance selection and breeding efforts. Switchgrass, an important perennial plant in soil conservation and for bioenergy feedstock production, can be found from southern Texas to Canada. Within this area, numerous varieties of switchgrass exist which are adapted to the specific agronomic and ecological characteristics of their geographic home. ARS researchers developed the first system to classify switchgrass varieties into gene pools, based on region-specific characteristics such as temperature, moisture, day length, soil type and pre-settlement vegetation. Since varieties in the same gene pool should have comparable growth potential and survivability in each other's geographic home, this classification system will be a very useful tool in switchgrass breeding and for prairie restoration/conservation projects.

Mapping the sorghum photoperiod-sensitivity gene will improve yields. Sorghum shows great potential as a bioenergy crop, but new varieties with higher biomass yield are needed. Much valuable sorghum germplasm is of tropical origin, but these lines do not successfully flower or produce seed in the U.S., where daylight is shorter. Through high-resolution mapping, ARS scientists, in collaboration with university, company and international partners, identified candidate genes in sorghum for photoperiod sensitivity. This accomplishment will allow breeders to exploit previously unusable sorghum germplasm and so will enable rapid development of high-yield sorghum varieties for U.S. farmers.

Component II: Feedstock Production

Economic, energy, and environmental impacts of biomass feedstock production systems. Switchgrass and alfalfa are promising feedstocks for biorefining, but their energy balance, environmental impacts and economics have not been quantified compared to corn. ARS scientists assessed production costs, farm income, net energy use, and environmental impacts of cellulosic ethanol production in the Upper Midwest for 4 crop systems: continuous corn (with and without stover harvest); continuous switchgrass; and an alfalfa-corn rotation. Although continuous corn (CC) had the highest ethanol yield and profit, it was the least energy efficient and led to the most erosion and N leaching. Alfalfa-corn (AC) produced less ethanol and lower profits, but was more energy efficient, had less erosion, and virtually eliminated N fertilizer use and leaching. Switchgrass created almost no erosion, was the most energy efficient, and was between CC and AC in N fertilizer use and leaching; but it was profitable only when selling prices or yields are high.

On-farm pretreatment of biomass. Effective on-farm pretreatment of herbaceous biomass to improve its enzymatic digestibility could reduce overall conversion costs and provide additional value to farmers. ARS scientists developed on-farm pretreatment practices utilizing dilute acid or alkali. By performing these pretreatments during storage, the prolonged reaction times were found to significantly deconstruct plant cell wall tissue, even at ambient temperatures.

Natural variation in alfalfa lignin content does not impact syngas yield. It is generally assumed that gasification of lignin-rich biomass will produce a more BTU-rich syngas product; and so would justify breeding lignin-rich varieties to supply gasification-based biorefineries. ARS scientists assessed the impact of the natural range of lignin content in alfalfa stems (8–20%) on syngas quantity and quality. This analysis found that lignin content (at least in this range) had little impact on the overall yield or energy content of syngas. These results are in stark contrast to the very strong negative impact of lignin content on biological fermentation.

Component III: Conversion and Co-Products

Low-cost switchgrass pretreatment. ARS scientists developed a low-cost alkaline pretreatment for deconstructing the cell wall structure in biomass prior to enzymatic hydrolysis. Use of this process in a cellulosic biorefinery would allow 80% of the glucose and xylose in switchgrass to be converted into ethanol.

Efficient xylose-to-ethanol biocatalyst. Although xylose is a major sugar in ligno-cellulosic biomass, yeasts are incapable of converting xylose to ethanol. ARS scientists introduced into yeast a number of genes that express both the enzymes to produce ethanol from xylose and the transport proteins to pump xylose into the yeast cell. The engineered strain efficiently ferments xylose to ethanol and so will help make cellulosic ethanol biorefining commercially viable.

Highly-active hemicellulase. ARS researchers discovered and characterized a hemicellulase enzyme from *Selenomonas ruminantium*; this enzyme is an order of magnitude more active than other hemicellulases. The enzyme enables high yields of fermentable sugars from efficient, mild pretreatment processes.

High-productivity membrane bioreactor. ARS scientists developed a membrane bioreactor to recycle ethanologenic biocatalysts and thereby reduce the cost of cellulosic ethanol production. The membrane bioreactor was tested with a recombinant bacterium that fermented xylose, a major sugar component of cellulosic biomass. The bioreactor exhibited xylose-to-ethanol productivities 60 times better than a traditional batch reactor, showing that a commercial system would require significantly lower capital costs.

Corn oil coproduct of ethanol biorefining. Aqueous enzymatic oil extraction, a process developed by ARS scientists for separating corn oil from the germ, could be commercially viable, but centrifugation volumes are too high. To overcome this barrier, ARS researchers developed a foam fractionation system that recovers 70-80% of the oil and reduces the centrifugation volume by 75%. Use of ARS-developed aqueous enzymatic oil extraction and oil recovery technologies in a typical 50 million gallon/yr ethanol biorefinery could improve profitability by $1.2 million/yr. These ARS technologies for generating corn oil coproduct could be deployed in the more than 100 corn ethanol biorefineries operational today and in the 100 or more in various stages of planning.

Improving DDGS flowability. Most of the DDGS produced by corn ethanol biorefineries are transported via rail to livestock feeding operations outside the Corn Belt. At least 10% of these DDGS shipments (> 1.5 million tons) cannot be discharged because of caking and bridging in the rail cars, resulting in significant costs for the ethanol industry. ARS scientists investigated the underlying mechanisms associated with DDGS caking and determined that flowability worsened with increasing levels of lipids, soluble solids and moisture in DDGS. It was also found that soluble level, storage temperature, and relative humidity all had a significant influence on DDGS moisture content over time. These findings help corn biorefiners choose DDGS storage and transportation practices so as to avoid flowability problems.

Stable recombinant ethanologen. Continuous fermentation processes require significantly less capital investment because they have higher (as much as 2X) productivity (g-EtOH/L-hr) compared to traditional batch fermentation systems. However, it is often difficult to use genetically-engineered microorganisms in continuous fermentations because the plasmids which contain the exogenous genes lack sufficient stability. ARS researchers developed a stable, recombinant, ethanologenic bacterium which ferments both pentose and hexose sugars; and they tested the stability of this recombinant strain in a continuous fermentor fed with wheat straw hydrolyzate. The bacterium was found to produce ethanol continuously over four months without any loss in productivity, plasmid stability or cell viability.

Highly-active biomass saccharification enzymes. ARS scientists discovered and expressed 16 polygalacturonase genes from the fungus *Rhizopus oryzae*. Polygalacturonase helps break down the pectin in plant cell walls, and 14 of the discovered enzymes are highly active.

Key enzyme for cell wall deconstruction. ARS researchers discovered, cloned and expressed novel genes for ferulic acid esterase, a key enzyme for cleaving the chemical bonds which link hemicellulose with lignin. The discovered enzyme was characterized and found to enhance pretreatment efficiency and so will make the conversion of lignocellulosic biomass to biofuels more efficient.

Cellulosic ethanol from barley hulls. ARS researchers developed a simultaneous saccharification and fermentation (SSF) process to convert cellulose-rich barley hulls into ethanol. By converting the hulls into ethanol, a barley biorefinery that processes barley starch could increase its ethanol output by 6-10%. In addition, the equipment it would use for converting the hulls could also process other cellulosic biomass that might be available, such as switchgrass.

Inhibitor removal from cellulosic biomass hydrolyzates. Pretreatment of ligno-cellulosic biomass often produces byproducts which inhibit subsequent fermentation of the sugars to ethanol. ARS researchers discovered a microorganism that removes these inhibitory chemicals, but the microorganism grew on xylose and so its use would significantly reduce the overall yield of ethanol from biomass. ARS scientists then constructed a new strain of the microorganism that retained its ability to metabolize only the inhibitors but not the xylose.

Biodiesel coproduct for aquaculture feed. In-situ biodiesel biorefining, a process developed by ARS researchers, produces biodiesel from any lipid-bearing material without the need for an oil-extraction step. In-situ processing both simplifies biodiesel synthesis and substantially expands the sources of oils for producing biofuel. However, for the process to be economically viable, economical uses must be found for the lipid-free meal coproduct left after the in-situ reaction. Testing by ARS scientists showed the spent meal to be quite suitable as feed in aquaculture.