United States Department of Agriculture

Research, Education, and Economics

AGRICULTURAL RESEARCH SERVICE

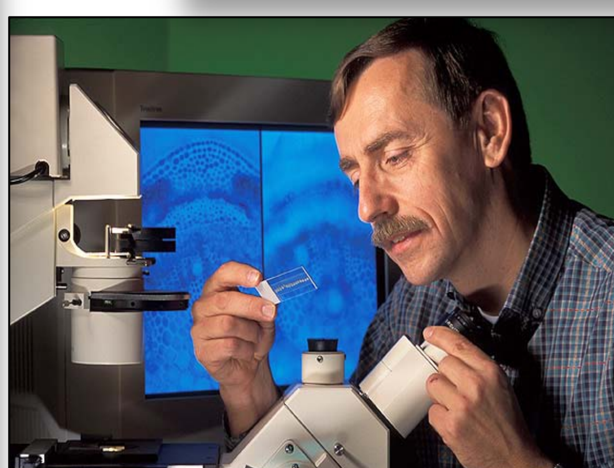
Office of National Programs

FEBRUARY 2013

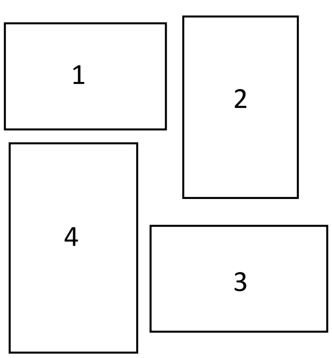
National Program 213

BIOENERGY

ACCOMPLISHMENT REPORT 2008-2012



Captions of front page photos, clockwise from upper left:



1. Soil scientist Doug Karlen instructs technician Tanya Ferguson (accompanied by her hearing guide dog) on how to visually assess soil quality impacts of harvesting crop residue as feedstock for bioenergy production. The foreground shows signs of severe soil erosion where about 90 percent of the stover was harvested. *Photo by Stephen Ausmus/ARS.*

2. At Wyndmoor, Pennsylvania, chemical engineer Akwasi Boateng (right) and mechanical engineer Neil Goldberg (center) adjust pyrolysis conditions while chemist Charles Mullen loads the reactor with bioenergy feedstock. *Photo by Peggy Greg/ARS.*

3. Dairy scientist Hans Jung examines alfalfa stem sections before and after digestion by rumen bacteria. Genetic modification of nondigestible xylem tissue would make stems better cattle feed and enhance their conversion to ethanol. *Photo by Keith Weller/ARS.*

4. A column filled with poultry litter-based activated chars is put to the test by chemist Isabel Lima (right) and Bonnie Dillon by letting a solution of copper ion pass through. The solution turns clear as the copper ions are absorbed by the activated chars. *Photo by Stephen Ausmus/ARS.*

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TABLE OF CONTENTS

The National Program 213 Action Plan 2008-2012
Components and Problem Statements

Component 1: Feedstock Development

PROBLEM STATEMENT 1A: *Breeding and evaluation of new germplasm*

PROBLEM STATEMENT 1B: *Biological and molecular basis of plant traits*

Component 2: Feedstock Production

PROBLEM STATEMENT 2A: *Accurate information at different levels of scale to help plan for future bioenergy production needs in ways that maintain the integrity of U.S. agriculture.*

PROBLEM STATEMENT 2B: *Greater amounts of biomass feedstocks are needed to achieve U.S. goals for replacing transportation fuels with renewable sources. This increase will require greater production from each acre of land used, and new kinds of production systems for the next generations of feedstocks that compliment food, feed, and fiber production.*

PROBLEM STATEMENT 2C: *Strategies are needed to use conversion co-products from agricultural-based energy production on farms to reduce the need for purchased inputs for crop production systems.*

Component 3: Biorefining

SUBCOMPONENT 3A: Biocatalytic Conversion

PROBLEM STATEMENT 3A-1: *Cost-effective conversion of lignocellulosic feedstocks to ethanol or butanol.*

PROBLEM STATEMENT 3A-2: *Biological production of hydrogen and methane from lignocellulosic feedstocks.*

PROBLEM STATEMENT 3A-3: *New and improved processes for biocatalytic conversion of starches and sugars to ethanol or butanol.*

PROBLEM STATEMENT 3A-4: *Biorefinery co-products.*

SUBCOMPONENT 3B: Thermochemical Conversion

PROBLEM STATEMENT 3B-1: *Managing biomass feedstocks for thermochemical processing.*

PROBLEM STATEMENT 3B-2: *On-farm production of heat and/or power.*

PROBLEM STATEMENT 3B-3: *Commercially viable thermochemical processes for producing liquid fuels from agricultural feedstocks.*

PROBLEM STATEMENT 3B-4: *Biorefinery co-products.*

SUBCOMPONENT 3C: Biodiesel

PROBLEM STATEMENT 3C-1: *Increasing feedstock (fatty acid) availability.*

PROBLEM STATEMENT 3C-2: *Biodiesel production processes.*

PROBLEM STATEMENT 3C-3: *Improving the inherent performance of fatty acid esters as fuels.*

PROBLEM STATEMENT 3C-4: *Biodiesel fuel quality assurance.*

PROBLEM STATEMENT 3C-5: *Biodiesel co-products.*

SUBCOMPONENT 3D: Process Economics and Life Cycle Analyses

PROBLEM STATEMENT 3D-1: *Estimating process costs and externalized costs.*

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BACKGROUND AND GENERAL INFORMATION

U.S. agriculture is expected to provide a significant portion of the nation's future energy requirements. Broadly interpreted, bioenergy encompasses agriculture in its entirety as a summation of energy transformations within the global carbon cycle.

The USDA Agricultural Research Service implicitly recognizes this; therefore, the Bioenergy National Program (NP 213) serves as the organizational structure where planning, coordinating, and executing research for the conversion of plant and animal inputs into biofuels and other products takes place.

In helping to optimize the development and the production of those feedstocks and the processes of their biorefinement, NP 213 research focuses on strengthening rural economies, providing increased supplies of renewable transportation fuel, enhancing energy security, and improving the U.S. balance of trade. A strong emphasis is made on distributed scale production of bioenergy from both pre-collected material that would otherwise be considered waste, and diverse, regionally appropriate feedstocks that can be used for the commercial production of energy and transportation fuels.

The 5 years covered by this report has been a period of change in the bioenergy landscape, as well as changes in national policy on bioenergy. In 2007, the Energy Independence and Security Act (EISA) set out the U.S. Renewable Fuels Standards (RFS-2), establishing a 36-billion gallon biofuel target by 2022**,** expanding the need for new sources of raw materials and new, more efficient production methods. In 2008, the Biomass Research and Development Board released its *National Biofuels Action Plan* for achieving RFS-2, and in 2009, the President formed the Biofuels Interagency Working Group, co-chaired by the Secretaries of Energy and Agriculture, and the EPA administrator.

In 2010, this Working Group released *Growing America's Fuel*[1] that establishes an outcome-driven strategy to meet RFS-2 goals. Also in 2010, the USDA produced *A USDA Regional Roadmap to*

[1] www.whitehouse.gov/sites/default/files/rss_viewer/growing_americas_fuels.PDF

Meeting the Biofuels Goals of the Renewable Fuels Standard by 2022 (USDA's Roadmap[2]). This document and the accompanying stakeholder feedback specifically define the roles that USDA agencies will play in achieving these goals.

In addition, the Secretary of Agriculture established two USDA Forest Service and five ARS Regional Biomass Research Centers[3] to conduct and coordinate research to help ensure the availability of dependable supplies of feedstocks needed for the production of advanced biofuels. These centers have led USDA's supply chain-oriented research activities to identify, improve, and efficiently produce regionally appropriate bioenergy feedstocks.

At the same time as these policies were being developed, the focus on renewable fuel production has shifted, in part because of the dramatic increase in the domestic production of shale-based natural gas. This has altered many of the economic assumptions for agriculture-based bioenergy production.

Though the current bioenergy landscape is quite different now from when the NP 213 Action Plan was written, this Action Plan had anticipated many of these changes and is consistent with the Nation's goals over the period of 2008-2012. By developing technologies that enable sustainable commercial production of biofuels in ways that enhance our natural resources without disruption to our existing food, feed, and fiber markets, NP 213 has helped overcome the technical barriers to RFS-2 goals.

As this report documents, during the period from 2008 to 2012, NP 213 has made significant contributions to addressing these barriers. These contributions include new approaches to reducing the recalcitrance of lignocellulosic material to biocatalytic saccharification, methods to improve performance of fermentations, new technologies for purifying and upgrading the products themselves, and development of value-added biorefining co-products.

PLANNING AND COORDINATION FOR THE NP 213 5-YEAR CYCLE

Customer and stakeholder interactions play key roles in helping ARS guide its research. NP 213 researchers and program leaders held a planning workshop in September 2007 to engage customers, stakeholders, and research partners in identifying issues and priorities that would be a match for the resources and expertise of ARS scientists. Incorporating input from customers and stakeholders, the NPLs' knowledge of the science subject matter, and input from other scientists, a team of ARS researchers identified and articulated the priority needs that could be realistically addressed with ARS resources and base funding. These individual research needs were aggregated into Problem Statements under three research Components. The resulting Action Plan guided development of new NP 213 research projects that began the current 5-year research cycle in 2008.

The Project Plan for each individual project includes statements about the agricultural problem being addressed; the anticipated products to be generated; the planned research contributions for mitigating or solving the larger NP 213 Problem Statements; and timelines and milestones for measuring progress toward achieving project objectives.

[2] www.usda.gov/documents/USDA_Biofuels_Report_6232010.pdf
[3] See the section for Component 1: Feedstock Development for more information about the Regional Biomass Research Centers.

In compliance with the Agricultural Research, Extension, and Education Reform Act of 1998, each of the 13 individual Project Plans were evaluated for scientific quality and feasibility by external peer review panels before research began. The next 5-year research cycle for NP 213 will begin in 2013 with a new Action Plan and new project plans.

STRUCTURE OF NP 213

NP 213 is a National Program with a vision and goals that reach beyond the normal ARS National Program structure. This is because the great bulk of ARS research that contributes to the first two components of NP 213's current Action Plan (Component 1: Feedstock Development and Component 2: Feedstock Production) is being performed under a number of other ARS National Programs.

In most National Programs, research that contributes to that program's Action Plan goals is typically performed in projects assigned specifically to that National Program. An administrative decision was made by the ARS Office of National Programs that the 2008-2013 Action Plan for NP 213 should cover all ARS research contributing to USDA goals for bioenergy. By expanding the scope of the NP 213 Action Plan, ARS ensured that its bioenergy research was documented and well coordinated across all aspects of ARS research so as to maximize both its effectiveness and resulting economic impact.

As detailed in Appendix 1, NP 213 consists of 14 active projects located in five states. The 52 scientists working in NP 213 are a multi-disciplinary group, and most are specialists in microbiology, molecular biology, chemistry, or chemical engineering. Significant contributions to NP 213 also come through multidisciplinary teams of scientists with specialties in genetics, agronomy, plant physiology, and plant molecular biology.

The NP 213 Action Plan is divided into three distinct components with collaboration between each.

Component 1: Feedstock Development

This component is focused on a vision of furnishing genetic, genomic, and bioinformatic tools, information, genetic resources, and crop varieties to enhance feedstock yield, quality, and production systems and to ensure an adequate supply of material for fuel and industrial products. To attain that vision, this component encompasses breeding improved germplasm and superior crop varieties; developing and applying new genetic and analytical bioinformatic tools; and safeguarding and utilizing plant genetic resources and associated genetic and genomic databases. Most of the ARS research that contributes to bioenergy feedstock development is actually managed under National Programs other than NP 213; these include:

- NP 215 (Pasture, Forage, and Rangeland Systems);
- NP 301 (Plant Genetic Resources, Genomics and Genetic Improvement);
- NP 303 (Plant Diseases); and
- NP 304 (Crop Protection and Quarantine).

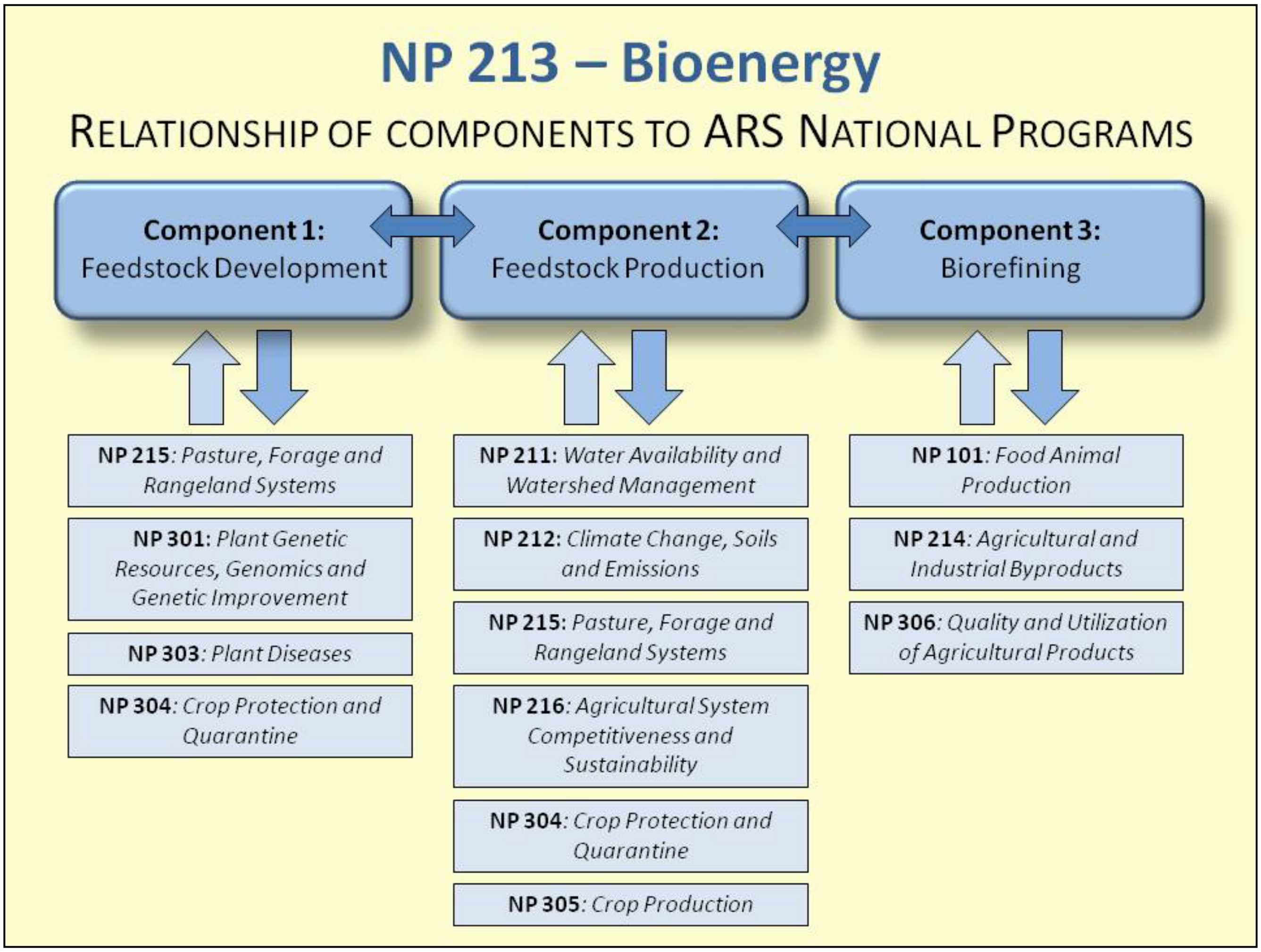

FIGURE 1: This graphic shows the close association among ARS National Programs both contributing to and receiving assistance from the three components of National Program 213, Bioenergy.

Component 2: Feedstock Production

This component encompasses ARS' efforts to improve existing or develop new production systems for many traditional and potential new crops that represent annual and perennial oilseed, starch, sugar, and cellulosic feedstocks. These enterprises are complex and depend on highly integrated management components that address crop production and protection, resource management, and mechanization. New technologies must address the need for lower-cost, higher-efficiency inputs that foster conservation of energy and natural resources, while maintaining profitability and promoting environmental sustainability. Furthermore, production systems must address the needs of small, intermediate, and large farming enterprises. Most of the ARS research that contributes to sustainable feedstock production systems is managed under National Programs other than NP 213; these include:

- NP 211 (Water Availability and Watershed Management);
- NP 212 (Climate Change, Soils and Emissions);
- NP 215 (Pasture, Forage, and Rangeland Systems);

- NP 216 (Agricultural System Competiveness and Sustainability);
- NP 304 (Crop Protection and Quarantine); and
- NP 305 (Crop Production).

Component 3: Biorefining
This component comprises research in four specific areas: biocatalytic conversion; thermochemical conversion; biodiesel; and process economics/life cycle analysis. Collectively, the research efforts seek to discover and apply novel processes with advanced materials, chemistries, products of protein and metabolic engineering, and thermochemical systems to agricultural products and byproducts. This research also integrates efforts in process systems engineering and economics to assess the potential impact of new technologies and to identify specific research targets that could maximize impact. ARS works on a variety of scales to convert a wide variety of feedstocks to useable energy, heat, and transportation fuels. Most of the biorefining research in ARS is managed under NP 213, but some biorefining research is also conducted under two other ARS National Programs:

- NP 214 (Agricultural and Industrial Byproducts); and
- NP 306 (Quality and Utilization of Agricultural Products).

Almost all the research projects assigned to NP 213 focus on the Biorefining component, whereas most of the research for feedstock development and feedstock production is conducted primarily in other National Programs. In fact, there are 70 projects assigned to other National Programs whose research contributes to NP 213, but are not being considered in this review.

In addition, some of the research done by projects within NP 213 contributes to the goals of other National Programs, such as:

- **NP 101—Food Animal Production:** Co-product research conducted by ARS biorefining scientists helps to develop and improve the quality and cost-effectiveness of livestock feeds.

- **NP 215, Pasture, Forage, and Rangeland Systems; NP 216, Agricultural System Competitiveness and Sustainability; and NP 305, Crop Production.** Technical capabilities provided by NP 213 scientists generate data that allow prediction of net energy yields and economic returns from new production systems, as well as how regional environmental and management conditions influence feedstock composition, quality, and value.

- **NP 215, Pasture, Forage, and Rangeland Systems; and NP 301, Plant Genetic Resources, Genomics and Genetic Improvement.** By characterizing the quality attributes of new varieties and hybrids, NP 213 scientists enable scientists in NP 215 and NP 301 to produce feedstocks with improved conversion efficiency, value-added traits, and co-products.

- **NP 214, Agricultural and Industrial Byproducts.** A portion of NP 213 research contributes to identifying solutions for converting manures into viable energy sources such as methane gas, pyrolysis oil, syngas, or biochar.

- **NP 306, Quality and Utilization of Agricultural Products.** Technologies developed by NP 213 scientists for hydrolyzing and refining feedstocks such as cellulosic biomass also enable the production of fermentable sugars that can be commercially converted into non-fuel industrial bioproducts.

COLLABORATIONS BETWEEN NP 213 AND PARTNERS OUTSIDE OF ARS

The National Program Leaders (NPLs) for NP 213 are responsible for coordinating ARS research activities with bioenergy efforts across USDA and other Federal departments. For instance, ARS is an active member of the USDA BioEnergy Science Team (BEST), which leads the Biomass Research Centers. Additional inter-agency coordination is accomplished through the USDA Energy Council Coordinating Committee (ECCC), which includes representatives from all USDA mission areas.

One key to success in bioenergy research is developing collaborations and partnerships with other government agencies (Federal, State, and local), for-profit companies, university researchers, non-governmental organizations, commodity groups, landowners, and farmers. NP 213 accomplishments are often achieved in close cooperation with public and private sector collaborators. Support can come in the form of in-kind contributions, trust agreements, cooperative research and development agreements (CRADAs), or non-funded cooperative agreements. For instance, during the period between 2008 and 2012, NP 213 scientists were involved in 30 CRADAs and 41 reimbursable-type research agreements with outside entities.

In addition, partnerships with universities enable ARS researchers to participate in and contribute to the training of America’s future agricultural researchers and entrepreneurs.

In-kind support through non-funded cooperative agreements also enables international collaboration between NP 213 scientists and Embrapa, the Brazilian counterpart of ARS. This collaboration has resulted in ARS hosting Brazilian scientists to enhance progress on common goals around bioenergy and expand Embrapa Agroenergy’s research efforts. The ARS-Embrapa collaborations have focused on developing pyrolysis technologies to produce bio-oil with stable properties, biochar co-products with potential application to soil amendment, and non-condensable combustible gases.

NP 213 NPLs and scientists often participate in multi-agency strategic planning and coordination efforts to integrate public and private sector partners into cooperative research. An example of this is the USDA's Farm-to-Fly initiative, a partnership among the USDA, Airlines for America, Inc., the Boeing Company, and others to advance the development and production of aviation biofuel. ARS, in cooperation with the DOE Office of Energy Efficiency and Renewable Energy, provided technical input to the Defense Logistics Agency (DLA)-Energy about the potential availability of different regional feedstocks and conversion technologies. The information was used by DLA-Energy to develop a Biofuels Business Case Analysis. ARS researchers and the ARS Office of Technology Transfer have also been providing support to business development projects in central California and Oklahoma that are determining the feasibility of region-based jet fuel from biomass production. This is in association with the California Association for Local Economic Development and the Center for Innovation at Arlington, Texas. These partnerships enable NP 213 to effectively leverage additional resources to conduct and transfer research that addresses critical agricultural problems.

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

The NP 213 Accomplishment Report is a distillation of the most significant research accomplishments of the past 5 years by ARS scientists working on the goals of this National Program. It is a 5-year snapshot that encompasses the research over this period and the early benefits of that research. In a report on the value of agricultural research, ERS pointed out that the benefits of research usually trail the completion of the research by 5 to 10 years (www.ers.usda.gov/publications/eb10/eb10.pdf), as it often takes that long before the technology is completely developed, transferred, and adopted by end users. It is important to recognize that benefits often increase over a number of years after the completion of the research, remain constant for a significant time before declining as newer technologies become available. Thus the accomplishments and breakthroughs in this report are unlikely to reflect all of the resulting impact.

Most of the accomplishments comprising this report were cited in NP 213 annual reports issued over the past 5 years, but this report stresses the impacts of those accomplishments and, where relevant, cites key publications or Web links associated with those accomplishments. The accomplishments are organized under the relevant Components and Problem Statements described in the NP 213 Action Plan. When those accomplishments result from research conducted in National Programs other than NP 213, it is so noted.

A list of the 17 research projects in NP 213 is in Appendix 1, organized by ARS project number. Publications associated with each of the NP 213 projects are listed in Appendix 2 by project. Appendix 3 contains selected information and documentation supporting the accomplishments and impact of NP 213 research.

This report was prepared for an external (to ARS) retrospective review of NP 213 to assess how well this National Program attained its projected goals, as outlined in its current Action Plan. The purpose of the retrospective review is not to judge the performance of individual NP 213 research projects, but rather to gauge the overall impact of the National Program. Consequently, the report does not attempt to catalogue all the individual accomplishments reported by the scientists assigned to NP 213's research projects.

In the same way that only selected accomplishments are reported, details of those accomplishments are selected and/or summarized to illustrate the overall variety of products and knowledge generated by this National Program. In some instances, the results from an individual study focusing on a specific problem are described, while in other instances, similar research or achievements are aggregated across the National Program. Individual researchers or projects are not identified by name in the narrative text; instead, their achievements are described in the context of contributions towards accomplishing the National Program's stated commitments to U.S. agriculture.

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COMPONENT 1: Feedstock Development

Development of new germplasm, parental stocks, and cultivars with value-added traits to enhance biomass yields, conversion efficiencies, and biorefinery co-product value are critical for the developing bioenergy industry. ARS has played a significant role in feedstock development with fundamental research on molecular, biochemical, and genetic control of key plant traits and has released improved plant varieties specifically for bioenergy. ARS conducts genetic and genomic research to enhance the value of bioenergy crops, identify molecular markers and functional gene sequences to facilitate selection of desired traits in breeding programs, and develop innovative and efficient breeding strategies and evaluation tools to support the bioenergy and agriculture industries.

However, as discussed in this Report's Introduction section, much of the work on feedstock development is managed outside the NP 213 administrative structure, albeit in a highly coordinated effort with the ARS Bioenergy National Program.

Only two projects within NP 213 directly address feedstock development. Other research that contributes to this Component's goals is managed through other National Programs such as NP 215, Rangeland, Pasture, and Forage Systems; NP 301, Plant Genetic Resources, Genomics, and Genetic Improvement; NP 303, Plant Diseases; and NP 304, Crop Protection and Quarantine.

These collaborative efforts are coordinated through the five USDA Regional Biomass Research Centers that were established in 2010 to help ensure that dependable supplies of needed feedstocks are available for the production of advanced biofuels to meet legislated goals and market demand. The Biomass Research Centers are networks of existing ARS and Forest Service facilities and scientists nationwide. The Centers provide a long-term leadership structure focused on harmonizing ARS and Forest Service intramural research throughout the United States to accelerate establishment of commercial, region-based biofuel supply chains based on agricultural and forestry based feedstocks and value-added co-products.

The Centers and their specific objectives are:

> **Northern-East Regional Center; Madison, Wisconsin.** This center is coordinated by the Forest Service and focuses on production of woody biomass for biofuels with research directed at screening for superior traits; short-rotation woody crops; sustainable management systems; life-cycle analysis; quantifying sustainable supply and demand; conversion of woody biomass to advanced fuels and co-products; and design of biofuels and co-product deployment.
>
> **Southeastern Regional Center; Booneville, Arkansas, and Tifton, Georgia.** The highest priority research needed for the Southeastern region is the development of superior performing herbaceous feedstocks that include energy cane, biomass sorghum (including sweet sorghum), other subtropical/tropical perennial grasses (such as napiergrass), and purposely grown woody biomass. Research needs include identifying

the best strategies to incorporate dedicated biomass crops into existing annual row crop, pasture, agroforestry, and forest-based systems, as well as to develop long-term strategies for using perennial energy grasses to meet the needs of emerging advanced-biofuel-producing facilities in the region.

Western Regional Center, Maricopa, Arizona. With the relatively low precipitation in much of the western United States, this Center's feedstock research focuses on development of new industrial oilseed crops. Oilseed crop research is conducted in conjunction with the Northwestern Regional Center and includes genomic modifications to optimize fatty acid genes and breed new oilseed cultivars, characterizations of germplasm collections to identify new feedstock types, and population phenotyping. New cropping systems are needed that fit specific local and regional niches for available resources and economic development, especially under limited water availability. Woody biomass research efforts include management and use of invasive eastern red cedar, pinyon pine, and western juniper to restore degraded rangelands; use of insect-, fire-, or disease-killed wood; sustainable productivity and residue removal; economics of in-woods pyrolysis and biochar and assessment of ecological outcomes; and the logistics and costs of handling and transportation.

Northwestern Regional Center, Pullman, Washington. This center's oilseed crop efforts are coordinated with those of the Western Regional Center, with an emphasis on integrating expanded oilseed production and minimizing its impact on existing wheat-based production systems. The center is also focused on restoration of western rangelands through harvest and removal of invasive western juniper and pinyon pine trees. The woody biomass emphasis is on wood utilization; poplar genomics, genetics, and short rotation management; forest resource supply and characterization; production standards for sustainable forest management systems; alternative energy policy evaluation; and economic feasibility of feedstock supply alternatives.

Central-East Regional Center, Lincoln, Nebraska. The main research focus for this Center is on the development of perennial grasses and biomass sorghum, along with significant coordination of research on corn grain ethanol and corn stover cellulosic biomass. Emphasis is on integrating dedicated feedstock production into central-eastern agricultural production systems to enhance water and air quality and to minimize the adverse affects of bioenergy on existing agricultural markets.

The research coordinated through the Regional Biomass Centers has generated most of the accomplishments for this Component and most of the research conducted at the Regional Biomass Centers is managed through ARS National Programs other than NP 213.

The NP 213 Action Plan includes two Problem Statements that were expected to guide the 5-year research plan and the development of the anticipated products in this Component. Here are lists of significant ARS research accomplishments that address each of these two Problem Statements.

PROBLEM STATEMENT 1A: *Breeding and evaluation of new germplasm.*

Breeding corn stover for higher biofuel yields. Corn stover, the most abundant biomass resource today, can be an attractive feedstock for biofuel production. However, research is needed to enable breeding of corn with stover that provides higher yields of ethanol. ARS researchers in St. Paul, Minnesota, with their colleagues at the University of Minnesota, showed that genetic traits of corn affecting cellulosic ethanol yield had moderate to high heritability and did not show an accompanying decrease in grain yield. Further, the researchers identified genetic markers for cell wall traits important for cellulosic ethanol production. Their work enables the use of marker-assisted selection to breed corn exhibiting both higher yields of cellulosic ethanol from stover and higher yields of grain. Also, University of Minnesota scientists discovered a corn mutant with reduced ferulate cross-linking in stover, and ARS scientists showed that stover from the mutant variety was more easily digested into fermentable sugars. Research is continuing to isolate the mutated gene that will allow researchers to breed a superior feedstock for both biofuels production and corn silage production. *[National Program 215]*

Barros-Rios, J., Malvar, R.A., Jung, H.G., Bunzel, M., and Santiago, R. 2012. Divergent selection for ester-linked diferulates in maize pith stalk tissues. Effects on cell wall composition and degradability. Phytochemistry 83:43-50.

Genetic resources for mining maize diversity. Maize is the most diverse crop in the world, but much of that useful genetic variation is found in maize not adapted to U.S. agriculture. ARS researchers in Ithaca, New York, genetically characterized the largest set of mapping lines for complex trait dissection in any species; more than 5,000 diverse maize inbred lines. Seed for those lines were deposited at the ARS Maize Stock Center in Urbana, Illinois, where they have been distributed to dozens of scientific groups who are determining the genetic bases for numerous agronomically important maize traits. *[National Program 301]*

Sachs, M.M. 2009. Cereal germplasm resources. Plant Physiology 149(1):148-151.

McMullen, M.D., Kresovich, S., Sanchez-Villeda, H., Bradbury, P., Li, H., Sun, Q., Flint Garcia, S.A., Thornsberry, J., Acharya, C., Bottoms, C., Brown, P., Browne, C.J., Eller, M.S., Guill, K.E., Harjes, C., Kroon, D., Lepak, N.K., Mitchell, S., Peterson, B.E., Pressoir, G., Romero, S.M., Oropeza Rosas, M., Salvo, S.A., Yates, H., Hanson, M., Jones, E., Smith, S., Glaubitz, J., Goodman, M., Ware, D., Holland, J.B., and Buckler IV, E.S. 2009. Genetic Properties of the Maize Nested Association Mapping Population. Science 325:737-740.

Leadership in switchgrass improvement. Until recently there were no switchgrass varieties specifically for bioenergy use. However, ARS scientists in Lincoln, Nebraska, Madison, Wisconsin, and Raleigh, North Carolina, have addressed this in a coordinated fashion. Switchgrass breeding and genetics research by ARS in Lincoln and Madison forms the foundation for all switchgrass breeding and genetics research now underway worldwide. This ongoing research has provided the basic genetic information for using self-incompatibility for producing switchgrass hybrid cultivars and hybrid cultivars of other grasses to improve yield over the past decade. Based on foundation and certified seed records, the switchgrass and intermediate wheatgrass cultivars developed by ARS in Lincoln and their collaborators are the most widely utilized cultivars of these species in the Great Plains and Midwest, with seeded acreage of the switchgrass and wheatgrass cultivars estimated at one million acres, which add an estimated $20 million annually to the U.S. agricultural economy. ARS scientists in Raleigh, collaborating with North Carolina State University, have improved and evaluated switchgrass as a multipurpose crop for the Southeast that can be grazed, harvested as hay or silage, or used as biofuel feedstock. Selecting switchgrass germplasm using criteria of yield potential, in vitro dry matter disappearance, and crude protein in a weighted index has resulted in the release of three cultivars: 'BoMaster' for yield; 'Colony' for improved cellulose concentration and yield; and 'Performer' for improved forage quality. Releases of the cultivars have been made and seed will be available commercially soon. ARS scientists have since developed a strain of switchgrass that, when grown in eastern Nebraska, produced a potential ethanol yield of 355 gallons per acre—20 gallons per acre greater than that of the previous best cultivar. This is the first publicized example of a switchgrass strain specifically bred for improved conversion to ethanol. *[National Program 215]*

Sarath, G., Dien, B.S., Saathoff, A.J., Vogel, K.P., Mitchell, R., and Chen, H. 2011. Ethanol yields and cell wall properties in divergently bred switchgrass genotypes. Bioresource Technology 102:9579-9585.

Burns, J.C., Godshalk, E., and Timothy, D.H. 2010. Registration of Colony Switchgrass. Journal of Plant Registrations 4:189-194.

Burns, J.C., Godshalk, E.B., and Timothy, D.H. 2008. Registration of 'BoMaster' switchgrass. Crop Science 2:31-32.

Expansion of the switchgrass and other warm-season grass germplasm collections. The ARS warm-season grass collection for species native to the United States did not contain adequate genetic diversity to meet the needs of researchers and breeders. The switchgrass (*Panicum*) collection was expanded with 94 accessions collected by ARS researchers in Griffin, Georgia, from areas in the United States previously underrepresented. Each newly collected switchgrass accession was tested for germination, genotypically characterized using simple sequence repeat markers, and assessed for ploidy level. These efforts provided well-characterized, readily available, and viable material to scientists for bioenergy feedstock development. In addition, germplasm for several other native warm season grass species (e.g., Andropogon, Sorghastrum, Schizachyrium, and Bouteloua) were made available through seed increases. The researchers also collected site data from historical records for more than a thousand Andropogon accessions and made that available online (www.ars-grin.gov/npgs/acc/acc_queries.html). These efforts collectively have increased the availability, broadened the diversity, and enhanced associated information for the native warm season grasses important for forage and bioenergy applications. *[National Program 301]*

Harrison Dunn, M.L. and Pederson, G.A. 2010. Expansion of the USDA Switchgrass Germplasm Collection. ASA-CSSA-SSSA Annual Meeting Abstracts.

Rapid estimation of conversion potential enables efficient breeding of dedicated bioenergy feedstocks. Although traditional wet-chemistry methods for analysis of feedstock properties to predict sugar composition, ethanol yield, and digestibility are laborious and expensive, ARS scientists in Lincoln, Nebraska, Peoria, Illinois, St. Paul, Minnesota, and Madison, Wisconsin, tested the actual and theoretical ethanol yields of over 100 different samples of switchgrass that varied widely in environmental growth conditions and genetic traits. Genetically related switchgrass plants with comparable lignin content displayed marked differences in plant anatomy, cell wall architecture, and subsequently ethanol yields. The researchers showed that breeding switchgrass for increased *in vitro* dry matter digestibility resulted in genotypes with reduced lignin content and variability in other cell wall and tissue properties that together influenced cellulose digestion and actual ethanol yields. This work demonstrated that heritable genetic differences exist among switchgrass for conversion efficiency, indicating that a focused breeding effort to develop switchgrass varieties specifically for ethanol biorefining could result in additional improvements.

Extensions of these research efforts produced near infrared spectroscopy predictive equations that, along with biomass yield data, enabled the same group of ARS researchers to make an accurate estimation of more than 20 biomass components, including cell wall and soluble sugars, ethanol, and released pentose sugars from a laboratory simultaneous saccharification and fermentation procedure. With this information, the researchers calculated an addition 13 complex feedstock traits, including theoretical ethanol yield from hexoses and simultaneous saccharification and fermentation hexose ethanol conversion efficiency. The predictive calibration equations were transferred to the Near Infrared Spectroscopy Consortium, which makes it available to other laboratories. The research led to the ARS being invited to join an interlaboratory study to measure the composition of four biomass NIST reference materials, co-sponsored by the National Institute of Standards and Technology and the National Renewable Energy Laboratory. It also led to further support from a large industrial partner to extend these results to Napier grass. Further research extending these results is being supported with a large interagency grant to develop an integrated supply chain approach to switchgrass production in the upper Great Plains. *[National Programs 213 and 215]*

Vogel, K.P., Dien, B.S., Jung, H.G., Casler, M.D., Masterson, S.D., and Mitchell, R.B. 2011. Quantifying actual and theoretical ethanol yields for switchgrass strains using NIRS analyses. Bioenergy Research 4(2), 96-110.

Doran-Peterson, J., Jangid, A., Brandon, S.K., DeCrescenzo-Henriksen E., Dien B., and Ingram, B.O. 2009. Simultaneous saccharification and fermentation and partial saccharification and co-fermentation of lignocellulosic biomass for ethanol production in biofuels, editor Jonathan R. Mielenz. Methods in Molecular Biology Vol. 581, pp 263-268

Dien, B.S., Jung, H.G., Vogel, K.P., Casler, M.D., Lamb, J.F., Iten, L.B., Mitchell, R., and Sarath, G. 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. Biomass and Bioenergy 30:880-891.

New sugarcane and energycane cultivars. New sugarcane cultivars are continuously being developed for sustained or improved yields, resistance to intense disease pressures, and for improved adaptability to freezes, high water tables, and muck and sandy soil. Energy cane is one of the crops that will be needed in the southeastern region of the United States to meet legislated biofuel targets. ARS researchers in Canal Point, Florida, in cooperation with the University of Florida and the Florida Sugar Cane League, Inc., bred superior sugarcane varieties that are high-yielding in both soil types and with improved tolerance to limiting sugarcane diseases. The products of the ARS sugarcane program have contributed significantly to U.S. sugar production and to those of several developing countries. With an ever-increasing interest in sugarcane as an energy crop, sugarcane breeders around the world are developing “energy cane” cultivars as a biofuel feedstock, while continuing to genetically improve sugarcane primarily as a sucrose source. Collaborating with Louisiana State University colleagues, ARS researchers in Houma, Louisiana, released three high-fiber sugarcane varieties that excel in total solids accumulation. These new varieties provide various market system options: very high-fiber, low-sugar; high-fiber, moderate-sugar; and traditional moderate-fiber, high-sucrose sugarcane that satisfy individual interests and regional constraints. *[National Program 301]*

Davidson, R., Milligan, S.B., Glaz, B.S., Comstock, J.C., Hu, C., Glynn, N.C., Edme, S.J., Holder, D.G., Gilbert, R.A., Sood, S.G., Del Blanco, I.A., and Zhao, D. 2011. Registration of ‘CPCL 99-4455’ sugarcane. Journal of Plant Registrations 5:54-61

Hale, A.L. 2010. Notice of release of a high fiber sugarcane variety Ho 02-113. Sugar Bulletin 88(10):28-29.

New genetic diversity cooperative for saccharum and perennial grasses. Elite sugarcane hybrids are derived from a very narrow genetic base that lacks key traits, such as disease/pest resistance and cold tolerance. Despite the high quality of current ARS sugarcane varieties, new genetic diversity is needed to overcome constraints to dependable feedstock production. ARS scientists from Canal Point, Florida, Houma, Louisiana, Tifton, Georgia, and Lincoln, Nebraska have begun a public-private cooperative for improving sugarcane and energy cane that will provide important new sources of genetic diversity to help meet the feedstock demands of both the sugarcane and the emerging biomass industry in the southeastern United States. Based on the successful ARS Germplasm Enhancement of Maize (GEM) Project, this cooperative will develop a pipeline of superior and genetically diverse sugarcane varieties and related energy grass feedstocks. A pilot program has been initiated involving ARS, university, and industry partners for sugarcane and energy cane germplasm enhancement. Resulting hybrid clones from crosses will be released as public germplasm and shared among public institutions and private industry members. Additional commercialization agreement opportunities are likely beyond this initial cooperative effort, which will be coordinated through the Southeastern Regional Biomass Research Center. *[National Program 301]*

Sorghum recombinant inbred mapping population. Recombinant populations with differing characteristics are needed to identify and utilize quantitative trait loci. ARS scientists in Lubbock and College Station, Texas, and Griffin, Georgia, with their university collaborators, developed and released a sorghum recombinant inbred mapping population derived from a cross between IS3620C and BTx623. Of the 430-member mapping population, 137 members served as the basis for the construction of landmark sorghum high-density genetic and physical maps, with the integrated genome map subsequently used to aid in assembly of the sorghum genome sequence. Since its 2009 public release, this population has served as an important resource for mapping key agronomic traits in sorghum including tillering, floral architecture, plant height, flowering date, grain size, and abiotic stress tolerance. *[National Program 301]*

Burow, G.B., Klein, R.R., Franks, C.D., Klein, P.E., Schertz, K.F., Pederson, G.A., Xin, Z., and Burke, J.J. 2011. Registration of the BTx623/IS3620C recombinant inbred mapping population of sorghum. Journal of Plant Registrations 5(1): 141-145.

Development of sorghum genetic stocks with reduced lignin and higher digestibility. Lignin is a barrier to digestibility in grasses. ARS scientists in Lincoln, Nebraska, developed three sorghum genetic stocks—BN611, A/BN612, and RN613—with the stacked brown midrib genes *bmr*-6 and *bmr*-12 and made them available for basic research on lignin synthesis, and for breeding new animal and bioenergy feedstocks. These brown midrib genes are known to be associated with reduced lignin and higher digestibility, making brown midrib sorghum better livestock feed and a better bioenergy feedstock. Previously, the ARS researchers had developed and released genetic stocks containing the individual *bmr*-6 and *bmr*-12 genes, and demonstrated the effect these genes had on lignin content and other cell wall-related traits. Since these genes affect different enzymes involved in lignin synthesis, it is expected their combined effects could result in even greater changes in lignin content and other traits. In another study, ARS scientists in Peoria, Illinois, and at the Central-East Regional Biomass Research Center conclusively showed the potential utility of sorghum lignin mutants as a cellulosic feedstock by analyzing the role of lignin as a barrier for cellulose hydrolysis. Glucose yields for the sorghum biomass of *bmr* mutants were improved compared to wild type. The ARS researchers determined that reducing lignin content can greatly benefit conversion efficiencies of lignocellulose to sugars and ethanol. Furthermore, the researchers determined that conversion efficiencies were strongly correlated with lignin content. This research demonstrated the value of *bmr* alleles in sorghum breeding programs for feedstock improvement. *[National Program 301]*

Sattler, S.E., Funnell-Harris, D.L., and Pedersen, J.F. 2010. Efficacy of singular and stacked brown midrib 6 and 12 in modification of lignocellulose and grain chemistry. Journal of Agricultural and Food Chemistry 58:3611-3616.

Dien, B.S., Sarath, G., Pedersen, J.F., Sattler, S.E., Chen, H., Funnell-Harris, D.L., and Cotta, M.A. 2009. Improved sugar conversion and ethanol yield for forage sorghum (*sorghum bicolor* L. moench) lines with reduced lignin contents. Bioenergy Research 2(3), 153-164.

Sattler, S.E., Saathoff, A.J., Haas, E.J., Palmer, N.A., Funnell-Harris, D.L., Sarath, G., and Pedersen, J.F. 2009. A nonsense mutation in a cinnamyl alcohol dehydrogenase gene is responsible for the sorghum brown midrib-6 phenotype. Plant Physiology 150 (2):584-95.

Pedersen, J.F., Toy, J.J., Funnell-Harris, D.L., Sattler, S.E., and Oliver, A.L. 2008. Registration of BN611, A/BN612, RN613 sorghum genetic stocks with stacked bmr-6 and bmr-12 genes. Journal of Plant Registrations 2:258-262.

Sorghum maturity locus gene cloned. Floral induction is the main determinant of biomass yield in sorghum, but is poorly understood. Maturity1 (ma1) is the major gene that permits the transition from vegetative growth to flowering under long-day conditions in grain sorghum. ARS scientists in College Station, Texas, together with university and industry scientists, cloned the wild-type (tropical) *ma*1 gene, along with naturally occurring sequence variants that enable flowering under long-day conditions in temperate latitudes. These sequences are now applied to molecular and genetic screening of tropical sorghum germplasm for photoperiodic response, and for marker-assisted selection for this important trait in sorghum grain and biofuel research programs. The ARS breeders are utilizing *ma*1 as a marker for conventional breeding to adapt ARS tropical sorghum germplasm accessions to temperate latitudes, and thus enhance its utility to sorghum breeders seeking desirable traits. *[National Program 301]*

Murphy, R.L., Klein, R.R., Morishige, D.T, Brady, J.A., Rooney, W.L., Miller, F.R., Dugas, D.V., Klein, P.E., and Mullet, J. E. 2011. Coincident light and clock regulation of pseudoresponse regulator protein 37 (PRR37) controls photoperiodic flowering in sorghum. Proceedings of the National Academy of Sciences 108:16469-16474.

Identifying key sorghum lines for bioethanol production. Sorghum with high sugar content (sweet sorghum) is an important biofuel crop, but its genetic content must be characterized before it can be bred and incorporated into genetic research for the development of efficient feedstock for biofuel production. ARS researchers in Griffin, Georgia, and Stoneville, Mississippi, analyzed 96 sweet sorghum samples with DNA genetic markers to determine their genetic variability and population structure. This new genetic information enables curators to manage sweet sorghum accessions more effectively and accelerates breeding of superior sweet sorghum cultivars for biofuel production. *[National Program 301]*

Wang, M.L., Zhu, C., Barkley, N.L., Chen, Z., Erpelding, J.E., Murray, S., Tesso, T., Pederson, G.A., and Yu, J. 2009. Genetic diversity and population structure analysis of accessions in the U.S. historic sweet sorghum collection. Theoretical and Applied Genetics Online DOI 10.1007/s00122-009-1155-6. 120:13-23.

Napier grass for biomass production. Napier grass is a perennial grass used for forage and has considerable potential as a biofuel feedstock for the Southeast primarily because of its high biomass yield. ARS researchers in Tifton, Georgia, assessed the genetic variation and genetic relatedness among 89 accessions of Naiper grass. The accessions clustered into five groups, including three groups from Kenya, a group from Puerto Rico, and accessions derived from the cultivar Merkeron. This research was the first molecular characterization of the Tifton accessions, which represent 30 years of plant collection and breeding. Furthermore, this work provides germplasm for Napier grass and pearl millet breeding improvement that is underway in collaboration with the University of Florida and industry collaborators. *[National Program 215]*

Harris-Shultz, K.R., Anderson, W.F., and Malik, R. 2009. Genetic diversity among napiergrass (*Pennisetum purpureum* Schum.) nursery accessions using AFLP markers. Plant Genetic Resources: Characterization and Utilization 8:63-70.

Improved high-starch winter barley varieties. Winter barley varieties are typically higher yielding feed varieties, but need to be improved further for use in a bioethanol refinery. ARS scientists in Wyndmoor, Pennsylvania, worked closely with plant breeders at Virginia Polytechnic Institute and State University to provide compositional data that has enabled the selection of advanced breeding lines and characterization of existing released varieties of improved winter hulled (e.g. 'Thoroughbred') and hulless (e.g. 'Dan') barley varieties. These winter varieties were shown to have significantly higher starch and β-glucans, as well as significantly lower kernel weights, fiber, and ash contents that make them more valuable for ethanol production than most hulled malting or feed varieties. The hulless varieties also have lower processing costs at the refinery. Regional interest in these varieties has increased because of the construction of a 65 million-gallon per year commercial ethanol facility in Hopewell, Virginia. *[National Program 213]*

Brooks, W.S., Vaughn, M.E., Griffey, C.A., Thomason, W.E., Paling, J.J., Pitman, R.M., and Hicks, K.B. 2011. Registration of 'dan' winter hulless barley. Journal of Plant Registrations 5(1),

1-4.

Griffey, C., Brooks, W., Kurantz, M., Thomason, W., Taylor, F., Obert, D., and Hicks, K. 2010. Grain composition of virginia winter barley and implications for use in feed, food, and biofuels production. Journal of Cereal Science 51(1), 41-49.

PROBLEM STATEMENT 1B: *Biological and molecular basis of plant traits.*

A model for grass cell wall genetics. Fundamental research in plant biology can provide a better understanding of the molecular processes underlying synthesis of grass cell walls—the material that will provide a substantial portion of the biomass required by the developing lignocellulosic fuel industry. ARS scientists in Albany, California, have focused on molecular genetic approaches in the model grass *Brachypodium distachyon*, as it is viewed as a simple system for studying grass cell walls and because of its close relationships among grass family members. Rapid discoveries in Brachypodium can efficiently identify target genes or genomic regions that regulate important cell wall traits with functional orthologs in bioenergy grasses. The ARS scientists, in collaboration with the U.S. Department of Energy-and other researchers, completed linkage mapping, physical mapping, sequencing and annotation of the entire Brachypodium genome. The genomic information is now publicly available on several databases, including at www.brachypodium.org. In addition, the scientists have initiated a project to resequence additional accessions. To date, four lines have been resequenced and the analysis of the sequences has begun. Knowledge of the genome sequence of Brachypodium and the linear order of genes in the genome relative to other grasses is a fundamental prerequisite for efficiently identifying and inferring the function of genes and associating genetic and phenotypic variation.

Huo, N., Garvin, D.F., You, F.M., McMahon, S., Luo, M., Gu, Y.Q., and Vogel, J.P. 2011. Comparison of a high-density genetic linkage map to genome features in the model grass *Brachypodium distachyon*. Theoretical and Applied Genetics 123(3), 455-464.

Vogel, J.P., Garvin, D.F., Mockler, T.C., Schmutz, J., Rokhsar, D., Bevan, M.W., et al. 2010. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463(7282), 763-768.

Gu, Y.Q., Ma, Y., Huo, N., Vogel, J.P., You, F.M., Lazo, G.R., and Luo, M. 2009. A BAC-based physical map of *Brachypodium distachyon* and its comparative analysis with rice and wheat. BMC Genomics 10, 496.

Glycoside-hydrolases in Brachypodium. To better understand carbohydrate synthesis and breakdown in plants, ARS and U.S. Department of Energy scientists in Albany, California, manually annotated the entire genome complement of glycoside-hydrolases in *Brachypodium distachyon* and sorghum, comparing them to existing sequences. The glycoside-hydrolases genes are fundamental in cleavage of bonds between carbohydrates and other carbohydrates, proteins, and lipids. A total of 356 glycoside-hydrolase genes that exist in Brachypodium correspond to 34 distinct families that encompass and define a glycoside-hydrolase profile unique to flowering plants. Specific gene functions of most are not known, but this work is a neccessary step toward further functional characterization using genetic and biochemical approaches.

Tyler, L., Bragg, J.N., Wu, J., Yang, X., Tuskan, G.A., and Vogel, J.P. 2010. Annotation and comparative analysis of the glycoside hydrolase genes in *Brachypodium distachyon*. BMC Genomics 11: 600

New tools for studying plant cell wall and other traits in grasses. ARS researchers from Albany, California, with scientists from Namik Kemal University in Turkey, have collected and now released over 200 inbred Brachypodium varieties. A T-DNA tagging/mutagenesis project has produced over 8,000 T-DNA tagged lines, 4,000 of which have already been released. Many of these lines are associated with sequence data and/or a visible mutant phenotype that can be searched at http://brachypodium.pw.usda.gov/. This Web interface greatly increases the utility of the collection. Collectively, this ARS-led research on Brachypodium has provided new tools and approaches to elucidate the biological processes underpinning production of lignocellulosic feedstocks that could not be accomplished directly in any other system. The T-DNA tagged lines have been submitted to the National Plant Germplasm System and have been distributed to researchers in Australia for phenotypic analysis.

Bragg, J.N., Wu, J., Gordon, S.P., Guttman, M.E., Thilmony, R., Lazo, G.R., and Vogel, J.P. 2012. Generation and characterization of the western regional research center brachypodium T-DNA insertional mutant collection. PLoS ONE 7(9)

Tyler, L., Bragg, J.N., Wu, J., Yang, X., Tuskan, G.A., and Vogel, J.P. 2010. Annotation and comparative analysis of the glycoside hydrolase genes in *Brachypodium distachyon*. BMC Genomics 11(1)

Filiz, E., Ozdemir, B.S., Budak, F., Vogel, J.P., Tuna, M., and Budak, H. 2009. Molecular, morphological, and cytological analysis of diverse *Brachypodium distachyon* inbred lines. Genome 52(10), 876-890.

Switchgrass genomic research provides basis for comparative approaches to molecular breeding. Rapid breeding of switchgrass varieties with desired traits for bioenergy use will require sequence information about the switchgrass genome. ARS scientists in Albany, California, led a national effort involving the U.S. Department of Energy Joint Genome Institute to produce 500,000 switchgrass sequence tags which have been released to the public through GenBank. Analysis of the sequences has identified approximately 76,000 unique genes. A separate switchgrass sequencing effort coordinated with ARS scientists at the Central-East Regional Biomass Research Center produced the complete chloroplast genome sequence and characterized sequence variation between upland and lowland ecotypes. Polymorphic sequences discovered through these efforts have enabled mapping, comparative genomics with other grass species, and characterization of genetic diversity at the subspecific level between switchgrass populations with differing ploidy and geographic origins. In addition, the ARS scientists are developing new sequence-based genotyping and RNA expression analysis resources for breeding material; these resources are being utilized in the feedstock development program at the Central-East Regional Biomass Research Center.

In related research, ARS scientists in Albany, California, working with the Samuel Roberts Noble Foundation, published the first genetic map for switchgrass. This achievement enables scientists to genetically dissect, identify, and assemble genes responsible for many high-value traits, enabling breeders to better recombine, evaluate, and enhance switchgrass germplasm that exhibits desired traits. Although this mapping project used a tetraploid population, the results demonstrated very little genetic exchange between subgenomes, and also demonstrated complete collinearity between the genetic maps of switchgrass and the closely related grass foxtail millet genome. The mapping population that was developed for this project is currently being used to dissect quantitative trait loci for yield, maturity, and cell-wall composition.

Young, H.A., Lanzatella, C.L., Sarath, G., and Tobias, C.M. 2011. Chloroplast genome variation in upland and lowland switchgrass. PLoS ONE 6(8)

Okada, M., Lanzatella, C., Saha, M.C., Bouton, J., Wu, R., and Tobias, C.M. 2010. Complete switchgrass genetic maps reveal subgenome collinearity, preferential pairing and multilocus interactions. Genetics 185(3) 745-760.

Tobias, C.M., Sarath, G., Twigg, P., Lindquist, E., Pangilinan, J., Penning, B., Barry, K., Carpita, N., and Lazo, G.R. 2008. Comparative Genomics in Switchgrass Using 61,585 High-Quality EST. The Plant Genome 1:111-124.

Genetic diversity in switchgrass is critical to future high-performance. As a dedicated biomass industry expands and matures, it will be critical to have a range of switchgrass varieties adapted to different production conditions across the region. Today's North American populations are the result of refuge environments that have preserved the ancient genetic diversity for thousands of years. ARS scientists in Madison, Wisconsin, and Albany, California, utilized marker systems they previously produced to provide the capability of assigning plants to lineages based on genotype alone, and to identify five distinct lineages of switchgrass that are derived from three glacial refugia that served as tallgrass prairie reserves during the Pleistocene. A dryland refuge in the U.S. southwest and Mexico is the only source of upland plants adapted to northern climates and dry soils. A western Gulf Coast coastal plains population and a lowland plains refuge along the northern and eastern areas of the Gulf Coast serve as sources of plants adapted to lowland warm climates and wet soils, and are also a source of remnant hybrids between upland and lowland ecotypes. The Gulf Coast region continues to serve as a rich resource of genetic variability for switchgrass genetic improvement. This work has provided the genetic framework that will guide preservation and breeding efforts, and was performed in collaboration with the U.S. Department of Energy, university collaborators, and the Central-East Regional Biomass Research Center.

Okada, M., Lanzatella, C., and Tobias, C.M. 2011. Single-locus EST-SSR markers for characterization of population genetic diversity and structure across ploidy levels in switchgrass (*Panicum virgatum* L.). Genetic Resources and Crop Evolution 58(6), 919-931.

Zalapa, J.E., Price, D.L., Kaeppler, S.M., Tobias, C.M., Okada, M., and Casler, M.D. 2011. Hierarchical classification of switchgrass genotypes using SSR and chloroplast sequences: Ecotypes, ploidies, gene pools, and cultivars. Theoretical and Applied Genetics 122(4), 805-817.

Unique switchgrass genetic stocks provide new genetic resources and insights into genome evolution. Identifying beneficial genes and gene combinations for bioenergy production is especially difficult in complex, polyploid genomes such as switchgrass. ARS scientists in Albany, California, have identified a diploid derivative of switchgrass that was the result of spontaneous genome reduction from a tetraploid line. This genotype could simplify large scale genome assembly due to its reduced complexity. With a complement of 18, rather than 36, individual chromosomes, the genotype was used to create a genetic karyotype based on chromosome arm length ratios, condensation patterns, and the locations of repetitive rDNA regions. Additional cytogenetic work consistently identified differences at specific repetitive rDNA and centromeric loci between tetraploid upland and lowland ecotypes. These differences indicate rapid evolution through gain or loss of these repeats by recombination, or gene conversion events. The plant material has been given to scientists at Oklahoma State University who will attempt to sequence the genome and that will assist with genome assembly efforts.

Young, H.A., Sarath, G., and Tobias, C.M. 2012. Karyotype variation is indicative of subgenomic and ecotypic differentiation in switchgrass. BMC Plant Biology 117.

Young, H.A., Hernlem, B.J., Anderton, A.L., Lanzatella, C.L., and Tobias, C.M. 2010. Dihaploid stocks of switchgrass isolated by a screening approach. Bioenergy Research 3(4), 305-313.

Biotech approaches to feedstock improvement. Designing bioenergy feedstocks with new properties can potentially improve the economics of production. ARS scientists in Albany, California, in collaboration with scientists at the Department of Energy, Energy Biosciences and Joint BioEnergy Institutes, have increased starch production in switchgrass by up to 250 percent using a novel form of the corn gene, corngrass1 (*cg1*). Starch produced by *cg1* switchgrass was converted into simple sugars, such as glucose, without energy intensive and expensive pretreatment of biomass. Switchgrass with c*g1* does not produce seeds or pollen, thus preventing the inadvertent movement of this gene by pollen to native switchgrass populations and protecting natural sources of genetic variation. This research demonstrates that perennial grasses could be a source of storage carbohydrates that could be readily converted to biofuels.

In related research, cinnamyl alcohol dehydrogenase (CAD) deficiency in grasses decreases overall lignin, alters lignin structure**,** and increases enzymatic recovery of sugars. To ascertain the effect of CAD down regulation in switchgrass, ARS scientists at the Central East Regional Biomass Research Center and in Albany California, down regulated the switchgrass CAD gene via RNA silencing. The resulting plants accumulated less CAD protein than control transformants and contained significantly less overall lignin and cutin. Glucose release from ground samples pretreated with ammonium hydroxide and digested with cellulases was also greater than in control transformants. Together with the *cg1* switchgrass, these results demonstrate the potential of biotechnological approaches to genetically improve biomass feedstocks, such as switchgrass, for conversion into biofuels.

Chuck, G.S., Tobias, C., Sun, L., Kraemer, F., Li, C., Dibble, D., and Hake, S. 2011. Overexpression of the maize Corngrass1 microRNA prevents flowering, improves digestibility, and increases starch content of switchgrass. Proceedings of the National Academy of Sciences of the United States of America 108(42), 17550-17555.

Saathoff, A. J., Sarath, G., Chow, E.K., Dien, B.S., and Tobias, C.M. 2011. Downregulation of cinnamyl-alcohol dehydrogenase in switchgrass by RNA silencing results in enhanced glucose release after cellulase treatment. PLoS ONE 6(1).

COMPONENT 2: Sustainable Feedstock Production Systems

Agricultural producers, government agencies, energy companies, and policy makers around the country need to know with certainty what kinds of bioenergy feedstocks can be produced, how much feedstock can be dependably harvested, and what will be the likely impacts of the bioenergy economy on whole-farm economic return and natural resources quality. ARS research seeks to develop new sustainable production strategies and decision tools to produce abundant amounts of next-generation feedstocks for the emerging biorefinery industries. These production systems should be designed to protect the natural resources base to ensure that long-term, sustainable production needs are met, while maintaining the integrity of U.S. food, feed, and fiber production.

The research conducted through USDA's Regional Biomass Research Centers (See Component 1) has generated most of the accomplishments listed below for Component 2. Many of those accomplishments, in turn, were the results from research managed in conjunction with other National Programs, such as: NP 211, Water Availability and Watershed Management; NP 212, Climate Change, Soils, and Emissions; NP 215, Rangeland, Pasture, and Forage Systems; NP 216, Agricultural System Competitiveness and Sustainability; NP 304, Crop Protection and Quarantine; NP 305, Crop Production; and NP 308, Methyl Bromide Alternatives.

The NP 213 Action Plan identified three Problem Statements that were expected to guide the 5-year research plan and the development of the anticipated products in this Component. Major accomplishments addressing each of these three problem statements are listed below; accomplishments from projects not in NP 213 will be identified as to the contributing National Program.

PROBLEM STATEMENT 2A: *Accurate information at different levels of scale to help plan for future bioenergy production needs in ways that maintain the integrity of U.S. agriculture.*

Comparing corn grain/stover versus a perennial grass as feedstocks for bioenergy production. It has been assumed that corn stover, an abundant and inexpensive source of biomass, can be removed from fields for bioenergy production with no deleterious productivity effects. A field study by ARS scientists in Lincoln, Nebraska, found that when half the stover was removed from non-irrigated, no-till fields, the cumulative yield of corn grain over the first 10 years dropped significantly even though nitrogen fertilizer was added to optimize yields. The study also showed that over the same time period, growing switchgrass as a biomass energy crop would produce as much or more ethanol per acre than the amount of ethanol per acre produced from both corn grain and harvested stover. *[National Program 215]*

Follett, R.F., Vogel, K.P., Varvel, G.E., Mitchell, R.B., and Kimble, J. 2012. Soil carbon sequestration by switchgrass and no-till maize grown for bioenergy. Bioenergy Research 5(4), 866-875.

Varvel, G.E., Vogel, K.P., Mitchell, R.B., Follett, R.F., and Kimble, J.M. 2008. Comparison of corn and switchgrass on marginal soils for bioenergy. Biomass and Bioenergy 32(1), 18-21.

Switchgrass production environment affects ethanol yields. Feedstock composition in perennial grasses as it affects ethanol yield at a biorefinery has previously not been available. Switchgrass biomass composition from farmer fields can be expected to have significant annual and field-to-field variation in a production region, and this variation may significantly affect ethanol or other liquid fuel yields. Near-infrared reflectance spectroscopy (NIRS) prediction equations developed by ARS scientists from the Central-East Regional Biomass Research Center in Lincoln, Nebraska, with their university collaborators, were used to analyze switchgrass biomass samples collected from switchgrass production fields on 10 farms for a 5-year period in Nebraska and South and North Dakota, and to calculate the biomass composition and expected ethanol yield. Expected ethanol yield varied significantly by year and field. Variability within established switchgrass fields ranged from 1 to 4 percent for expected ethanol yield and 14 to 38 percent for expected ethanol production. Cellulosic biorefineries will need to consider this potential variation in biofuel yields when developing their business plans considering the yearly variation that can occur in biomass production across a region. This research shows the multidisciplinary efforts to address feedstock production issues through the coordination of the Biomass Research Centers. As a result of this effort, ARS and the Near Infrared Spectrophotometry Consortium established a cooperative agreement to transfer switchgrass composition NIRS calibrations to public and private laboratories and industries developing switchgrass as a biofuel biomass crop. *[National Programs 213 and 215]*

Schmer, M.R., Vogel, K.P., Mitchell, R.B., Dien, B.S., Jung, H.G., and Casler, M.D. 2012. Temporal and spatial variation in switchgrass biomass composition and theoretical ethanol yield. Agronomy Journal 104(1), 54-64.

Switchgrass grown for biomass energy results in significant soil carbon sequestration. It has been speculated that perennial grasses for bioenergy production could sequester a significant amount of carbon dioxide in their extensive root systems, and hence be a carbon-negative bioenergy crop. In research that extended over 9 years, ARS scientists in Lincoln, Nebraska, Fort Collins, Colorado, Mandan, North Dakota, and University Park, Pennsylvania, documented carbon sequestration in soils where switchgrass was grown at rate of 1,800 pounds per acre per year. The amount of sequestered soil carbon exceeded the levels used in previous switchgrass lifecycle assessments for greenhouse gases. Data collected at multiple locations indicated a large variation in sequestered carbon, and this variation needs to be considered in life cycle analyses and carbon trading policies. *[National Program 215]*

Skinner, R.H. and Adler, P.R. 2010. Carbon dioxide and water fluxes from switchgrass managed for bioenergy production. Agriculture, Ecosystems and Environment 138(3-4), 257-264.

Sanderson, M.A. and Adler, P.R. 2008. Perennial forages as second generation bioenergy crops. International Journal of Molecular Sciences 9(5), 768-788.

Invasive trees on western rangelands could be used to produce jet fuel. Eastern redcedar, pinyon pine, and Western Juniper trees are native, but have become an invasive nuisance and fire hazard on formerly productive rangelands in the western United States. Clearing these trees will help to restore rangeland productivity for native wildlife habitat and cattle grazing. ARS scientists in Burns, Oregon, Reno, Nevada, and El Reno, Oklahoma, working through the Western and Northwestern Regional Biomass Research Centers, have assessed the biomass amounts of these species of trees across several states, and are developing restoration management plans and estimating the economic and natural resources benefits of tree removal. Using a remote sensing technique developed by ARS scientists in El Reno with their collaborators at the USDA Natural Resources Conservation Service (NRCS), the scientists estimated that the 12 million tons of redcedar growing in the 17 counties most affected is enough to produce 800 million gallons of biofuel or 9 million megawatt hours of electricity. ARS, NRCS, and the Forest Service in Reno have produced an inventory of pinyon pine and juniper infestations on public and private lands in Nevada, Arizona, New Mexico, Utah, and California. In addition to making an inventory of Western Juniper in Oregon, ARS scientists in Burns have completed their assessment of the impact of biomass removal and have developed restoration management methods to improve habitat for endangered sage grouse, while improving grazing for cattle. Commercial business developers are using this information to develop business plans for building the first aviation biofuel production facility. *[National Program 215]*

Sankey, T.T., Glenn, N.F., Ehinger, S., Boehm, A., Seyfried, M.S., and Hardegree, S.P. 2010. Characterizing Western Juniper (*Juniperus occidentalis*) expansion via fusion of Landsat TM5 and LiDAR data. Rangeland Ecology and Management 63:514-523.

PROBLEM STATEMENT 2B: *Greater amounts of biomass feedstocks are needed to achieve U.S. goals for replacing transportation fuels with renewable sources.*

Assessment of growing switchgrass as a bioenergy crop. To determine the economic feasibility of producing switchgrass as a bioenergy crop on farms in the eastern Great Plains, ARS scientists in Lincoln, Nebraska, and Mandan, North Dakota, in cooperation with the University of Nebraska, studied switchgrass production over 5 years on 10 farms spread across Nebraska and the Dakotas. Average production cost was found to be $33 a ton, plus $17 a ton for land rent, while yield averaged 3.4 tons an acre. Prorating the establishment costs over 9 years would reduce costs by $6 ton. Two farmers experienced in switchgrass production were able to produce the biomass for less than $40 a ton including land costs, which represents a farm-gate feedstock cost for ethanol production of $.50 per gallon. The study also determined that the net energy gain for the production of ethanol from switchgrass averaged 540 percent more than the nonrenewable (fossil fuel-based) energy consumed in making it. This figure is 20 times greater than the net energy for corn-grain ethanol. *[National Program 215]*

Schmer, M.R., Mitchell, R., Vogel, K.P., Schacht, W.R., and Marx, D.A. 2010. Spatial and temporal effects on switchgrass stands and yields in the Great Plains. Bioenergy Research 3:159-171.

Perrin, R.K., Vogel, K.P., Schmer, M.R., and Mitchell, R.B. 2008. Farm-scale production cost of switchgrass for biomass. BioEnergy Research 1:91-97. 2008.

Herbicides for improving switchgrass establishment. Weeds limit switchgrass establishment from seed, but few herbicides are labeled for use in establishing switchgrass. ARS scientists in Lincoln, Nebraska, and Mandan, North Dakota, tested selected herbicides on stand establishment and subsequent yields of adapted upland switchgrass cultivars in Nebraska, South Dakota, and North Dakota, as well as on lowland ecotypes in Nebraska. Applying quinclorac, which provides effective control of grassy weeds, plus atrazine, which provides good broadleaf weed control, resulted in acceptable stands and high yields at all locations for all ecotypes. With the help of herbicides, switchgrass can produce yields equivalent to half of full production in the establishment year and can be at full production the second year following planting. Without herbicides, the time to full production can be as long as 3 years. *[National Program 215]*

Mitchell, R.B., Vogel, K.P., Berdahl, J., and Masters, R.A. 2010. Herbicides for establishing switchgrass in the central and northern Great Plains. Bioenergy Research 3(4), 321-327.

High-biomass alfalfa as a means of increasing biomass yields with fixed nitrogen. Utilizing nitrogen-fixing legumes in a bioenergy crop system could provide nitrogen for grass or tree growth, plus additional bioenergy feedstocks. ARS researchers in St, Paul, Minnesota, developed high-biomass alfalfa germplasm and a crop management/collection system for bioenergy production where the forage is separated into a leaf fraction and sold separately as livestock feed, while the stems are used to produce ethanol. Both leaf and stem yields were maximized by reducing the number of plants seeded per field and by a delayed and less frequent cutting schedule. The high-biomass alfalfa germplasm and management system had comparable leaf protein yields, a third greater stem sugar yields, and nearly double the potential ethanol production compared to the hay-type alfalfas. *[National Program 215]*

Rock, K.P., Thelemann, R.T., Jung, H.G., Tschirner, U.W., Sheaffer, C.C., and Johnson, G.A. 2009. Variation due to growth environment in alfalfa yield, cellulosic ethanol traits, and paper pulp characteristics. BioEnergy Research 2:79-89.

Lamb, J.F., Jung, H.G., Sheaffer, C.C., and Samac, D.A. 2007. Alfalfa leaf protein and stem cell wall polysaccharide yields under hay and biomass management systems. Crop Science 47:1407-1415.

Response of Napier grass to fertilizer. Napier grass, a high-yield perennial, is a promising feedstock for the emerging cellulosic biofuels industry in the southeastern United States. ARS scientists in Tifton, Georgia, studied the rain-fed growth of Napier grass under three fertilizer treatments —no fertilizer; poultry litter; and inorganic fertilizer. Relative to the unfertilized control, Napier grass grown with either poultry litter or inorganic fertilizers exhibited yields that were 17 percent and 48 percent greater in the second and third year of growth, respectively. In a companion study, the researchers assessed the performance of perennial warm-season grasses under rain-fed conditions with no fertilizer inputs. Dry matter yield was highest in the second year for all species. When averaged over 3 years, the yields of energy cane and Napier grass were significantly higher than switchgrass, but switchgrass had higher carbon-to-nitrogen uptake. These results contribute to the development of best management practices for viable biomass feedstock production systems in the southeast. *[National Program 215]*

Knoll, J.E., Anderson, W.F., Strickland, T.M., and Hubbard, R.K. 2010. Field performance of potential biomass feedstocks under no inputs in South Georgia. Proceedings of 32nd Symposium on Biotechnology for Fuels and Chemicals, Clearwater, FL. April 19-22, 2010.

Developing canola as a winter rotation crop in wheat fields. Some farmers in the Pacific Northwest want to diversify their crop production so that they can produce either winter wheat (the traditional crop) or canola for biodiesel production. However, canola seeds cannot survive the hot summer temperatures typical of this region. ARS scientists in Pullman, Washington, developed a strategy for establishing canola by moving up planting time so the plants can become established in autumn and survive the winter. The strategy is based on: 1) using shovels to move hot soil away from the seed row, and 2) using weather forecasts to predict time periods of cool (less than 85 degrees F) after-planting temperatures. These strategies allow plants to attain sufficient size to ensure winter survival. In addition, the scientists showed that canola crop rotations inhibit weeds in wheat fields, reduce soil erosion, and utilize excess nitrogen that could be lost to ground and surface waters. The Colville Confederated Tribes are implementing these practices and producing winter oil seed crops on tribal lands as part of their strategy to extract the seed oil and make biodiesel for their school buses, and sell the crushed seeds to local farmers as a livestock feed supplement. In addition, the USDA Risk Management Agency has used the research findings as the basis for providing crop insurance to canola growers in northern Washington. This research is timely since the EPA has determined that canola-based biodiesel is considered an advanced biofuel under RFS-2 guidelines. *[National Programs 213 and 216]*

Production systems for new crops in the upper Midwest. Farmers in the upper Midwest need economically and environmentally sustainable crops to improve the profitability of their farms. ARS researchers in Morris, Minnesota, discovered that camelina, an alternative oilseed that can serve as feedstock for biofuels, can be successfully grown as a low-input cash cover crop in the northern Corn Belt. The researchers determined that the greatest seed and oil yields were achieved by planting camelina in late September to early October into no-tilled soil without herbicide and only a low amount of nitrogen fertilizer. Winter camelina is harvested early enough to enable production of a second seasonal crop. This cropping strategy provides farmers with an additional economic opportunity and information for better management decisions of camelina plantings. The ARS researchers also found that another new crop, cuphea, requires little nitrogen compared to corn. Residual soil nitrogen after corn or soybean harvests is sufficient for a subsequent cuphea crop. Cuphea harvest by swathing is cheaper than direct combining because it reduces seed drying costs. Their findings indicated that corn and soybean were more profitable when grown the year following cuphea than when they were each grown year after year. These findings are included in the growers' guide supplied by Technology Crops International (TCI), a specialty seed company that contracts with farmers in the northern United States, Canada, and northern Europe for cuphea seed production. SarTec Corporation/Ever Cat Fuels, a biodiesel manufacturer, is contracting with farmers to grow around 2,500 acres of camelina in Minnesota for biodiesel feedstock in 2012, and TCI has contracted with farmers to grow cuphea in the upper Midwest, Canada, and Europe. Aveda Corporation is beginning to use cuphea oil in the development of new products to replace petroleum-based products. *[National Program 305]*

Gesch, R.W. and Cermak S. 2011. Sowing date and tillage effects on fall-seeded camelina in the northern Corn Belt. Agron. Journal 103:980-987.

Gesch, R.W., Archer, D.W., and Forcella, F. 2010. Rotational effects of cuphea on corn, spring wheat, and soybean. Agron. Journal 102:145-153.

Berti, M., Johnson, B., Gesch, R. and Forcella, F. 2008. Cuphea nitrogen uptake and seed yield response to nitrogen fertilization. Agron. Journal 100:628-634.

Management strategy for a new oilseed crop, Lesquerella. Lesquerella is a new oilseed crop being developed for production in the southwestern United States. For maximum yields, growers need to know optimum planting and harvest dates for the crop. ARS researchers in

Maricopa, Arizona, evaluated different planting dates (fall, winter, and spring) to assess the effect on reproductive and vegetative growth. Fall planting dates were determined to be optimum for maximum yield. The researchers also developed an imaging system using a multi-spectral camera to accurately monitor crop status. The system helps growers to optimize fertilizer application times and harvest dates for Lesquerella. The University of Arizona is incorporating these data into guidelines for Lesquerella production systems in the southwest. *[National Program 305]*

Dierig, D.A., Wang, G., McCloskey, W.B., Thorp, K.R., Isbell, T.A., Ray, D.T., and Foster, M.A. 2011. Lesquerella: new crop development and commercialization in the U.S. Industrial Crops and Products 34:1381-1385.

Thorp, K.R., Dierig, D.A., French, A.N., and Hunsaker, D.J. 2011. Analysis of hyperspectral reflectance data for monitoring growth and development of Lesquerella. Industrial Crops and Products 33:524-531.

Billet planting of sugarcane is economical. Sugarcane producers prefer to use a chopper harvester, which cuts stalks of seed cane into short pieces (billets) as an alternative to traditional whole-stalk harvesters. However, billet plantings require more seed cane to ensure adequate cane stands and equivalent yields. ARS researchers in Houma, Louisiana, demonstrated that billet planting with sugarcane variety LCP 85-384 is economical and does not negatively influence cane or sugar yields. Growers are beginning to adopt this strategy, which can save about $24 per acre in labor costs and up to $100 per acre in seed costs. *[National Program 305]*

Johnson, R.M., Viator, R.P., and Richard Jr, E.P. 2011. Effects of billet planting rate and position on sugarcane yields in Louisiana. Journal of the American Society of Sugar Cane Technologists 31:79-90.

Sugarcane yield monitor predicts field cane yields. Louisiana sugarcane producers continue to search for ways to increase yields and profitability. One way to increase profitability is to accurately predict and map cane yields at harvest so that transportation costs are minimized and in-field variability is more effectively managed. In a cooperative research effort, ARS researchers in Houma, Louisiana, with their colleagues at Kansas State University, developed and validated an optical yield monitor for predicting cane yields under field harvest conditions. The yield monitor is insensitive to variety and harvester speed, and requires minimal maintenance. This technology allows sugarcane producers to map within field variability, identify areas requiring additional inputs, and maximize harvester efficiency. *[National Program 305]*

Price, R.R., Johnson, R.M., Viator, R.P., Larsen, J., and Peters, A. 2011. Fiber optic yield monitor for a sugarcane chopper harvester. Transactions of the ASABE 54(1):31-39.

Potential biomass production from Conservation Reserve Program land. Marginal croplands may have potential as a biomass source for bioenergy, but there is limited information about the species composition on these lands and their suitability for ethanol production. ARS researchers in University Park, Pennsylvania, found that northeastern U.S. Conservation Reserve Program grasslands with the highest number of species had the lowest potential ethanol yields per acre. However, sites dominated by a small number of native tall prairie grass species, such as switchgrass, big bluestem, and indiangrass, had the highest potential yields. The results from this study demonstrated that the species composition of plant mixtures used in low-input, high-diversity systems affects both biomass production and chemical composition of the resulting feedstock, and that including a large number of species with undesirable fermentation characteristics could reduce ethanol yields. These findings have been used by non-government organizations (Chesapeake Bay Commission) to quantify the biomass feedstock yield potential of abandoned farmlands in the eastern United States. In addition, ARS scientists in El Reno, Oklahoma, showed that Old World bluestem produced an average of 3,380 pounds an acre, and a native mix produced 1,710 pounds an acre of dry biomass feedstock on Oklahoma Conservation Reserve Program land evaluated across all years, locations, and harvest dates. At the native mixed species sites, 3 years of annual harvest did not alter species composition or soil characteristics, but biomass production consistently declined at all sites and for all harvest dates over the period. The scientists determined that maintaining sustainable production requires some form of nutrient replacement such as chemical fertilizers, manure, or conversion-process byproducts, and that it could be beneficial to add a legume component to the grasslands. *[National Program 215]*

Adler, P.R., Sanderson, M.A., Weimer, P.J., and Vogel, K.P. 2009. Plant species composition and biofuel yields of conservation grasslands. Ecological Applications 19:2202-2209.

Venuto, B. C. and Daniel, J. A. 2010. Biomass feedstock harvest from conservation reserve program land in northwestern Oklahoma. Crop Science 50:737-743.

PROBLEM STATEMENT 2C: *Strategies are needed to use conversion co-products from agricultural-based energy production on farms to reduce the need for purchased inputs for crop production systems.*

Finding grass straw that produces less gasification ash. The relatively high mineral (especially silicon) content of grass straws makes it more likely that gasification for energy production will result in ‘slag’ material which can plug gasification equipment. ARS scientists in Corvallis, Oregon, and Wyndmoor, Pennsylvania, evaluated straws from different varieties of Kentucky bluegrass, perennial ryegrass, and tall fescue and determined that each contains different concentrations of minerals. For example, very significant differences in mineral content (i.e., calcium, chlorine, potassium, sulfur, and silicon) were observed between different genotypes of Kentucky bluegrass, whereas differences in the concentration of phosphate—a mineral with fertilizer value—were smaller in magnitude. In addition, the researchers analyzed the mineral content (heavy metals and dioxin) of ash produced from gasification of Kentucky bluegrass straw to assess its suitability as a soil amendment. These findings help biomass producers to select better quality varieties with specific mineral residues needed for processing in gasification-based biorefineries. *[National Programs 213 and 216]*

Banowetz, G.M., Griffith, S.M., and El Nashaar, H.M. 2009. Mineral content of grasses grown for seed in low rainfall areas of the Pacific Northwest and analysis of ash from gasification of bluegrass (*Poa pratensis* L.) straw. Energy and Fuels 23:502-506.

Mustard seed meal amendments for suppression of rootknot nematodes. Mustard seed meals result from the extraction of oil from seeds and are waste by-products of the biodiesel industry.

Methods are being developed to utilize these seed meals in agricultural applications, thereby eliminating waste disposal issues and augmenting profits, while enhancing agricultural practices. The seed meals contain naturally occurring chemicals that make them of interest as management agents for weeds and soilborne pathogens. Previous studies indicated that seed meals from two species of mustard, *Brassica juncea* and *Sinapis alba*, are nematotoxic. ARS scientists in Prosser, Washington, determined that treatment with seed meal from *B. juncea* tended to be the least toxic to pepper seedlings, indicating that nematotoxic rates of *B. juncea* could be applied relatively close to the time of pepper transplant. Germinating lettuce seeds were not as sensitive as pepper seedlings to *S. alba* seed meal. This research is valuable to scientists optimizing the use of seed meal amendments for managing plant-parasitic nematodes without toxicity to crop plants. In additional studies, *B. juncea* and *S. alba* were applied to soil individually at equal rates and as 1:1 and 1:3 combinations. Longer pepper shoots, greater plant weights, and lower nematode galling indices tended to result from an application of *B. juncea* seed meal and one of the seed meal combinations. These treatments also tended to result in low numbers of nematode eggs per root weight. This demonstrated that a combination of seed meals could be as effective against nematodes as an individual seed meal, potentially allowing for greater weed suppression, along with reductions in nematode populations. *[National Program 308]*

Zasada, I.A., Meyer, S.L., and Morra, M.J. 2010. Brassicaceous seed meals as soil amendments to suppress the plant-parasitic nematodes *Pratylenchus penetrans* and *Meloidogyne incognita*. Journal of Nematology 41:221-227.

COMPONENT 3: Biorefining

ARS research under the Biorefining component of National Program 213 is intended to address known problems and knowledge gaps that were identified during stakeholder meetings at the beginning of the current project cycle. This research supports the Administration's current bioenergy strategy (Growing America's Fuels) to advance multiple conversion routes in parallel, including biochemical, thermochemical, and hybrid designs, and to diversify product options and risks through the development of value-added co-products.

In addressing these challenges, ARS scientists have used interdisciplinary and creative approaches to discover ways to improve existing processes for energy production from a wide variety of agricultural materials. They have pioneered new processes that circumvent technical and economic impediments to distributed scale energy production, and have devised new uses for byproducts and co-products of bioenergy production systems.

Unlike the research contributing to Component 1 and Component 2, the great bulk of the research contributing to Component 3 is managed directly under the NP 213 National Program. The accomplishments for Component 3 are aligned under four Subcomponents, each with one or more Problem Statements, as noted in the Action Plan.

- 3A: Biocatalytic Conversion
- 3B: Thermochemical Conversion
- 3C: Biodiesel
- 3D: Process Economics and Life Cycle Analyses

SUBCOMPONENT 3A: Biocatalytic Conversion

In this Subcomponent, ARS researchers specifically focused on improving prefermentative and fermentative processes that are used to convert complex lignocellulosic biomass or grains into transportation fuels, such as ethanol and butanol. Researchers also discovered new ways of improving anaerobic processes producing hydrogen and methane and appropriate ways to utilize residual material or byproducts derived from these fermentations toward new products, energy, and feed.

PROBLEM STATEMENT 3A-1: *Cost-effective conversion of lignocellulosic feedstocks to ethanol or butanol.*

Inhibitor removal from cellulosic biomass hydrolyzates. Pretreatment of lignocellulosic biomass during the biochemical conversion process often produces chemical byproducts such as furfural and 5-hydroxymethylfurfural (HMF) that inhibit subsequent fermentation of the sugars to ethanol. ARS researchers in Peoria, Illinois, discovered a microorganism (*Coniochaeta ligniaria* NRRL30616) that removes these inhibitory chemicals when added prior to fermentation by *Saccharomyces cereviseae.* However, native *C. ligniaria* grows on xylose, a sugar resulting from the conversion process, and would significantly reduce the overall yield of ethanol from biomass. Therefore, the scientists developed a transformation system and constructed a new strain of *C. ligniaria* that retained its ability to metabolize only the inhibitors, but not the xylose. This bioabatement method has specific advantages compared to alternate methods, including suitability for treating liquid-solid mixtures, minimal generation of waste streams, no need for chemical inputs, and no requirement for recharging (as might be required by adsorption resins). Biological abatement could also facilitate recycling of process water, since concentration of inhibitors through the process could be a limiting factor in water re-use. A patent based on this research has been granted. The scientists are planning to sequence the genome of *C. ligniaria* and its relatives and to analyze global patterns of gene expression with support from the Department of Energy. The bioabatement has potential at the 100-liter scale to be attractive enough to companies that they might consider applying some form of it once their commercial-scale sites are operational.

Nichols, N.N., Dien, B.S., and Cotta, M.A. 2010. Fermentation of bioenergy crops into ethanol using biological abatement for removal of inhibitors. Bioresource Technology 101(19), 7545-7550.

Nichols, N.N., Sharma, L.N., Mowery, R.A., Chambliss, C.K., van Walsum, G.P., Dien, B.S., and Iten, L.B. 2008. Fungal metabolism of fermentation inhibitors present in corn stover dilute acid hydrolysate. Enzyme and Microbial Technology 42(7), 624-630.

Increasing yield of ethanol from corn stover. One reason that cellulosic ethanol is more expensive than corn-based ethanol is that biomass contains both six-carbon sugars (hexoses, such as glucose), and five-carbon sugars (pentoses). Corn-based ethanol is produced with brewer’s yeast, which converts only glucose. Although new recombinant microorganisms have been developed to convert hexoses and pentoses to ethanol, these organisms ferment glucose preferentially and do not begin to metabolize any pentoses until low glucose concentrations have been reached. As a result, fermentations times are long, and the pentoses are not fully converted. To overcome these hurdles, ARS researchers in Wyndmoor, Pennsylvania, with collaborators at Iowa State University and an industrial partner, co-developed a two-stage simultaneous saccharification and fermentation process that employed aqueous ammonia pretreated corn stover. In the first phase, xylanase and endo-glucanase are used to produce xylose and fermented to ethanol by the recombinant organism *Escherichia coli* KO11. In the second phase, cellulase and β-glucosidase are used to produce glucose, which is fermented to ethanol by the yeast *Saccharomyces cerevisiae*. Using this process, the scientists were able to achieve an ethanol yield of 85 gallons per ton from corn stover. If the traditional process that ferments only glucose had been used, the yield would have been only 65 gallons per ton. This process addresses several problems with the conventional processes involving pentose utilization from three aspects:

1. Pentose fermentation can be integrated into a single-reactor using xylan-rich pretreated corn stover;
2. The inhibition of glucose on xylose fermentation, which is always the case in conventional simultaneous saccharification and fermentation process, can be circumvented by converting xylan prior to glucan; and
3. The application of *S. cerevisiae* in the second phase ensures the stable and efficient utilization of glucose and overcomes the relatively low ethanol yield of genetically engineered strains.

This research finding is currently being evaluated by a large biorefining company for its potential in their processes. One option that the company is investigating is diversion of the high-xylose stream for production of high-value co-products.

Li, X., Kim, T. H., and Nghiem, N.P. 2010. Bioethanol production from corn stover using aqueous ammonia pretreatment and two-phase simultaneous saccharification and fermentation (TPSSF). Bioresource Technology 101(15), 5910-5916.

Increasing energy efficiency of bioethanol production. A major concern associated with corn ethanol fuel is the relatively low energy efficiency of its life-cycle production, a situation resulting in large part from the high energy input required to distill ethanol from fermentation broth. In addition, the fermentative conversion of biomass to ethanol involves especially low concentrations of ethanol, so the distillation step requires even larger amounts of energy. ARS scientists in Albany, California, invented a new method to make thin-film composite membranes for use in recovering ethanol or other volatile compounds from fermentation broths. The novel fabrication method applies an ultra-thin, ethanol-selective active layer to a highly permeable, large-pore support layer. The result is a membrane with double the enhanced selectivity for ethanol and flux of commercially available membranes. A conventional active layer application method that relied on directly coating the support would have difficulty utilizing the low mass transport resistance supports employed by this membrane because of intrusion of the coating solution into the support. Thus, this method, which enables an energy efficient alternative for ethanol production, represents a distinct improvement. A patent application has been filed on the technique (U.S. patent application no. 12/941,776). As a direct result of this work, further development of block co-polymer membranes for recovery of ethanol by pervaporation is being pursued by several university collaborators through an Energy Biosciences Institute award.

Offeman, R.D. and Ludvik, C.N. 2011. A novel method to fabricate high permeance, high selectivity thin-film composite membranes. Journal of Membrane Science 380(1-2), 163-170.

New process for producing hydrocarbon fuels from biomass. A commercially viable process for converting cellulosic biomass into drop-in replacements for petroleum-derived fuels would be a significant advancement for the biofuels industry. Recent heavy investments in such advanced biofuels have focused either on thermochemical platforms that utilize high temperatures and pressures, often with expensive catalysts, or on biological conversions using pure (and often genetically modified) microbial cultures that would face challenges at an industrial scale. A relatively unexplored alternative route to advanced biofuels is a volatile fatty acid (VFA) platform in which stable mixed cultures convert biomass to C2-C6 organic acids that can subsequently be converted by various chemical or electrochemical routes to produce alkanes, alkenes, ketones, and alcohols. Most research on the VFA platform has utilized chemical inhibitors, such as iodoform, to prevent further conversion of VFA to methane that occurs at long (2 to 3 week) retention times. In collaboration with an industrial partner, ARS scientists in Madison, Wisconsin, combined the fermentative production of VFA from biomass by bacteria from the bovine rumen with subsequent electrolysis of the VFAs to produce C2-C6 alkanes and alkenes, with hydrogen gas as a co-product. The fermentation can be performed on ground biomass without additional pretreatment; without sterilization of the biomass, culture medium, or process equipment; and without additional enzyme addition. Because the microbial consortium in the bovine rumen is adapted to utilizing a wide variety of plant-based feedstocks, it converts not only the hexose and pentose fractions of polysaccharides, but also proteins, nucleic acids, plant organic acids, and some lipid components, thus increasing overall product yields. The process operates at short retention times (2 to 3 days), thus maximizing VFA yields (60-70 percent by weight) and concentrations (>20 g/L) by preventing VFA conversion to methane. Augmentation of the mixed culture with other bacterial species can alter the VFA product mix for specific applications. The electrolytic step can be conducted at low voltages with inexpensive graphite electrodes, although more advanced electrode systems can improve product yield and selectivity. A U.S. patent application has been filed, and additional patents are being sought through the industrial partnership and technology transfer process.

Weimer, P.J., Stevenson, D.M., Mertens, D.R., and Hall, M. 2011. Fiber digestion, VFA production, and microbial population changes during in vitro ruminal fermentations of mixed rations by monensin-adapted and unadapted microbes. Animal Feed Science and Technology 169:68-78.

Low-cost alkaline pretreatment of lignocelluloses. Switchgrass and other perennial grasses are being developed as bioenergy crops because of their high productivity. However, pretreatment is needed for their conversion to ethanol to allow the process enzymes (e.g. cellulases) to hydrolyze the plant structural carbohydrates into fermentable sugars at appreciable yield. ARS scientists in Peoria, Illinois, have developed an efficient pretreatment for herbaceous biomass that is compatible with ethanol fermentation. The scientists determined that treating with dilute-ammonia is an efficient pretreatment as it allows for good sugar yields upon enzymatic saccharification and the sugars that are produced are readily fermented by *Saccharomyces cerevisiae* yeast. Also, because ammonia is volatile, it can be removed following pretreatment by evaporation, thereby avoiding the added cost of a neutralizing chemical and allowing for potential recycling of the catalyst. Ammonium hydroxide pretreatment was determined to preserve 97 percent of the carbohydrates and still allow enzymatic conversion. The pretreated material was easily converted to ethanol using either a native or xylose-fermenting *S. cerevisiae*. Significantly, there was no lag phase observed for the fermentation, which indicated an absence of inhibitors that are commonly observed in acid catalyzed pretreatments.

Dien, B.S., Casler, M.D., Hector, R.E., Iten, L.B., Nichols, N.N., Mertens, J.A., and Cotta, M.A. 2011. Biochemical processing of reed canarygrass into fuel ethanol. Int. J. Low-Carbon Tech doi:10.1093/ijlct/ctr041.

Dien, B.S., Miller, D.J., Hector, R.E., Dixon, R.A., Chen, F., McCaslin, M., and Cotta, M.A. 2011. Enhancing alfalfa conversion efficiencies for sugar recovery and ethanol production by altering lignin composition. Bioresource Technology 102(11), 6479-6486.

On-farm pretreatment of biomass. Obtaining reasonable ethanol yields requires pretreatment of cellulosic biomass. However, because that requires expensive equipment, pretreatment represents a major cost component of cellulosic ethanol production. In addition, the narrow window for crop harvesting requires long-term storage of biomass feedstocks, which can lead to significant losses from spoilage. ARS scientists in Madison, Wisconsin, and Peoria, Illinois, developed simple, yet novel methods to combine these two steps—storage and pretreatment—

on-farm. The researchers demonstrated first at a pilot scale, then at farm scale, that acid pretreatment and anaerobic storage not only preserved the reed canarygrass or switchgrass substrates by limiting microbial activity, but also began to degrade the cellulose-hemicellulose-lignin cell wall matrix, thereby enhancing accessibility for enzymatic degradation. Acid-pretreated substrate produced more ethanol after simultaneous saccharification and fermentation than untreated reed canarygrass and switchgrass. Additionally, the researchers found that simply spraying the acid onto the biomass as it was moved by conveyor to the ensiling bag resulted in as good or better preservative and cell wall degradation than if the acid was added to the biomass in a feed mixer and mixed before bagging. Finally, the on-farm pretreatment method was found to be compatible with fermentation to ethanol by *Saccharomyces cerevisiae* yeast at a relatively high (10 percent) solids loading. On-farm pretreatment at the rates explored in this study did not yield complete conversion of available cellulose, but the residual sulfuric acid could be used to further degrade the cell wall matrix at the biorefinery through an additional thermal process. The scale of this work demonstrated that this approach could be a viable storage and pretreatment option for existing and future biomass feedstock producers.

Digman, M.F., Dien, B.S., and Hatfield, R.D. 2012. On-farm acidification and anaerobic storage for preservation and improved conversion of switchgrass into ethanol. Biological Engineering Transactions 5(1), 47-58.

Digman, M.F., Shinners, K.J., Casler, M.D., Dien, B.S., Hatfield, R.D., Jung, H.G., and Weimer, P.J. 2010. Optimizing on-farm pretreatment of perennial grasses for fuel ethanol production. Bioresource Technology 101(14), 5305-5314.

Digman, M.F., Shinners, K.J., Muck, R.E., and Dien, B.S. 2010. Full-scale on-farm pretreatment of perennial grasses with dilute acid for fuel ethanol production. Bioenergy Research 3(4), 335-341.

Single bottle determination of feedstock composition. Traditional methods for evaluating feedstocks for potential ethanol yield are laborious, expensive processes. To reduce time for this analysis and allow calibration of spectroscopic methods for estimation of feedstock composition, ARS researchers in Peoria, Illinois, Madison, Wisconsin, and St. Paul, Minnesota, developed a sequential method for laboratory analysis of feedstocks that is simple to perform. The throughput was sufficient to analyze hundreds of switchgrass samples from a diversity of environments. The samples were also analyzed using near-infrared spectroscopy. Together, the data allowed development of a highly accurate prediction equation based on the near-infrared spectroscopy spectra that could estimate a number of feedstock properties. The predictive calibration equations were transferred to the Near Infrared Spectroscopy Consortium, which makes them available to other laboratories. The research led to ARS being invited to join an inter-laboratory study in collaboration with the National Institute of Standards and Technology and the National Renewable Energy Laboratory to measure the composition of four biomass NIST reference materials. The research is being extended to Napier grass in collaboration with a large industrial partner. The scientists are applying this research toward development of an integrated supply chain approach to switchgrass production in the Upper Great Plains with university and industrial partners and support from the USDA National Institute of Food and Agriculture.

Doran-Peterson, J., Jangid, A., Brandon, S.K., DeCrescenzo-Henriksen E., Dien B., and Ingram, B.O. 2009. Simultaneous saccharification and fermentation and partial

saccharification and co-fermentation of lignocellulosic biomass for ethanol production in biofuels, editor Jonathan R. Mielenz, Methods in Molecular Biology Vol. 581, pp 263-268

Designer yeasts for cellulosic ethanol. Although xylose is a major sugar in lignocellulosic biomass, yeasts (*Saccharomyces cereviseae*) are incapable of converting xylose to ethanol. ARS scientists in Peoria, Illinois, introduced genes required to produce ethanol from xylose and the transport proteins to pump xylose into the yeast cell—xylose reductase, xylitol dehydrogenase, and xylulokinase—into six industrial yeasts. All six strains were isolated from distillery-type operations or otherwise recommended for lignocellulose conversion. The engineered strains were compared for their growth and fermentation rates of xylose and xylose/glucose mixtures in aerobic and anaerobic cultures. The group demonstrated that these yeasts efficiently ferment xylose to ethanol. The three best strains produced 13 to 17 percent more ethanol than the parental control strains with ammonium pretreated switchgrass as a feedstock. These improved strains should enhance the commercial viability of cellulosic ethanol biorefining. The *S. cereviseae* strain, as well as the molecular tools used to create the strain, has been widely distributed to 14 different university and private sector research laboratories in 5 different countries, as well as to other ARS scientists. Further metabolic engineering of this yeast strain has also begun to show promising results that have resulted in a patent filing.

Another yeast, *Scheffersomyces (Pichia) stipitis* NRRL Y-7124, is naturally able to ferment both hexoses and pentoses. However, byproducts created when fermentable sugars are produced from cellulosic biomass severely cripples this yeast's ability to utilize xylose, which constitutes about one-third of the sugars in cellulosic biomass. Using adaptation and genetic engineering techniques, another group of ARS scientists in Peoria developed a new strain of industrial yeast that both tolerates the toxic byproducts and efficiently ferments xylose to ethanol. The scientists have applied for a patent, and several industrial partners plan to test the yeast for possible commercial production. In addition, research collaborators at Michigan State University plan to test top strains in combination with different pretreatment options on different feedstocks. Process optimization for economical ethanol production is currently underway using this promising strain.

Hector, R.E., Dien, B.S., Cotta, M.A., and Qureshi, N. 2011. Engineering industrial *Saccharomyces cerevisiae* strains for xylose fermentation and comparison for switchgrass conversion. Journal of Industrial Microbiology and Biotechnology 38(9), 1193-1202.

Slininger, P.J., Thompson, S.R., Weber, S., Liu, Z.L., and Moon, J. 2011. Repression of xylose-specific enzymes by ethanol in *Scheffersomyces* (*Pichia*) *stipitis* and utility of repitching xylose-grown populations to eliminate diauxic lag. Biotechnology and Bioengineering 108(8), 1801-1815.

More efficient hemicellulases. Enzymes that degrade plant cell wall polymers to simple molecules represent a substantial fraction of the cost to produce liquid biofuels from lignocellulosic material. ARS researchers in Peoria, Illinois, and Albany, California, discovered and characterized a glycoside hydrolase from *Selenomonas ruminantium* (SXA). This enzyme was an order of magnitude more active than other hemicellulases at catalyzing the hydrolysis of xylooligosaccharides to xylose. Its high activity, stability at low pH and higher temperatures, and ease of protein production in *Escherichia coli* recommend employment of SXA in industrial processes where it would serve in the hydrolysis of herbaceous biomass (xylan fraction) to simple sugars for fermentation to fuel ethanol and other bioproducts. The enzyme enables high yields of fermentable sugars from efficient, mild pretreatment processes. One problem with the native SXA hydrolose enzyme is that high concentrations of xylose or glucose inhibit its effectiveness. Consequently, ARS scientists developed a mutated enzyme by using error-prone polymerase chain reaction (PCR) and saturation mutagenesis. The mutated enzyme tolerates 300 percent higher sugar concentrations and lowers production costs for cellulosic ethanol. A patent application has been filed.

As the SXA enzyme was superior in many respects, the same scientists analyzed structurally similar enzymes from *Bacillus halodurans*, the thermophilic *Geobacillus thermoleovorans*, *Alkaliphilus metalliredigens*, *Bacillus pumilus*, *Bacillus subtilis subsp. Subtilis*, and *Lactobacillus brevis* for comparative purposes and for possible protein engineering approaches. These were expressed in *E. coli* and found to have unique affinities, pH, and temperature optima against xylooligosaccharide substrates. One enzyme from *L. brevis* was found to have even higher activity than SXA against xylobiose.

Wagschal, K., Jordan, D.B., and Braker, J.D. 2012. Catalytic properties of β-d-xylosidase XylBH43 from *Bacillus halodurans* C-125 and mutant XylBH43-W147G. Process Biochemistry 47(3), 366-372.

Jordan, D.B., Wagschal, K., Fan, Z., Yuan, L., Braker, J.D., and Heng, C. 2011. Engineering lower inhibitor affinities in β-D-xylosidase of *Selenomonas ruminantium* by site-directed mutagenesis of Trp145. Journal of Industrial Microbiology and Biotechnology 38(11), 1821-1835.

Wong, D.W.S., Chan, V.J., McCormack, A.A., and Batt, S.B. 2010. A novel xyloglucan-specific endo-β-1,4-glucanase: Biochemical properties and inhibition studies. Applied Microbiology and Biotechnology 86(5), 1463-1471.

Ferulic acid esterase improves efficiency of biomass conversion. To convert corn stover and switchgrass into biofuel, the plant fibers must first be broken down into sugars. But cell wall polymers such as hemicellulose, lignin, and cell wall proteins are cross-linked via ferulic acid in various ways that make them very resistant to breaking down. ARS researchers in Albany, California, and Lincoln, Nebraska, discovered, cloned, and expressed novel genes for ferulic acid esterase in yeast. This represents a key enzyme for cleaving the chemical bonds in plant cell wall material and the cell walls of grasses in particular. The study represents the first report on engineering yeast for the breakdown of ferulic acid crosslinks to facilitate consolidated bioprocessing. The discovered enzyme was characterized and found to produce ferulic acid efficiently from pretreated switchgrass cell wall material that may result in reducing the recalcitrance of biofuel feedstocks to biochemical breakdown. A patent has been granted for this work that was developed in collaboration with an industrial partner. In addition to increasing the efficiency of biomass conversion to biofuel, the enzymes could also be used to enhance the digestibility and the nutritional qualities of animal feeds, aid in the development of nutritional supplements, and prove useful in the development of other value-added products.

Wong, D.W.S., Chan, V.J., Batt, S.B., Sarath, G., and Liao, H. 2011. Engineering *Saccharomyces cerevisiae* to produce feruloyl esterase for the release of ferulic acid from switchgrass. Journal of Industrial Microbiology and Biotechnology 38(12), 1961-1967.

New, industrially useful polygalacturonase/arabinase enzymes. Pectin-degrading enzymes, such as polygalacturonases and methylesterases, are useful industrial catalysts that can assist conversion of biomass waste, such as sugar beet pulp and citrus peels, to simple sugars, as well as serving specific uses in food and textile industries. However, due to its complex structure, pectin requires several activities to completely degrade. *Rhizopus oryzae* is an industrially important Mucormycotine fungus that is a source of a wide array of pectinases that have not yet been completely characterized. To better understand the various polygalacturonases from *R. oryzae,* ARS researchers in Peoria, Illinois, expressed 15 of them in *Pichia pastoris*. These all belong to the same family of glycolytic enzymes that are used industrially in the rhetting of flax and for juice clarification. Fourteen of the enzymes were found to be highly active. A number of the enzymes have now been used in combination with ultrasound for bioscouring cotton, which can eliminate current harsh chemical processes currently used by the textile industry.

Arabinans are also constituents of pectin, and using a different, metagenomic approach, ARS scientists in Albany, California, isolated arabinases from the microflora of the cow rumen. In the process of doing so, the scientists discovered a new, highly active exo-1,5-α-arabinanase. Very few enzymes have been found to have its activity and this particular enzyme displayed a unique mode of action in the release of monosaccharides rather than di- or tri-saccharides from arabinans and arabino-oligosaccharides.

Mertens, J.A., Hector, R.E., and Bowman, M.J. 2012. Subsite binding energies of an exo-polygalacturonase using isothermal titration calorimetry. Thermochimica Acta 527:219-222.

Mertens, J.A. and Bowman, M.J. 2011. Expression and characterization of 15 *Rhizopus oryzae* 99-880 polygalacturonase enzymes in *Pichia pastoris*. Current Microbiology 62(4), 1173-1178.

Wong, D.W.S., Chan, V.J., and Batt, S.B. 2008. Cloning and characterization of a novel exo-α-1,5-L-arabinanase gene and the enzyme. Applied Microbiology and Biotechnology 79(6), 941-949.

A process-oriented approach to bio-ethanol production from agricultural residues. Wheat straw is an abundant byproduct from wheat production. The average yield of wheat straw is 1.3–1.4 times that of wheat grain, making it an attractive feedstock for ethanol production from wheat-growing areas. ARS scientists in Peoria, Illinois, have focused on both ethanol and bio-butanol production from wheat straw using different processes and different fermentative organisms. They produced a stable, recombinant, ethanologenic *E. coli* (strain FBR5) that ferments both pentose and hexose sugars. Continuous fermentation processes require significantly less capital investment because they have higher (as much as two times) productivity compared to traditional batch fermentation systems. However, it is often difficult to use genetically engineered microorganisms in continuous fermentations because the plasmids that contain the exogenous genes lack sufficient stability. The scientists investigated the stability of this recombinant strain in a continuous fermentation fed with wheat straw hydrolyzate. The strain was found to produce ethanol continuously over 4 months without any loss in productivity, plasmid stability, or cell viability. The scientists found that fed-batch simultaneous saccharification and fermentation processes that included bioabatement using *Coniochaeta ligniaria* performed the best. The fed-batch method mitigated problems associated with mixing and heat transfer that result from high solids loading. The scientists were able to obtain a final ethanol concentration of 4.5 percent using the above methods while minimizing the time and quantity of enzyme required.

Saha, B.C. and Cotta, M.A. 2011. Continuous ethanol production from wheat straw hydrolysate by recombinant ethanologenic *Escherichia coli* strain FBR5. Applied Microbiology and Biotechnology 90(2), 477-487.

Saha, B.C., Nichols, N.N., and Cotta, M.A. 2011. Ethanol production from wheat straw by recombinant *Escherichia coli* strain FBR5 at high solid loading. Bioresource Technology 102(23), 10892-10897.

Saha, B.C., Nichols, N.N., Qureshi, N., and Cotta, M.A. 2011. Comparison of separate hydrolysis and fermentation and simultaneous saccharification and fermentation processes for ethanol production from wheat straw by recombinant *Escherichia coli* strain FBR5. Applied Microbiology and Biotechnology 92(4), 865-874.

A process-oriented approach to bio-butanol production from agricultural residues. Bio-butanol is an advanced biofuel more compatible with the U.S. transportation fuels infrastructure than ethanol. Bio-butanol has higher energy content than ethanol, and due to its similarities with gasoline, it can be blended at higher rates than ethanol. Its production along with acetone and ethanol via fermentation of starch by *Clostridium* ssp. is one of the oldest industrial fermentations. To convert wheat straw to butanol, ARS scientists in Peoria, Illinois, developed a simultaneous saccharification and fermentation process using *Clostridium beijerinckii*. The process was the first to employ acid pretreatment steps for butanol production and was shown not to require a post-pretreatment detoxification step, thus resulting in significant cost savings. Further, gas-stripping the fermentation broth to remove butanol avoided product inhibition of the fermentation and maintained high rates of butanol production. Interestingly, it was found that the common fermentation inhibitors furfural and hydroxymethyl furfural, which are produced when biomass is pretreated with dilute acids, stimulated the rate of acetone, butanol and ethanol production. The integrated saccharification, fermentation, and recovery process significantly reduces the cost of producing butanol from lignocellulosic feedstock. This work is currently being extended by the scientists to improve product recovery and butanol tolerance, and to study process economics of butanol production.

Also, using *C. beijerinckii,* ARS researchers in Peoria, Illinois, were able to convert switchgrass, corn stover, and barley straw into bio-butanol in a manner similar to wheat straw (see the previous accomplishment). However, in the case of switchgrass and corn stover, unidentified fermentation inhibitors were present. Inhibition was alleviated somewhat by overliming, but more research is required to make this process commercially feasible.

Qureshi, N., Bowman, M.J., Saha, B.C., Hector, R., Berhow, M.A., and Cotta, M.A. 2012. Effect of cellulosic sugar degradation products (furfural and hydroxymethyl furfural) on acetone-butanol-ethanol (ABE) fermentation using *Clostridium beijerinckii* P260. Food and Bioproducts Processing 90(3), 533-540.

Qureshi, N., Saha, B.C., Dien, B., Hector, R.E., and Cotta, M.A. 2010. Production of butanol (a biofuel) from agricultural residues: Part I - Use of barley straw hydrolysate. Biomass and Bioenergy 34(4), 559-565.

Qureshi, N., Saha, B.C., Dien, B., Hector, R.E., and Cotta, M.A. 2010. Production of butanol (a biofuel) from agricultural residues: Part II – Use of corn stover and switchgrass hydrolysates. Biomass and Bioenergy 34(4), 566–571.

Qureshi, N., Saha, B.C., and Cotta, M.A. 2008. Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii.*: Part II-fed-batch fermentation. Biomass and Bioenergy 32(2), 176-183.

Ethanol and biobutanol from other agricultural residues and feedstocks. Waste from barley starch to ethanol processes includes grain hulls and barley straw which represent potentially fermentable sources of cellulose-rich material. ARS researchers in Wyndmoor, Pennsylvania, developed a simultaneous saccharification and fermentation process to ferment barley hulls into ethanol. By converting hulls into ethanol, a barley biorefinery that processes barley starch could increase ethanol output by 6 to 10 percent. In addition, the equipment the biorefinery would use in converting hulls could process other available cellulosic biomass, such as switchgrass or straw. A demonstration scale project is currently being extended in partnership with industry to evaluate whether this process would be successful on a larger scale.

Kim, T.H., Taylor, F., and Hicks, K.B. 2008. Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment. Bioresource Technology 99(13), 5694-5702.

High-productivity membrane bioreactor. A major problem challenging the economical production of ethanol is low reactor productivity. To address this shortcoming, ARS scientists in Peoria, Illinois, developed a membrane bioreactor to recycle ethanologenic biocatalysts, thereby reducing the cost of cellulosic ethanol production. The membrane bioreactor was tested with ethanologenic *E. coli* FBR5 using xylose, a major sugar component of cellulosic biomass. The bioreactor exhibited xylose-to-ethanol productivities 60 times better than a traditional batch reactor and demonstrated that a commercial system would require significantly lower capital costs. At the high product concentrations produced, inhibition can be a problem, so gas-stripping was used to remove the ethanol product continuously, resulting in greatly improved productivity.

Qureshi, N., Dien, B.S., Liu, S., Saha, B.C., Hector, R., Cotta, M.A., and Hughes, S. 2012. Genetically engineered *Escherichia coli* FBR5: Part I. comparison of high cell density bioreactors for enhanced ethanol production from xylose. Biotechnology Progress published online DOI:10.1002/btpr.1585

Qureshi, N., Dien, B.S., Liu, S., Saha, B.C., Cotta, M.A., Hughes, S., and Hector, R. 2012. Genetically engineered Escherichia coli FBR5: Part II. ethanol production from xylose and simultaneous product recovery. Biotechnology Progress, published online DOI:10.1002/btpr.1584

PROBLEM STATEMENT 3A-2: *Biological production of hydrogen and methane from lignocellulosic feedstocks.*

Increasing the profitability of manure digestion by adding dewatered food waste or switchgrass. Methane, the main component of biogas, is currently used as a “clean,” energy-dense transportation fuel. Livestock farmers that use anaerobic digesters to produce methane and other biogases can increase their income by adding food waste to the digesters and charging fees for accepting food waste from restaurants and other sources. ARS scientists in Beltsville, Maryland, set out to determine how much food waste farmers could accept and still maintain maximal efficiency during anaerobic digestion. The scientists showed that anaerobically digesting mixtures of swine (or dairy) manure and 5 percent pulped food waste (the maximum allowed) produced about 20 percent more biogas than mixtures containing only 1 percent food waste. However, the researchers also found that the ability to achieve higher biogas production required good control of digester pH at approximately 7. They also showed that adding switchgrass to a high-solids anaerobic digester while controlling pH increased the biogas yield from dairy manure about four-fold. In addition, the scientists found that increased biogas production is the same for green switchgrass (harvested in July) or senescent/brown switchgrass (harvested in January), although the point of harvest changes the timing of optimal biogas production. This work has the potential to improve manure management strategies to maximize economic returns in diary operations.

Ahn, H., Smith, M.C., Ingram, S.K., and White, J.W. 2010. Evaluation of biogas production by dry anaerobic digestion of switchgrass-animal manure mixtures. Applied Biochemistry and Biotechnology 160:965-975.

PROBLEM STATEMENT 3A-3: *New and improved processes for biocatalytic conversion of starches and sugars to ethanol or butanol.*

Aqueous enzymatic oil extraction process for bioethanol plants. Most corn oil is extracted from corn in wet mill refineries because they are large enough to justify the use of expensive solvent extraction. In contrast, most bioethanol plants use a dry grind process and do not produce edible corn oil as a co-product. ARS scientists from Wyndmoor, Pennsylvania, developed a process called aqueous enzymatic oil extraction to separate corn oil from the germ that can potentially be produced at the front end of a dry-grind biorefinery. The process recovers 80-90 percent of the corn germ and it does not require heat, but the extraction process needed a pretreatment step to increase oil yields. Consequently, ARS scientists developed an enzyme-based pretreatment step to increase oil yields by 90 percent. Preliminary cost estimates indicate that with the pretreatment step, the aqueous enzymatic oil extraction process will allow ethanol biorefineries to produce edible corn oil economically, and may even replace hexane extraction in wet mill refineries as a source of corn oil production. This advancement could help the approximately 200 U.S. corn ethanol refineries produce a valuable co-product (corn oil) and be more economically resilient to volatile corn or ethanol prices. A large industrial partner is evaluating use of this technology under a cooperative research and development agreement.

Dickey, L.C., Johnston, D.B., Kurantz, M.J., McAloon, A., and Moreau, R.A. 2011. Modification of aqueous enzymatic oil extraction to increase the yield of corn oil from dry fractionated corn germ. Industrial Crops and Products 34(1), 845-850.

Enhanced drying process improves efficiency of distillers grain production. A major criticism of corn-based ethanol is its relatively low life-cycle energy efficiency, because the bulk of the energy produced is consumed at the biorefinery, almost half of which goes into drying the distillers grain co-product. Using commercial enzymes during the fermentation process, ARS scientists in Wyndmoor, Pennsylvania, significantly increased water removal during centrifugation in corn-to-ethanol operations resulting in lower energy usage. A large-scale demonstration project of the enzymatic dewatering process was conducted at a 54 million-gallon per year dry grind ethanol facility. Data collected from the trial was used to develop process engineering and cost models for comparison to conventional processing to determine differences in energy consumption, water utilization, greenhouse gas production, and potential economic impacts. The results showed a significant improvement in water removal during centrifugation. The efficiency of the drier increased significantly and resulted in a 12 percent overall reduction in natural gas used by the plant. The dried distillers grains with solubles composition were not found to be affected by the enzyme treatment, but may potentially be more digestible due to the enzymes used. Process models developed from the plant trial results for the baseline and enzymatic dewatering process showed a 14 percent reduction in water use for the process and a 10 percent water use reduction overall. Electricity and steam use were also reduced slightly. Overall, the improvements decreased greenhouse gas output of the ethanol facility by approximately 16 million pound carbon dioxide equivalents per year. Although tradeoffs between enzyme and natural gas costs occurred, the researchers found economically viable conditions for the dewatering enzyme application. This technology can easily be adopted by existing ethanol plants to reduce their energy usage and operating costs that could improve profitability by an estimated $1.2 million a year, mainly through reduction in natural gas usage for drying.

Henriques, A.B., Johnston, D., Mcaloon, A.J., and Dudukovic, M.P. 2011. Reduction in energy usage during dry grind ethanol production by enhanced enzymatic dewatering of whole stillage: plant trial, process model and economic analysis. Industrial Biotechnology 7(4)288-297.

Winter barley as an alternative bioenergy feedstock. Barley can be grown in areas not normally used for corn, and is often used as a winter crop. Developing a barley based ethanol industry could benefit rural economies where corn-based ethanol production is not viable. Stakeholder-driven ARS research on barley starch conversion to ethanol in collaboration with Virginia Tech University, Drexel University, and multiple industrial partners has helped bring this process to commercial scale.

- Most varieties of barley contain a hull made up of non-fermentable lignocellulosic biomass that covers and protects the starch-rich endosperm part of the kernel. If ethanol producers use the entire kernel, hull, and endosperm to make ethanol, the presence of the hull does nothing to produce ethanol, but it decreases the efficiency of the system by taking up fermentor space and increases the energy needed to stir and transport the “mash.” ARS researchers in Wyndmoor, Pennsylvania, have developed a new two-step method to remove the hull from barley before it is used to make fuel ethanol. In this new process, barley kernels are passed through the same type of “roller mills” that are used to convert wheat into flour. The barley flour produced is then treated with a new process that separates out the unwanted barley hull using a combined sieving and air-classification (density) system. The process results in barley flour much improved for ethanol feedstock over traditionally ground barley. The researchers also developed an alternative roller milling process that produced improved barley flour without having to use the combined sieving and air-classification steps. This information has been implemented into the process design of an ethanol plant that plans to use barley as its primary feedstock.

- Barley grain also contains oil that is extremely rich in valuable and health-promoting phytosterols, tocotrienols, and tocopherols. The scientists demonstrated that the dehulling process also yielded oil-rich small kernel fragments and larger starch-rich fragments for fuel ethanol production. The ability to produce nutraceutical-rich barley oil as a co-product will improve the profitability of a barley-to-ethanol biorefinery.
- Another hurdle in commercial production of fuel ethanol from barley is the high content of β-glucans in the barley grains. During the mashing process these polysaccharides are solubilized, causing the viscosity of the mash to increase to levels that prevent the adequate mixing needed to properly distribute yeast and the required enzymes and nutrients. The ARS scientists, together with private industrial stakeholders, developed an enzymatic treatment and fermentation process, which solves the traditional technical problems with ethanol production from barley. The process uses β-glucosidase, in combination with β-glucanases, to further reduce viscosity and create more fermentable glucose, resulting in higher ethanol yields. A techno-economic study of this process is described under Subcomponent 3D. Influenced by this work, a private ethanol producer constructed a 65 million gallon per year barley ethanol facility in Hopewell, Virginia.

Nghiem, N.P., Taylor, F., Johnston, D.B., Shetty, J.K., and Hicks, K.B. 2011. Scale-up of ethanol production from winter barley by the EDGE (enhanced dry grind enzymatic) process in fermentors up to 300 l. Applied Biochemistry and Biotechnology 165(3-4), 870-882.

Nghiem, N.P., Hicks, K.B., Johnston, D.B., Senske, G., Kurantz, M., Li, M., and Konieczny-Janda, G. 2010. Production of ethanol from winter barley by the EDGE (enhanced dry grind enzymatic) process. Biotechnology for Biofuels 3:8.

PROBLEM STATEMENT 3A-4: *Biorefinery co-products.*

Fate of antibiotics used in ethanol production. Antibiotics are used to control bacterial contamination at commercial fuel ethanol facilities in order to promote the growth of healthy and beneficial yeasts to rapidly ferment materials to desired products, but the fate of these drugs had not been documented. A significant concern has been whether there are antibiotic residues in dried distillers grains (DDG) that are used for livestock feed. ARS scientists in Peoria, Illinois, in collaboration with the National Corn-to-Ethanol Research Center in St. Louis, Missouri, measured the activity of a common antibiotic, virginiamycin, used by ethanol producers in ethanol process streams. The trial included three runs: one control run with no virginiamycin; one run with virginiamycin dosed within the FDA approved range (2 ppm); and one run with virginiamycin dosed 10 times above the FDA approved range (20 ppm). The scientists collected a total of 54 samples, including process intermediates, such as whole stillage, wet cake, thin stillage, syrup, and dried distillers grains with solubles (DDGS), for analysis of antibiotic residues. Low antimicrobial activity was observed in fermentation samples and DDG and DDGS samples from the run with 2 ppm virginiamycin dosage, with the activity lower than the equivalent of 1 ppm virginiamycin. However, antimicrobial activity was observed in every sample collected from the run with 20 ppm virginiamycin dosage, with the highest activity observed in DDGS samples near the equivalent of 9 ppm virginiamycin. Using feeding trials, the scientists also determined that although biologically active antibiotics can persist in distillers grains derived from fermentors treated with virginiamycin, the antibiotic is not biologically active the livestock feed.

Bischoff, K.M., Rich, J.O., and Zhang, Y. 2012. Determining the fate of virginiamycin in the fuel ethanol production process [abstract]. Fuel Ethanol Workshop Track 3. Paper 1. p. 23.

Using ethanol to lower energy costs for food refining processors. ARS researchers in Albany, California, found that ethanol can be used to dehydrate food processing or refining products, such as wheat gluten, resulting in up to 60 percent lower capital cost and energy consumption compared to standard industry practices such as fluidized bed, flash, or rotary drying. The researchers developed general criteria using best-case assumptions for this replacement, and determined that the techniques could be employed in a system that combined low-load solvent drying with distillation. Distillation energy for regenerating ethanol solvent to 90 percent or less and producing water more efficiently was equivalent to a series of 3 to 4 thin-film evaporators when compared on a separation-energy per unit-of-water-removed basis. The study considered total system costs, including capital costs for ethanol recovery (via distillation), and also showed that dehydrating with ethanol can improve product quality.

Robertson, G.H., Offeman, R.D., Cao, T.K., and Orts, W.J. 2011. Ethanol in biorefining and dehydration of agricultural materials: Energy, capital cost, and product quality implications. Biofuels, Bioproducts and Biorefining 5(1), 37-53.

Improved dry distillers grains with solubles flowability alleviates processing/handling problem. Most of the DDGS produced by corn ethanol biorefineries are transported via rail to livestock feeding operations outside the Corn Belt. At least 10 percent of these shipments (more than 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 in Wyndmoor, Pennsylvania, in collaboration with researchers at University of Illinois and University of Missouri, investigated the underlying mechanisms associated with DDGS caking and determined that flowability worsened with increased 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. Caking can be minimized during transport by ensuring lower initial moisture content. ARS scientists in Brookings, South Dakota, had previously developed a process for pelleting DDGS on a commercial scale. Utilizing traditional feed milling equipment, high quality pellets were produced without the use of binders and without affecting nutrient composition. Pelleting significantly increased the bulk density of DDGS and decreased the angle of repose, thereby increasing DDGS flowability. The researchers then extended the research by conducting a transportation logistics study and found that in addition to increasing the value of DDGS to the industry, pelleting could expand DDGS use into rangeland settings. These findings have helped corn biorefiners choose DDGS storage and transportation practices to avoid flowability problems and potentially reduce transportation costs, especially for plants that ship their DDGS via rail, by utilizing carrier capacity more efficiently. A private company is currently marketing pelletized DDGS.

Belyea, R.L., Rausch, K.D., Clevenger, T.E., Singh, V., Johnston, D.B., and Tumbleson, M.E. 2010. Sources of variation in composition of DDGS. Animal Feed Science and Technology 159(3-4), 122-130.

Wood, C.R. and Rosentrater, K.A. 2010. Granular packing influences the bulk density of DDGS. Cereal Chemistry 87(16):586-596.

Rosentrater, K.A. and Kongar, E. 2009. Modeling the effects of pelleting on the logistics of distillers grains shipping. Bioresource Technology 100:6550-6558.

SUBCOMPONENT 3B: Thermochemical Conversion

In this Subcomponent, ARS researchers specifically focused on thermochemical means of generating pyrolysis oils or syngas from a variety of feedstocks, as well as developing methods of utilizing this technology for adding value to agricultural byproducts.

PROBLEM STATEMENT 3B-1: *Managing biomass feedstocks for thermochemical processing*.

Protein-rich press cake produces more stable pyrolysis oils. Biodiesel is often produced from non-food oil crops such as camelina, jatropha, and pennycress. Some crops, such as pennycress, can produce yields in the range of 2,200 pounds of seed per acre within a conventional soybean/corn rotation and do not displace a food crop or require additional land. Unlike press cakes from food-based feedstocks, such as soy and palm fruits, the press cakes of biodiesel crops are often not suitable for consumption as animal feed. However, oilseed press cakes make an ideal thermochemical conversion feedstock because of their inherently high initial calorific value. ARS researchers in Wyndmoor, Pennsylvania, with support from an industrial partner, showed that when protein-rich oilseed press cake is used as a feedstock, overall pyrolysis oil yield remains unchanged, but more oxygen is released as water and fewer acids are produced than with typical low-protein feedstocks. Consequently, the resulting pyrolysis oil is less acidic, less corrosive, and has greater energy content. This offers significant advantages over pyrolysis oil derived from purely lignocellulosic biomass. Owing to the fact that stability is a major barrier to commercialization of pyrolysis liquids as second-generation biofuels, the results are encouraging, with the potential to use some press cakes not suitable for use as animal feed to produce advanced biofuels, such as jet-fuel additives. A patent has been filed pertaining to this research and follow-on work indicates that this effect is generally true of pyrolysis oils from other high-protein feedstocks.

Boateng, A.A., Mullen, C.A., and Goldberg, N.M. 2010. Producing stable pyrolysis liquids from the oil-seed press cakes of mustard family plants: Pennycress (*Thlaspi arvense* L.) and camelina (*Camelina sativa*). Energy and Fuels 24(12), 6624-6632.

PROBLEM STATEMENT 3B-2: *On-farm production of heat and/or power.*

***Improved composition of pyrolysis oils by catalytic pyrolysis utilizing a fluidized-bed system*.** Fast pyrolysis is a process that could potentially enable on- or near-farm production of fuel intermediates. Fast pyrolysis oils are not chemically and thermally as stable as conventional petroleum fuels due to their high content of reactive oxygen-containing compounds and low boiling volatiles. ARS researchers in Wyndmoor, Pennsylvania, in partnership with industry and with support from the Department of Energy, investigated production of partially deoxygenated pyrolysis liquids through incorporation of catalysts in the pyrolysis process. The researchers utilized a 5 kg/h bubbling fluidized bed pyrolysis system to study *in situ* catalytic upgrading at different points in the process from pyrolytic decomposition of the biomass to condensation of pyrolysis liquids. They found that by locating the upgrading step at different points during condensation, the composition of the vapor stream changed through removal of various components, including water and small, highly oxygenated water-soluble components. One tested catalyst lowered the acetic acid yield by 70 percent and generated a product stream much richer in aromatics such as benzene, toluene, xylenes, and naphthalenes. The scientists found that selectivity for single aromatic ring compounds (benzenes) versus two aromatic ring compounds (naphthalenes) increased as the vapor stream became drier. Several patents have been filed pertaining to this work. Further work (including research under a Biomass Research and Development Initiative grant involving multiple partners with ARS as the lead agency) is underway to improve the process, which has the potential to drastically reduce transportation costs associated with feedstock collection, to take this work from development to a demonstration scale of two-ton per day capacity.

Compton, D.L., Jackson, M.A., Mihalcik, D.J., Mullen, C.A., and Boateng, A.A. 2011. Catalytic pyrolysis of oak via pyroprobe and bench scale, packed bed pyrolysis reactors. Journal of Analytical and Applied Pyrolysis 90(2), 174-181.

Mullen, C.A., Boateng, A.A., Mihalcik, D.J., and Goldberg, N.M. 2011. Catalytic fast pyrolysis of white oak wood in a bubbling fluidized bed. Energy and Fuels 25(11), 5444-5451.

PROBLEM STATEMENT 3B-3: *Commercially viable thermochemical processes for producing liquid fuels from agricultural feedstocks.*

Pyrolysis economics. Unlike other biomass conversion technologies, pyrolysis may enable biorefining processes on or near the farm at a relatively small scale, thereby minimizing the costs associated with transporting large quantities of low-density biomass. By developing and using an ASPEN+ process cost and simulation model for biomass fast-pyrolysis, ARS researchers in Wyndmoor, Pennsylvania, determined that a 200-ton biomass per day plant is the smallest size that would be cost-competitive with $85 a barrel of petroleum. This work has since spurred development of a more comprehensive model in collaboration with researchers at the University of Maine and is also being extended for utilization of equine waste for local hot water heating via fast-pyrolysis. The work has provided more accurate decision tools to energy producers considering using fast-pyrolysis for small and medium-scale applications.

Taylor, F., Mullen, C., McAloon, A., and Boateng, A.A. 2008. A simplified approach to aspen-plus© mass and energy balances for preliminary process analysis and economic feasibility of pyrolysis of switchgrass to bio-oil. Paper presented at the AIChE 100 - 2008 AIChE Annual Meeting, Conference Proceedings.

PROBLEM STATEMENT 3B-4: *Biorefinery co-products.*

Phosphate-rich biochar from poultry litter. Together, Mississippi, Alabama, and Georgia produce more than one-third of the broiler chickens in the United States. While poultry waste has a traditional value as fertilizer, amended soils can become too saturated with manure, and the nitrogen and phosphorus in the waste can leach into nearby rivers, streams, and groundwater supplies. County Resource Conservation and Development Councils from these states are very concerned about overabundant and burdensome poultry litter from these farming operations, and pyrolysis of poultry litter represents a potential solution to this problem. ARS scientists in New Orleans, Louisiana, and Wyndmoor, Pennsylvania, have found that poultry char is perfectly suited for use in wastewater treatment systems, for scavenging metals discharged by industrial activities, and for absorbing excess fertilizer nutrients from farming operations. Improving soil fertility and sequestering carbon through soil amendment with poultry char is one possible use, but data were not available on the bioavailability of phosphorous from charred manure. The ARS scientists demonstrated that poultry litter chars used as soil amendments did in fact release a significant portion of their phosphorous. Thus charred manure can be used to sequester carbon while also acting as a source of phosphorous to plants. However, unexpectedly, the scientists found that biochar derived from poultry litter was toxic to earthworms, whereas earthworms were unaffected by biochar made from pine chips. Since a healthy earthworm population is a key indicator of productive soils, these results demonstrate that biochars produced from some materials can have detrimental impacts on soil quality, at least in the short-term. This work also illustrates the complexity of evaluating the ecological impact of using char for carbon sequestration or as a soil amendment. Variation in soil chemistry and char composition create uncertainty about long-term affects on soil fertility. The scientists have entered into a cooperative agreement with a poultry producer to resolve the remaining technical barriers to greater utilization of poultry litter biochar.

Spokas, K.A., Cantrell, K.B., Novak, J.M., Archer, D.W., Ippolito, J.A., Collins, H.P., and Nichols, K.A. 2012. Biochar: A synthesis of its agronomic impact beyond carbon sequestration. Journal of Environmental Quality 41(4), 973-989.

Lima, I.M., Boateng, A.A., and Klasson, K.T. 2010. Physicochemical and adsorptive properties of fast-pyrolysis bio-chars and their steam activated counterparts. Journal of Chemical Technology and Biotechnology 85(11), 1515-1521.

Lima, I.M., Boateng, A.A., and Klasson, K.T. 2009. Pyrolysis of broiler manure: Char and product gas characterization. Industrial and Engineering Chemistry Research 48(3), 1292-1297.

Lima, I.M., McAloon, A., and Boateng, A.A. 2008. Activated carbon from broiler litter: Process description and cost of production. Biomass and Bioenergy 32(6), 568-572.

SUBCOMPONENT 3C: Biodiesel

In this Subcomponent, ARS researchers specifically focused on identification and characterization of new feedstocks for biodiesel production and ways of improving biodiesel's cold-flow properties and stability. Research under this subcomponent also identified new uses for byproduct glycerol and press cake from biodiesel production and improved significantly on existing methods of biodiesel production by obtaining high rates of transesterification from minimally processed raw materials.

PROBLEM STATEMENT 3C-1: *Increasing feedstock (fatty acid) availability.*

A new salt-tolerant biodiesel feedstock. Seashore mallow (*Kosteletzkya pentacarpos*) is a non-invasive perennial halophytic (salt tolerant) oilseed-producing dicot. ARS scientists in Peoria, Illinois, with cooperators from the University of Delaware, investigated the Seashore mallow's potential as a feedstock for production of both biodiesel from its seeds and ethanol from the residual stem biomass. Their research demonstrated that seashore mallow seed mass contained 19.3 percent oil with maximum calculated oil yields of about 295 pounds an acre; and that after extraction, this oil can be efficiently converted into mixed methyl esters of methyl linoleate, palmitate, and oleate. The scientists characterized the fuel properties and compared them to biodiesel standards ASTM D6751 and EN 14214, which they met. As part of the research, the scientists also demonstrated that seashore mallow stems were rich in neutral carbohydrates. After simultaneous saccharification and fermentation employing a native *Saccharomyces cerevisiae* yeast strain, the stems provided very good ethanol and xylose yields. These results, together with Seashore mallow's compatibility with existing farm infrastructure, relative stress tolerance, and because it can be cultivated on saline or dry land that can be irrigated with brackish or seawater demonstrate its potential to increase biodiesel feedstock availability utilizing fallow land resources while liberating fresh water and high quality soil for traditional agriculture use. The scientists are now studying the use of residual biomass for hydromulch, animal litter, or bioabsorbent purposes. They are also performing ongoing fatty acid content analysis and further characterization of 20 diverse Seashore mallow lines for selection of improved materials for further research.

Moser, B.R., Dien, B.S., Seliskar, D.M., and Gallagher, J.L. 2013. Seashore mallow (*Kosteletzkya pentacarpos*) as a salt-tolerant feedstock for production of biodiesel and ethanol. Renewable Energy 50, 833-839.

PROBLEM STATEMENT 3C-2: *Biodiesel production processes.*

Biodiesel without the messy oil. To produce biodiesel from vegetable oils and animal fats, it is currently necessary to isolate and purify these lipids from the agricultural materials in which they are biosynthesized. The technologies for doing so also contribute to feedstock cost and involve the use of chemicals that are not legally permitted in all locations. To avoid these difficulties, ARS researchers in Wyndmoor, Pennsylvania, developed an alternate method of biodiesel production, termed '*in situ* transesterification.' In this approach, the feedstock lipid is directly converted to biodiesel while still residing in the raw agricultural material in which it is naturally found. Since 2008, the ARS team has further characterized and optimized the *in situ* transesterification reaction, showing the power of this approach for biodiesel production from a wide variety feedstocks. In addition, the researchers identified simple pretreatments that increased the efficiency of the reaction, examined alternate methods for its conduct, and collaborated with industrial and academic partners to successfully transesterify a ton of soybeans. The low-fat meal co-product of the latter process was then shown to be an acceptable protein source in diets for trout and poultry. This work established that *in situ* transesterification can be conducted on virtually any lipid-bearing material, at any scale, and in any location. This accomplishment has influenced a number of groups outside of the agency that have subsequently demonstrated the approach successfully with algae, peanuts, cottonseed meal, oil palm pulp, Jatropha, municipal sewage sludge, and corn.

Haas, M.J. and Wagner, K.M. 2011. Simplifying biodiesel production: the direct or *in situ* transesterification of algal biomass. Eur. J. Lipid Sci and Tech 113: 1219-1229.

Haas, M.J. and Wagner, K.M. 2011. Substrate pretreatment can reduce the alcohol requirement during biodiesel production via in situ transesterification. JAOCS, Journal of the American Oil Chemists' Society 88(8), 1203-1209.

Wyatt, V.T. and Haas, M.J. 2009. Production of fatty acid methyl esters via the in situ transesterification of soybean oil in carbon dioxide-expanded methanol. JAOCS, Journal of the American Oil Chemists' Society 86(10), 1009-1016.

PROBLEM STATEMENT 3C-3: *Improving the inherent performance of fatty acid esters as fuels.*

High oleic acid soybeans. Biodiesel fuel produced from soybean oil with high levels of oleic acid has significantly better cold-flow properties and higher oxidative and temperature stability than biodiesel produced from standard soybeans. ARS scientists in Columbia, Missouri, identified and combined mutant alleles of two soybean fatty acid-modifying genes, resulting in beans with high oleic acid content. The researchers also developed molecular markers for these genes, thereby facilitating the breeding of soybean varieties containing this valuable trait. These two alleles are currently being introgressed into elite germplasm for commercial release. *[National Program 301]*

Pham, A., Shannon, G.J., and Bilyeu, K.D. 2012. Combinations of mutant FAD2 and FAD3 genes to produce high oleic acid and low linolenic acid soybean oil. Theoretical and Applied Genetics 125:503-515.

Improving cold-flow performance of biodiesel. Biodiesel typically thickens in cold temperatures, a phenomenon that can lower engine performance and may even clog engine fuel filters. ARS scientists in Peoria, Illinois, determined that adding low levels (2.5 percent) of ethyl levulinate, a bio-based material, improves cold flow performance of biodiesel fuel derived from cottonseed and poultry wastes. Unlike fatty acid methyl esters, ethyl levulinate, with a larger head group and significantly different hydrocarbon tail disrupt the formation of crystal nuclei. The scientists also determined that enriching the vegetable oil feedstock with certain types of fatty acids (e.g., decanoic acid) results in biodiesel fuels with better cold flow properties. Together this knowledge will be useful to crop breeders attempting to produce oil crop varieties better suited for biodiesel production, and it will also promote the enhanced utilization of otherwise satisfactory biodiesel with poor cold-flow properties.

Joshi, H., Moser, B.R., and Walker, T. 2012. Mixed alkyl esters from cottonseed oil: Improved biodiesel properties and blends with diesel fuel. Journal of the American Oil Chemists' Society 89(1), 145-153.

Joshi, H., Moser, B.R., Toler, J., Smith, W.F., and Walker, T. 2011. Ethyl levulinate: A potential bio-based diluent for biodiesel which improves cold flow properties. Biomass and Bioenergy 35(7), 3262-3266.

PROBLEM STATEMENT 3C-4: *Biodiesel fuel quality assurance.*

Biodiesel from inexpensive grease. Most biodiesel is produced from refined vegetable oils. However, the high cost of these oils can make biodiesel production unprofitable. Greases are lower-cost feedstocks, but their high, free fatty acid content makes them difficult to use in a conventional biodiesel plant. Although approaches have been reported for the conversion of trap greases to biodiesel, achieving this conversion in an economically affordable manner is a challenge that has prevented general usage of trap greases for biodiesel production to date. In collaboration with university and industrial partners, ARS researchers in Wyndmoor, Pennsylvania, developed immobilized acid catalysts, such as diarylammonium salts, that are highly efficient in esterifying free fatty acids to biodiesel. The researchers demonstrated that greater than 90 percent weight of free fatty acids and less than 10 percent weight of acylglycerols can be converted to fatty acid methy esters at 257°F in 1 hour in a pressurized vessel. The product of the process came very close to meeting ASTM D6751 specifications, the accepted standard for biodiesel quality. Further optimization of the use of the new catalysts studied may lead to acceptably high degrees of esterification of remaining residual free fatty acids. This novel technology could enable economical production of biodiesel fuels from more types of greases and other inexpensive, second-use fats and oils.

Ngo, H.L., Xie, Z., Kasprzyk, S., Haas, M., and Lin, W. 2011. Catalytic synthesis of fatty acid methyl esters from extremely low quality greases. Journal of the American Oil Chemists' Society 88(9), 1417-1424.

Ngo, H.L., Zafiropoulos, N.A., Foglia, T.A., Samulski, E.T., and Lin, W. 2008. Efficient two-step synthesis of biodiesel from greases. Energy and Fuels 22(1), 626-634.

PROBLEM STATEMENT 3C-5: *Biodiesel co-products.*

Glycerol-based plastics. Glycerol is the major co-product produced from the process used to make biodiesel and continues to drop in price as biodiesel volumes increase and traditional markets for glycerol become highly saturated. To create new, value-added markets for glycerol, ARS scientists in Wyndmoor, Pennsylvania, developed a variety of polyesters by reacting glycerol with adipic, azelaic, suberic, and sebacic acids in a Lewis-acid catalyzed synthesis. The resulting highly branched polymers are biodegradable and can be used as weed barriers and for controlled-release of fertilizers or pesticides. Glycerol polyesters could also replace common petrochemical polymers such as polyethylene or polypropylene. Such biocompatible polymers may also be of use in cosmetics, and as food additives, surfactants, lubricants, and azeotrophic phase separators and for medical applications. The glycerol-based polyesters have been used for synthesis of new composite films by combination with plant cell wall-derived polysaccharides. The composites' viscoelastic properties are currently being characterized. The polyesters have also been transferred to a comprehensive applications screening program with a private company for potential use in the cosmetics industry.

Wyatt, V.T. and Strahan, G.D. 2012. Degree of branching in hyperbranched poly(glycerol-co-diacid)s synthesized in toluene. Polymers 4(1), 396-407.

Wyatt, V., Strahan, G.D., Nunez, A., and Haas, M.J. 2011. Characterization of thermal and mechanical properties of hyperbranched oligo(glycerol-glutaric acids). Journal of Biobased Materials and Bioenergy 5(1), 92-101.

Wyatt, V.T., Strahan, G.D., and Nuñez, A. 2010. The lewis acid-catalyzed synthesis of hyperbranched oligo(glycerol-diacid)s in aprotic polar media. Journal of the American Oil Chemists' Society 87(11), 1359-1369.

Biodiesel co-product for aquaculture feed. The cost of fish meal to meet the nutritional needs of farm raised fish has risen significantly, creating a need for economical alternatives to fish meal. ARS researchers in Wyndmoor, Pennsylvania, have previously demonstrated that it is possible to produce fatty acid esters by the direct exposure of soybeans to alkaline methanol (*in-situ* transesterification —see previous accomplishment). However, for the *in-situ* process to be commercially viable, economical uses must be found for the lipid-free meal co-product left after the *in-situ* reaction. Testing by ARS scientists in Aberdeen, Idaho, showed that the spent meal is suitable as a feed ration in aquaculture and as a replacement for fish meal. Encouraging results with this approach have resulted in new collaborations with researchers from Ohio State University and from the private sector, and the aquaculture results are being extended to feeding studies in poultry, as well as chemical analysis of the transesterified meal.

Barrows, F., Gaylord, T.G., Sealey, W.M., Haas, M.J., and Stroup, R.L. 2008. Processing soybean meal for biodiesel production; effect of a new processing method on growth performance of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 283:143-147.

SUBCOMPONENT 3D: Process Economics and Life Cycle Analyses

In this Subcomponent, ARS researchers specifically focused on looking at the effects of technology on economic and environmental factors. These studies have helped to quantify the impact of technology from a variety of dimensions, including economic and energy-based analyses.

PROBLEM STATEMENT 3D-1: *Estimating process costs and externalized costs.*

Process cost model for fuel ethanol production from winter barley. The advances to barley biorefining using new cultivars, fractionation processes, and the enhanced dry grind enzymatic process (EDGE) described earlier for barley based ethanol production require economic analysis to ensure they are cost-effective for producers. ARS scientists in Wyndmoor, Pennsylvania, analyzed the EDGE process to determine if use of additional enzymes would bring a net positive economic benefit. The researchers determined in all, but one scenario they examined, that the benefits were clearly demonstrated of obtaining higher ethanol yields through decreased viscosity resulting from addition of β-glucosidase. Values of the additional ethanol produced were shown to outweigh the increases in operating costs because of the enzymes. Utilizing this new process could help existing and future barley-to-ethanol plants to increase efficiency.

Nghiem, N.P., Ramírez, E.C., McAloon, A.J., Yee, W., Johnston, D.B., and Hicks, K.B. 2011. Economic analysis of fuel ethanol production from winter hulled barley by the EDGE (enhanced dry grind enzymatic) process. Bioresource Technology 102(12), 6696-6701.

Understanding the reduction in cost of U.S. corn ethanol production. Using experience-curve analyses, ARS researchers in Wyndmoor, Pennsylvania, showed that cumulative dry grind ethanol production has doubled seven times since 1983. Over the same time, costs have been reduced by 45 percent on average. This indicates that ethanol processing costs decline 13 percent per doubling in cumulative production. The calculations on the experience curve present a progress ratio of 0.55 for corn production costs over the period 1975–2005. A progress ratio of 0.87 is calculated for industrial processing costs (without costs for corn and capital) since 1983. A comparison with Brazilian sugarcane and ethanol production reveal similar progress ratios for feedstock and industrial processing cost reductions. This work has provided corn ethanol producers the ability to project future production costs and the results have been used by economists and academics to model competition scenarios between alternative renewable fuels.

Hettinga, W.G., Junginger, H.M., Dekker, S.C., Hoogwijk, M., McAloon, A.J., and Hicks, K.B. 2009. Understanding the reductions in U.S. corn ethanol production costs: An experience curve approach. Energy Policy 37(1) 190-203.

Biodiesel provides a five-to-one return on fossil fuel energy inputs. The value of different biofuels can be assessed from their total life-cycle net energy return. For instance, the fossil energy replacement ratio (which is the total energy output/fossil energy input) for corn ethanol is estimated to be 1.34, while that of petroleum-based fuels is about 0.9. ARS researchers in Wyndmoor, Pennsylvania, along with other USDA and university collaborators, completed a life-cycle analysis of soybean biodiesel production and showed that the fossil energy replacement ratio was 5.5. In other words, for every BTU of fossil energy used to produce biodiesel, 5.5 BTU of biodiesel are produced. A previous assessment based largely on pre-1990 data estimated a net ratio of 3.2. The new study also determined that the higher biodiesel energy return was due to improvements in soybean crushing facilities and biodiesel refinery facilities; soybean farmers adopting energy-saving farm practices such as reduced tillage; and improved soybean yields. This return is likely to improve over time as new process efficiencies are adopted and soybean yields increase.

Pradhan, A., Shrestha, D.S., McAloon, A., Yee, W., Haas, M., and Duffield, J.A. 2011. Energy life-cycle assessment of soybean biodiesel revisited. Transactions of the ASABE 54(3), 1031-1039.

APPENDIX 1

National Program 213 – Bioenergy

ACCOMPLISHMENT REPORT 2008-2012

Research Projects in NP 213

1935-41000-082-00D
Distributed-scale pyrolysis of agricultural biomass for production of refinable crude bio-oil and valuable coproducts; Akwasi Boateng (P), Kevin Hicks, and Neil Goldberg; Wyndmoor, Pennsylvania.

1935-41000-083-00D
From barley to biomass - the development of a regional multi-feedstock biorefinery; Kevin Hicks (P), Madhav Yadav, Nhuan Nghiem, Andrew McAloon, and Robert Moreau; Wyndmoor, Pennsylvania.

1935-41000-084-00D
Expanding the use of fats and oils as replacements for fossil-derived fuels, lubricants, and polymers; Michael Haas (P), Kevin Hicks, Helen Ngo, Victor Wyatt, and Jonathan Zerkowski; Wyndmoor, Pennsylvania.

1935-41000-085-00D
Value added coproducts for improving the economics and greenhouse gas emissions of corn and cellulosic fuel ethanol production; David Johnston (P), Madhav Yadav, Kevin Hicks, Robert Moreau, Andrew McAloon, and Nhuan Nghiem; Wyndmoor, Pennsylvania.

3620-41000-133-00D
Advanced conversion technologies for sugars and biofuels: superior feedstocks, pretreatments, inhibitor removal, and enzymes; Bruce Dien (P), Michael Bowman, Nancy Nichols, Jeffrey Mertens, Michael Cotta, and Douglas Jordan; Peoria, Illinois.

3620-41000-135-00D
Improving biochemical processes for the production of sustainable fuels and chemicals; Kenneth Bischoff (P), Timothy Leathers, Joseph Rich, Siqing Liu, and Stephen Hughes; Peoria, Illinois.

3620-41000-147-00D
Genomics and engineering of stress tolerant microbes for lower cost production of ethanol from lignocelluloses; Patricia Slininger (P) and Zonglin Liu; Peoria, Illinois.

3620-41000-148-00D
Vegetable oil-based fuels, additives and coproducts; Gerhard Knothe (P), Rex Murray, Robert Dunn, and Bryan Moser; Peoria, Illinois.

* Projects are listed and organized in Appendixes 1 and 2 according to the ARS project number used to track projects in the Agency's internal database. A (P) after a scientist's name indicates the project's principal investigator.

3620-41000-149-00D
Process technologies for producing biofuels and coproducts from lignocellulosic feedstocks; Badal Saha (P), Michael Cotta, Jeffrey Mertens, Nancy Nichols, Nasib Qureshi, and Ronald Hector; Peoria, Illinois.

3655-41000-006-00D
Adding value to biofuels production systems based on perennial forages; Paul Weimer (P), Matthew Digman, Michael Sullivan, Ronald Hatfield, and Michael Casler; Madison, Wisconsin.

5325-21000-017-00D
Genetic foundations for bioenergy feedstocks; Olin Anderson (P), Yong Qiang Gu, Christian Tobias, and John Vogel; Albany, California.

5325-41000-049-00D
Biorefining processes; William Orts (P), Delilah Wood, Kevin Holtman, Kurt Wagschal, Richard Offeman, Charles Lee, and Dominic Wong; Albany, California.

6209-13610-007-00D
Production of quality power and/or heat for on-farm operations; Brian Vick (P); Bushland, Texas.

6435-41000-089-00D
Thermochemical processing of agricultural wastes to value-added products and bioenergy; Thomas Klasson (P), Sophie Uchimiya, and Isabel Lima; New Orleans, Louisiana.

APPENDIX 2

National Program 213 – Bioenergy

ACCOMPLISHMENT REPORT 2008-2012

Publications by Research Projects

1935-41000-082-00D
Distributed-scale pyrolysis of agricultural biomass for production of refinable crude bio-oil and valuable coproducts; Akwasi Boateng (P), Kevin Hicks, and Neil Goldberg; Wyndmoor, Pennsylvania.

Boateng, A.A. and Mtui, P.L. 2012. CFD modeling of space-time evolution of fast pyrolysis products in a bench-scale fluidized-bed reactor. Applied Thermal Engineering 33-34:p.190-198.

Boateng, A.A., Mullen, C.A., Osgood-Jacobs, L., Carlson, P., and Macken, N. 2012. Mass balance, energy and exergy analysis of bio-oil production by fast pyrolysis. Journal of Energy Resources Technology 134/042001-1-9.

Dickey, L.C., Boateng, A.A., Goldberg, N.M., Mullen, C.A., and Mihalcik, D.J. 2012. Condensation of acetol and acetic acid vapor with sprayed liquid. Industrial and Engineering Chemistry Research 51:5067-5072.

Wise, J., Vietor, D., Provin, T., Capareda, S., Munster, C., and Boateng, A.A. 2012. Mineral nutrient recovery from pyrolysis systems. Journal of Environmental Progress and Sustainable Energy 31(2):251-255.

Mihalcik, D.J., Mullen, C.A., and Boateng, A.A. 2011. Screening acidic zeolites for catalytic fast pyrolysis of biomass and its components. Journal of Analytical and Applied Pyrolysis 92:224-232.

Mihalcik, D.J., Boateng, A.A., Mullen, C.A., and Goldberg, N.M. 2011. Packed-bed catalytic cracking of oak derived pyrolytic vapors. Industrial and Engineering Chemistry Research 50:13304-13312.

Mullen, C.A., Boateng, A.A., Mihalcik, D.J., and Goldberg, N.M. 2011. Catalytic fast pyrolysis of white oak wood in-situ using a bubbling fluidized bed reactor. Energy and Fuels 25:5444-5451.

Mullen, C.A. and Boateng, A.A. 2011. Production and analysis of fast pyrolysis oils from proteinaceous biomass. BioEnergy Research 4;303-311.

Mullen, C.A. and Boateng, A.A. 2011. Characterization of water insoluble solids isolated from various biomass fast pyrolysis oils. Journal of Analytical and Applied Pyrolysis 90:197-203.

Strahan, G.D., Mullen, C.A., and Boateng, A.A. 2011. Characterizing biomass fast pyrolysis oils by 13C-NMR and chemometric analysis. Energy and Fuels 25:5452-5461.

Boateng, A.A., Mullen, C.A., and Goldberg, N.M. 2010. Producing stable pyrolysis liquids from the oil-seed presscakes of mustard family plants: pennycress (*Thlaspi arvense L*.) and camelina (*Camelina sativa*). Energy and Fuels 24:6624-6632.

Boateng, A.A., Mullen, C.A., McMahan, C.M., Whalen, M.C., and Cornish, K. 2010. Guayule (*Parthenium argentatum*)pyrolysis and analysis by PY-GC/MS. Journal of Analytical and Applied Pyrolysis 87:14-23.

Mullen, C.A. and Boateng, A.A. 2010. Catalytic pyrolysis-GC/MS of lignin from several sources. Fuel Processing Technology 91:1446-1458.

* Projects are listed and organized in Appendixes 1 and 2 according to the ARS project number used to track projects in the Agency's internal database. A (P) after a scientist's name indicates the project's principal investigator.

Mullen, C.A., Boateng, A.A., Hicks, K.B., Goldberg, N.M., and Moreau, R.A. 2010. Analysis and comparison of bio-oil produced by fast pyrolysis from three barley biomass/byproduct streams. Energy and Fuels 24:699-706.

Boateng, A.A., Mullen, C.A., Goldberg, N.M., Hicks, K.B., McMahan, C.M., Whalen, M.C., and Cornish, K. 2009. Energy-dense liquid fuel intermediates by pyrolysis of guayule (*Parthenium argentatum*) shrub and bagasse. Fuel 88:2207-2215.

Boateng, A.A. 2009. Response of the devolatilization process to the lignin concentration in alfalfa stems. Energy and Fuels 23:2316-2318.

Mullen, C.A., Strahan, G.D., and Boateng, A.A. 2009. Characterization of various fast pyrolysis bio-oils by NMR spectroscopy. Energy and Fuels 23:2707-2718.

Boateng, A.A., Weimer, P.J., Jung, H.G., and Lamb, J.F. 2008. Response of thermochemical and biochemical conversion processes to lignin concentration in alfalfa stems. Energy and Fuels 22:2810-2815.

Boateng, A.A., Mullen, C.A., Goldberg, N.M., Hicks, K.B., Jung, H.G., and Lamb, J.F. 2008. Production of bio-oil from alfalfa stems by fluidized-bed fast pyrolysis. Industrial and Engineering Chemistry Research 47:4115-4122.

Mullen, C.A. and Boateng, A.A. 2008. Chemical composition of bio-oils produced by fast pyrolysis of two energy crops. Energy and Fuels 22:2104-2109.

1935-41000-083-00D

From barley to biomass - the development of a regional multi-feedstock biorefinery; Kevin Hicks (P), Madhav Yadav, Nhuan Nghiem, Andrew McAloon, and Robert Moreau; Wyndmoor, Pennsylvania.

Holt, M.D., Moreau, R.A., Dermarderosian, A., McKeown, N., and Jacques, P.F. 2012. Accelerated solvent extraction of alkylresorcinols in food products containing uncooked and cooked wheat. Journal of Agricultural and Food Chemistry 60:4799-4802.

Moreau, R.A., Bregitzer, P.P., Liu, K., and Hicks, K.B. 2012. Compositional equivalence of barleys differing only in low and normal phytate levels. Journal of Agricultural and Food Chemistry 60:6493-6498.

Srinivasan, R., Hicks, K.B., Wilson, J., and Challa, R.K. 2012. Effect of barley roller milling on fractionation of flour using sieving and air classification. Applied Engineering in Agriculture 28(2):225-230.

Yoo, C., Nghiem, N.P., Hicks, K.B., and Kim, T. 2011. Pretreatment of corn stover using low-moisture anhydrous ammonia (LMAA) process. Bioresource Technology 102:10028-10034.

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Advanced conversion technologies for sugars and biofuels: superior feedstocks, pretreatments, inhibitor removal, and enzymes; Bruce Dien (P), Michael Bowman, Nancy Nichols, Jeffrey Mertens, Michael Cotta, and Douglas Jordan; Peoria, Illinois.

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Improving biochemical processes for the production of sustainable fuels and chemicals; Kenneth Bischoff (P), Timothy Leathers, Joseph Rich, Siqing Liu, and Stephen Hughes; Peoria, Illinois.

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APPENDIX 3

National Program 213 – Bioenergy
ACCOMPLISHMENT REPORT 2007 – 2012

Selected Supporting Information and Documentation for Accomplishments and Impact of NP 213 Research

RELATIONSHIP OF THIS NATIONAL PROGRAM TO THE ARS STRATEGIC PLAN

Outputs of NP 213 research support the actionable strategies associated with the performance measures shown below from the *ARS Strategic Plan for 2006-2011,* Strategic Goal 2: Enhance the Competitiveness and Sustainability of Rural and Farm Economies; Objective 2.1: *Expand domestic market opportunities*.
(www.ars.usda.gov/SP2UserFiles/Place/00000000/ARSStrategicPlan2006-2011.pdf)

One performance measure sets the target for NP 213 research within the Strategic Plan:

Performance Measure 2.1.1: Create new scientific knowledge and innovative technologies that represent scientific/technological advancements or breakthroughs applicable to bioenergy.

> **Target:** Cumulatively, 24 technological breakthroughs or scientific advancements that make significant contributions toward reducing the cost and increasing profitability, improving the efficiency, increasing the yield, and increasing the sustainability of producing or converting biobased feedstocks into biofuels.

PATENTS

ARS scientists working in projects assigned to NP 213 have been successful in getting approval for eight U.S. patents based on their research during the 2008-2012 timeframe of this report. The patents granted are listed below in order of patents issued.

Weimer, P.; Wood adhesives containing solid residues of biomass fermentations. [no. 7,651,582]

Lima, I.; Pyrolytic products from poultry manure. [no. 7,794,601]

Hicks, K.; Porous polymeric matrices made of natural polymers and synthetic polymers and optionally at least one cation and methods of making. [no. 8,080,590]

Cotta, M.; Application of tannins to reduce odor emissions from animal waste. [no. 8,137,660]

Hicks, K.; Process for converting whole barley into fermentable sugars. [no. 8,173,404]

Hicks, K. and Nghiem, N.; Methods for improving the bioavailability of polysaccharidesin lignocellulosic materials. [no. 8,202,970]

Hicks, K.; A method for co-extrusion of high melting temperature thermoplastics with heat-sensitive biologically active substances. [no. 8,268,905]

Hughes, S.; Mutant lycotoxin-1 peptide sequences for insecticidal and cell membrane altering properties. [no. 8,334,366]

In addition to these eight patents, ARS research under Component 3 has generated another 19 innovations for which patent applications have been submitted, and the ARS Office of Technology Transfer is assessing potential patent applications to protect an additional 20 innovations. All these innovations have been disclosed since 2008 (inclusive).

EXTERNAL AWARDS

Peer-reviewed research grants and stakeholder awards are a useful indicator of the quality and value of the NP 213 program. From 2008 to 2012, NP 213 scientists were awarded 61 grants and awards that complemented and enhanced the objectives of the NP 213 Action Plan.

Table 1 shows that the largest portion of external funding sources was from industry—evidence that the research is valued by stakeholders. Other Federal grants were the second largest number. ARS scientists were also successful in competing for grants from university sources, and usually involved cooperative research projects jointly conducted with university partners. (18 total).

External Grant Sources for National Program 213 Projects - 2007-2012

UNIVERSITY	GOVERNMENT						INDUSTRY	NON-PROFIT & TRUST
	Corps of Engineers, Dept of the Army	Dept of Energy	Dept of the Navy	NIFA	OTHER FEDERAL	STATE/ LOCAL		
12	2	7	2	5	1	1	30	1

Table 1: Peer-reviewed research grants and awards given to NP 213 project scientists from 2007-2012.