Bioenergy & Energy Alternatives:

ARS National Program 307

Accomplishment Report 1999 – 2006

United States Department of Agriculture

Research, Education & Economics

Agricultural Research Service

National Program Staff
April 2007

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Introduction

Before 1999, the relatively limited scope of bioenergy research at ARS was managed under the National Program for Quality and Utilization Research. The formation of a separate National Program in bioenergy was initiated by a workshop in April, 1999 which provided the agency with input from stakeholders about the nature of the technical barriers to the growth of both biofuels and the use of alternative energy in agriculture. The barriers identified at this workshop are summarized beginning on page 6.

With the intention of addressing these barriers and considering its unique, but limited capabilities, ARS developed a set of goals that ARS research would target over the next 5-7 years. These goals, which constitute the Action Plan for the new National Program in Bioenergy and Energy Alternatives, are summarized on pp. 9-12.

The bulk of this report summarizes selected, technical accomplishments that resulted from the ensuing research. These accomplishments are categorized in this report by the Program's major components:

The success of the Bioenergy and Alternative Energy National Program, relative to the goals established in the Program's Action Plan, will be assessed by a panel of outside experts. Their assessment will be published on the ARS website in the summer of 2007 and will provide a basis on which to begin planning for a second 5-year research cycle in this National Program.

ARS is proud to submit this Accomplishments Report to the Assessment Panel, to our stakeholders and customers, and to the Nation. ARS National Program Staff are confident that the research performed under the Bioenergy and Energy Alternatives National Program reflects a productive research portfolio given the funding levels involved. The Agency anticipates the program to continue to serve as a source of innovation for our stakeholders and the bioenergy industry, and looks forward to many future success stories and accomplishments.

Background

GENERAL

The Agricultural Research Service (ARS; http://www.ars.usda.gov) is the intramural research agency for the United States Department of Agriculture (USDA; http://www.usda.gov), and is one of four agencies that comprise the Research, Education, and Economics mission area of the Department. In 1998, ARS organized its research under a National Program structure to better manage and coordinate research efforts into specific genres. These programs serve to bring coordination, communication, and empowerment to the more than 1200 research projects carried out by the Agency. Management of National Programs focuses on enhancing the relevance, quality, and impact of ARS research.

The Bioenergy and Energy Alternatives National Program (NP) 307 (http://www.ars.usda.gov/biofuels) was formed with the mission to develop alternative energy sources and increase the use of agricultural materials as feedstocks for biofuels, to create jobs and economic activity in America, reduce the Nation's dependence on foreign oil, and improve the environment. The National Program's mission follows the ARS Strategic Plan (http://www.ars.usda.gov/aboutus/docs.htm?docid=1766) which, in turn, is directed towards achieving the goals mandated by the USDA Research Education and Extension Mission Strategic Plan and the USDA Strategic Plan (http://www.usda.gov/ocfo/usdasp/usdasp.htm).

In working to solve agricultural problems, the boundaries of ARS national programs often overlap, and as a result, many of the projects contributing to NP 307 also contribute to achieving goals of other programs. Bioenergy research is very closely linked to biobased product research managed under the Quality and Utilization of Agricultural Products National Program (NP 306; http://www.ars.usda.gov/research/programs/programs.htm?NP_CODE=306), and several other National Programs in Natural Resources and Sustainable Agricultural Systems (NRSAS) and Crop Production and Protection (CPP).

The goals of NP 307 not only support ARS priorities, but strive to address problems of high national priority. There are growing concerns over the security and reliability of our energy supply. These concerns, compounded by the environmental effects of fossil energy use, the economic costs of importing petroleum, and a struggling rural economy, have spurred interest to develop alternative, renewable energy. The U.S. Congress has responded with legislation in support of developing alternative fuels derived from biomass. This includes the Biomass Research and Development Act of 2000 (H.R. 2559, Public Law No: 106-224; http://www.brdisolutions.com/default.aspx). The Act states that the Secretary of Agriculture and the Secretary of Energy shall coordinate and cooperate on policies and procedures that promote research and development leading to the production of biobased industrial products. The purposes of the coordination and cooperation are:

- to understand the key mechanisms underlying the difficulty in converting biomass into biobased industrial products;
- to develop new and cost-effective technologies that would result in large-scale commercial production of low-cost and sustainable biobased industrial products;

- to ensure that biobased industrial products are developed in a manner that enhances their economic, energy security, and environmental benefits; and
- to promote the development and use of agricultural and energy crops for conversion into biobased industrial products.

Furthermore, the Congress passed the 2002 Farm Bill (http://www.ers.usda.gov/Features/FarmBill/2002FarmAct.pdf) which contained the first energy title in farm bill history (Title IX) and the Energy Policy Act of 2005 (EPAct; http://www.epa.gov/OUST/fedlaws/publ_109-058.pdf). EPAct, signed into law on August 8th, 2005, was the first Federal energy law passed since 1992 and the first to mandate utilization of renewable fuel (7.5 billion gallons of renewable fuel by 2012).

The President introduced his Advanced Energy Initiative (AEI; http://www.whitehouse.gov/stateoftheunion/2006/energy/energy_booklet.pdf) on February 20th, 2006. Most recently during the 2007 State of the Union address, the President laid out a vision for the country to produce 35 billion gallons of ethanol per year as a renewable fuel by 2017. The President's goal is ambitious, and is largely believed to be attainable only through the development and commercialization of cellulosic ethanol.

Currently, the 110th U.S. Congress is working on numerous bills, including the 2007 Farm Bill, designed to spur the necessary research, development and commercialization of cellulosic ethanol.

PLANNING, REVIEW, AND IMPLEMENTATION

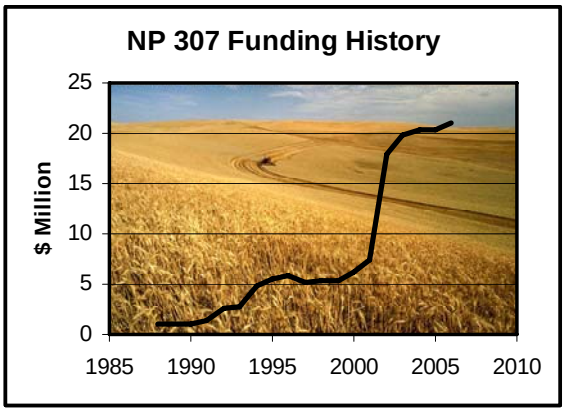
Management of each of the ARS National Programs follows a ~5-year program cycle consisting of planning, review, implementation, and assessment. The initial National Program Cycle for the Bioenergy & Energy Alternatives National Program began on April 15, 1999, with a Customer Workshop in Beltsville, Maryland. The primary purpose of the workshop was to identify problems and issues of concern to the bioenergy and energy alternatives industry. The workshop was attended by customers, stakeholders, and partners, each with an interest in biobased or alternative energy, along with ARS scientists, engineers, and managers.

Following the customer workshop, the National Program Leadership team overseeing NP 307, working with Agency scientists and engineers, developed an Action Plan to direct the research efforts of the program for five years by addressing priority researchable problems identified at the customer workshop (http://www.ars.usda.gov/research/programs/programs.htm?np_code=307&docid=281). The plan consisted of four components, each addressing an area of need identified by participants in the customer workshop. The mission of this national program effort is to create jobs and economic activity in America, reduce the Nation's dependence on foreign oil, and improve the environment by developing alternate energy sources and increasing the use of agricultural crops as feedstocks for biofuels.

A follow up planning and coordination workshop attended by ARS scientists and engineers was held in Laurel, Maryland in 2001 to assess and improve implementation of the 1999 Action Plan.

Additional workshops, attended by ARS engineers and scientists, were held for each component of the NP 307 Action Plan. The objective of these workshops was to enhance research outcomes by facilitating communication and coordination among researchers.

Once the Action Plan was completed, specific research Project Plans contributing to the Bioenergy and Energy Alternatives National Program were written to support the NP 307 actions. These new project plans were evaluated for scientific quality by a panel of external examiners convened by the Office of Scientific Quality Review in May 2004. Project Plans were revised in response to panel recommendations and then implemented. The success of the national program, as measured against the goals outlined in the Action Plan has been assessed by an external retrospective assessment panel.



The next five-year national program cycle will begin with a customer/stakeholder meeting in September 2007. National and Congressional interest in renewable energy has resulted in increased funding for bioenergy research (see figure at left). This increase in funding and support is projected to continue to rise and the program is poised for further growth.

Development of biobased, renewable energy to supply a significant portion of the nation's needs is a major effort that requires the coordinated application of the skills and resources of many groups. Bioenergy research both within and in support of NP 307 is being conducted at multiple ARS locations across the country (see map below). Efforts continue to be made to partner with others to leverage the research capabilities of ARS and to enhance the outcomes and impact of ARS research. Communication and partnering of ARS with other USDA agencies, the Department of Energy and its national laboratories, universities, and industry continue to grow. National Program 307 requires complementary research efforts across the Agency and requires cross-disciplinary collaboration.

PROBLEMS IDENTIFIED BY CUSTOMERS

Input from customers and stakeholders focused on ethanol, biodiesel, rural energy needs, and energy crops. Issues and needs included:

- **Ethanol**: The main problem associated with ethanol was that it could not be produced from agricultural feedstock at a selling cost competitive with petroleum fuels. Priority issues were:
  - Cost of producing ethanol from cornstarch. Stakeholders identified value-added coproducts as the number one research priority for the fuel ethanol industry. Though existing coproducts played an important role in the economics of fuel ethanol production, most were high-volume and relatively low-value and had potential for economic and process improvement;
  - Need for advances in fundamental science and engineering to support development of technology for ethanol production;
  - Need for conversion of cellulosic biomass into ethanol:
    - The first biomass target should be corn fiber, due to its ready availability at ethanol plants and extremely low value
    - Findings from corn fiber research would be applicable to other forms of biomass ranging from agricultural and forestry wastes to crops grown solely for energy production
    - Research should focus on, but not be limited to, developing new enzymatic processes for saccharification and improved microorganisms to ferment the multiple sugars found in cellulosic biomass
    - Development of biocatalysts for the biomass-to-ethanol industry; biocatalysts must be capable of fermenting lignocellulose to ethanol and work in industrial processes under industrial conditions.

- **Biodiesel:** Major biodiesel problems identified were high cost and the lack of adequate standards and tests. Hurdles to widespread commercialization of biodiesel included:
  - Excessive emissions;
  - Lack of fuel quality standards and on-line testing procedures;
  - High feedstock cost;
  - Poor cold flow properties of fuel; and
  - Inadequate storage stability.

- **Energy Alternatives for Rural Practices:** Major problems for rural areas were:
  - Inadequate energy to provide reliable, safe drinking water for livestock and humans, and inadequate basic electrical service.

- **Energy Crops:** Though biomass crops could provide a significant portion of the Nation's energy needs, the available cropping system and conversion technologies were not adequate to achieve that potential. Identified needs included:
  - Technology for integrated field-to-factory cellulosic biomass crop production systems;
  - Conversion methods that reduce the cost of deriving liquid fuels from biomass; and

- Procedures for determining the amount of crop residue that can be removed without deleterious effects.

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

In this report, information about NP 307 achievements and their impact is organized according to National Program research components and program goals, as described in the current National Program Action Plan. The report first outlines the four NP 307 Research Components. These are followed by selected accomplishments and include the impact and/or potential of those achievements to solve the problems and meet the needs identified by the customers and stakeholders in the NP 307 Action Plan.

For the most part, the content of this report is derived from NP 307 scientists who were asked to summarize their project's major accomplishments during the last five years in terms of impact, and provide key references documenting those accomplishments. Consequently this report does not include all accomplishments achieved in the national program but rather, those key accomplishments selected by the National Program Leaders who authored this report. As a result, this report encompasses a subset of the total spectrum of NP 307 accomplishments, chosen to illustrate and exemplify the total progress and achievements at the National Program level.

This Accomplishment Report was compiled by National Program Staff members, Dr. Chad Haynes, Dr. Robert Fireovid, and Mrs. Ellen Buckley.

Action Plan Components and Plans to Address Problems

Component I: Ethanol

To address the issues and needs expressed by customer workshop participants, it was determined that research would focus on increasing process efficiency and coproduct development with the following goals:

- *Process Efficiencies*
  Goal: To alter traditional unit operations, develop new ones, develop new organisms, and increase process conversions. To create enzymes with the capacity to convert starch to glucose without the need for cooking or for a multi-step conversion as is common in 'wet' grain process technologies.

- *Coproduct Development*
  Goal: To develop new, higher-valued coproducts or improve the existing ones from the non-starch portion of the grain, resulting in a reduced net cost of the starch used for producing ethanol.

Projected Outcomes of Ethanol Research

It was projected that the ethanol research would have the following outcomes:

- *Process Efficiencies*
  Intermediate: Ethanol yield will be improved by partial saccharification of corn fiber, by the use of new organisms which ferment pentose sugars, and/or do not produce succinate, or which ferment xylose without glucose repression. The cost to produce ethanol will be reduced by new unit operations which require less energy to separate the ethanol, or which facilitate greater conversion of the starch to ethanol.
  Long-Term: Technologies will be completed to eliminate competing fermentation products from the first generation of new ethanol-producing organisms. Membrane pervaporation will significantly improve the productivity of biomass hydrolysate fermentations. New technologies that integrate feedstock pretreatment, biological conversion and product recovery processes, as well as fundamental knowledge regarding fermentation, milling and membrane separations, will result in a reduction in capital and processing costs associated with biofuel production.

- *Coproduct Development*
  Intermediate: Aquaculture feeds that will provide outlets for excess, low valued corn gluten feed, distiller's dried grains and corn gluten meal. Technology to produce a low cost zein product for use as biodegradable and edible films. New fermentation products from fuel ethanol residues that will reduce the net cost of the ethanol.
  Long-Term: Higher value coproducts generated from current low-value production byproducts. Envisioned coproducts include specialty oils, novel polysaccharides that will compete with imported gums and sugar alcohol food additives, enzymes, and inexpensive aquaculture feeds.

Projected Impact of Ethanol Research

It was projected that the impact of the ethanol research would be that starch-derived ethanol would be a more competitive and profitable fuel.

Component II: Biodiesel

To address the issues and needs expressed by customer workshop participants, it was determined that biodiesel research would address the following topics with associated goals:

- *Combustion and exhaust emissions*
  Goal: Develop technology to meet exhaust emissions regulations.

- *Fuel quality testing and on-line process control*
  Goal: Develop technology for rapid, easy-to-use process control and for fuel quality analysis.

- *Reduce cost of feedstocks*
  Goal: Develop conversion technology for production of biodiesel from low-cost, non-conventional, agriculturally derived feedstocks.

- *Cold flow properties*
  Goal: Develop fundamental understanding of cold flow phenomena and develop technology to reduce cold flow operability problems.

- *Storage stability*
  Goal: Identify factors that reduce oxidative stability of biodiesel and develop technology that improves storage stability.

Projected Outcomes of Biodiesel Research

It was projected that the biodiesel research would have the following outcomes:

- *Combustion and exhaust emissions*
  Intermediate: Engine tests to evaluate cetane improvers, for performance, exhaust emissions, and formulations. Ignition quality characteristics of biodiesel fuel components and cetane improvers.
  Long-Term: A biodiesel fuel with reduced exhaust emissions, especially NOx, which will be in compliance with current and future environmental regulations.

- *Fuel quality testing and on-line process control*
  Intermediate: Rapid analytical methods to monitor biodiesel production, and quality.
  Long-Term: A more rapid, cost-effective, easy-to-conduct analytical method for assessing biodiesel fuel quality and for the on-line monitoring of biodiesel production.

- *Reduce cost of feedstocks*
  Intermediate: Processes for lower-cost production of biodiesel from vegetable oils and from non-conventional feedstocks
  Long-Term: Feedstocks and processes that allow production of cost-competitive biodiesel.

- *Cold flow properties*
  Intermediate: Strategies for synthesizing and evaluating cold flow improvers
  Long-Term: Temperature limiting cold flow operability for biodiesel is reduced by at least 10 degrees Celsius (18 degrees Fahrenheit). Cold flow operability of 20% biodiesel/petro-diesel blends (B-20) improved to a level equivalent to that of petro-diesel. Cold flow improvers that inhibit crystal nucleation in biodiesel fuels are identified.

- *Storage stability*
  Intermediate: A more accurate lipid quality index that predicts oxidative stability of biodiesel fuels. The potential for phenolic and other antioxidants to improve biodiesel resistance to oxidation will be determined. Technology to interpret oxidative and/or hydrolytic stability under accelerated conditions.
  Long-Term: Conditions are established for handling and storage of biodiesel and blends to safeguard compliance with industrial fuel specifications. Methods and instrumentation for evaluating oxidative stability of biodiesel under accelerated conditions are developed. Analytical methods are developed for tracking fuel quality of biodiesel during short- and long-term storage.

Projected Impact of Biodiesel Research

Biodiesel fuels produced from agricultural lipid sources are more uniform, environmentally beneficial, and cost-competitive.

Component III: Energy Alternatives for Rural Practices

To address the issues and needs expressed by customer workshop participants, it was determined that energy alternatives research would focus on meeting energy needs of remote rural areas with goals of developing:

- *On-site energy for agriculture*
  Goal: Autonomous pumping systems to supply water for irrigation, livestock, and farmstead needs, and renewable energy systems to supply needed electric power.

Projected Outcomes of Energy Alternatives for Rural Practices Research

It was projected that the energy alternatives for rural practices research would have the following outcomes:

- *On-site energy for agriculture*
  Long-Term: Solar and wind water pumping systems are sufficiently efficient and reliable to meet the water needs of livestock. Renewable (hybrid wind/diesel (biofuel)) electric generation systems are available in remote rural areas.

Projected Impact of Energy Alternatives for Rural Practices Research

It was projected that the impact of the energy alternatives for rural practices research would be the availability of technology to provide adequate and reliable electric power and water to meet remote rural needs.

Component IV: Energy Crops

As a result of the potential for energy crops to be a significant source of renewable energy and of needs expressed by stakeholders, an energy crops component was included in the Bioenergy and Energy Alternatives national program. Although several ARS scientists were conducting forage breeding and production research, at the time of the customer workshop none of their projects were funded as part of this national program but were included in NP 205 Rangeland, Pasture, and Forages (http://www.ars.usda.gov/research/programs/programs.htm?NP_CODE=205). In order to address the issues and needs expressed by customer workshop participants and using existing expertise in forage production research as a foundation, an enhanced energy crop research effort was formed with plans to:

- Establish coordinated research teams at locations representing principal biogeographic regions where production of biomass crops is feasible.
- Develop biomass plants and production systems that document environmental benefits of biomass crop production.
- Establish research to develop technology for biomass handling, storage, and transportation systems.

To avoid duplication and benefit from synergy, plans were made to partner with the U.S. Department of Energy (DOE), with ARS leading research on biomass production systems and on assessing their environmental benefits. The following goals were established:

- *Energy crop production*
  Goal: Develop biomass plants and production systems that maximize the environmental benefits of biomass crop production.
- *Feedstock logistics*
  Goal: Develop technology for biomass handling, storage, and transportation systems.
- *Crop residues*
  Goal: Determine the amount of crop residue that can be removed without deleterious effects.

Projected Outcomes of Energy Crops Research

It was projected that outcomes of the energy crops research would be:

- Switchgrass germplasm with improved yields and biomass fuel characteristics.
- Management strategies to improve switchgrass production.
- Quantitative assessment of residue removal effects

Projected Impact of Energy Crops Research

It was projected that the impact of the energy crops research would be to enable production of liquid fuel from biomass produced with systems that improve soil and water quality and reduce carbon in the atmosphere.

Selected Accomplishments and Impacts

Component I: Ethanol

Topic: Process Efficiencies
Goal: To alter traditional unit operations, develop new ones, develop new organisms, and increase process conversions. To create enzymes with the capacity to convert starch to glucose without the need for cooking or for a multi-step conversion as is common in 'wet' grain process technologies.

Summary: The U.S. corn grain to ethanol industry has grown considerably over recent years. 2006 output capacity was approximately 5 billion gallons, spurred largely by the use of ethanol as a gasoline fuel oxygenate additive and the potential for ethanol to be used in a concentrated form as a direct replacement for gasoline derived from petroleum. Indeed this growth has been positively influenced by numerous technological advances in corn grain handling and processing. ARS has specifically contributed to many of these technologies, including:

- Enhanced corn milling processes to decrease cost and increase ethanol and valuable byproduct yields
- Development of novel bacterial and yeast strains, and microbial enzymes for more efficient conversion of biomass to ethanol
- Development of pretreatment and conversion technologies for cellulosic biomass

These broadly stated accomplishments are validated by the specific accomplishments listed below.

Significant Accomplishments & Impact:

- **Developed and demonstrated an eco-friendly corn steeping processes for enhanced wet milling** Demonstrated a new corn steeping process using enzymes to reduce or eliminate the need for hazardous sulfur dioxide in wet milling of corn. The ARS research team also optimized the process so that enzyme requirements were reduced by a factor of ten. This process was patented (Johnston, D.B., Singh, V. and Eckhoff, S. (2003) "Use of Enzymes to Reduce Steep Time and SO_2 Requirements in a Maize Wet-Milling Process." U.S. Patent 6,566,125.). The process was successfully tested on a commercial scale; design and construction plans are now being developed to implement the process at a wet milling facility outside of the U.S. The process was considered so important that a Memorandum of Understanding was developed between ARS and the Corn Refiners' Association. David Johnston, a Lead Scientist at ARS, received a Presidential Young Scientist Award from the White House in 2006 for this work. It is anticipated that as the process gets developed off-shore, news of the higher starch yields, food grade proteins, and lower emissions of sulfur dioxide will drive U.S. Wet Millers to adopt the process. Adoption of this technology on a commercial scale will positively impact both the economic and environmental cost of wet milling of corn. The announcement of the licensing of the process to a major enzyme company is imminent.

  Johnston, D.B. and Singh, V. (2001) "The Use of Proteases to Reduce Steep Time and Eliminate SO_2 Use in a Corn Wet-Milling Process" Cereal Chemistry 78(4): 405-411.

Johnston, D.B. and Singh, V., (2004) "Enzymatic Milling of Corn: Optimization of Soaking, Grinding, and Enzyme Incubation Steps." Cereal Chemistry 81(5): 626-632.

Johnston, D.B. and Singh, V. (2005) "Enzymatic Milling Product Yield Comparison with Reduced Levels of Bromelain and Varying Levels of Sulfur Dioxide". Cereal Chemistry 82(5) 523-527.

Singh, V. and Johnston, D.B. (2002) "Pasting Properties and Surface Characteristics of Starch Obtained from an Enzymatic Corn Wet Milling Process." Cereal Chemistry 79(4): 523-527.

Singh, V. and Johnston, D.B. (2004) " An Enzymatic Process for Corn Wet Milling". Advances in Food and Nutrition Research. 48: 151-171.

- **Discovered the most active β-xylosidase for releasing sugars from biomass**
  Sources of β-xylosidase are needed for converting xylan to xylose, the second most common sugar present in biomass after glucose, for subsequent bioconversion to ethanol. Determined that the β-xylosidase from *Selenomonas ruminantium* is the most catalytically efficient enzyme known (at least 15-fold better than those reported in the literature) for catalyzing the hydrolysis of xylo-oligosaccharides to xylose and has good properties of temperature and pH stability. Additionally, the enzyme can be efficiently produced in *Escherichia coli* (>4 g enzyme/liter). These properties place this enzyme research at the forefront for development as a cellulose saccharification catalyst. The technology is patent pending.

  Jordan, D. B., X. Li, C. A. Dunlap, T. R. Whitehead, and M. A. Cotta. Structure-Function Relationships of a Catalytically Efficient Beta-D-Xylosidase. Biochemistry. Accepted 7-12-06.

  Jordan, D. B., X. Li, C. A. Dunlap, T. R. Whitehead, and M. A. Cotta. Beta-D-xylosidase from *Selenomonas ruminantium* of Glycoside Hydrolase Family 43. Appl. Biochem. and Biotechnology. Accepted 10-24-06.

  Jordan, D. B. and J. D. Braker. Inhibition of the Two-subsite β-D-xylosidase from *Selenomonas ruminantium* by Sugars: Competitive, Noncompetitive, Double Binding, and Slow Binding Modes. Submitted to Carbohydrate Research 2/20/07.
  Jordan, D. B., X.-L. Li, C. A. Dunlap, T. R. Whitehead, and M. A. Cotta. Beta-xylosidase for Conversion of Plant Cell Wall Carbohydrates to Simple Sugars. Provisional Patent Application 60,850,668. October 10, 2006.

  Qureshi, N., B. S. Dien, N. N. Nichols, B. C. Saha, and M. A. Cotta. Genetically Engineered *Escherichia coli* for Ethanol Production from Xylose: Substrate and Product Inhibition and Kinetic Parameters. 2006. Food and Bioproducts Processing 84:114-122.

  Saha, B. C. 2003. Purification and Properties of an Extracellular β-xylosidase from a Newly Isolated *Fusarium proliferatum*. Bioresource Technology 90:33-38.

- **Discovered and patented two novel microbial enzymes for cellulose processing.**
  Discovered and patented two novel microbial enzymes (high glucose tolerant β-glucosidase from *Candida peltata* and thermostable L-arabinofuranosidase from *Aureobasidium pullulans*) that greatly aided in the saccharification of the cellulose and hemicellulose components of biomass into fermentable sugars. Isolated a fungal (*Fusarium verticilloides*) strain capable of producing hemicellulase enzymes that are highly effective in hydrolyzing hemicellulose (complex heteroarabinoxylan) from corn fiber. No commercial hemicellulase preparations available to date can effectively saccharify solubilized hemicellulose from corn fiber. The research team has also engineered and patented a microorganism (*Escherichia coli*) that has enhanced stability and produces ethanol from mixed sugar substrates efficiently with excellent yield. The novel metabolic engineering method used for constructing these strains has been patented and the strains are being evaluated by numerous laboratories here and abroad in Europe and Australia. Use of this engineered organism promises to significantly reduce the cost of biomass processing by consolidating numerous

biochemical processing steps to a single, industrial organism. These *E. coli* strains have also been the subject of numerous publications by ARS as well as other laboratories.

Canakei, S., A. O. Belduz, B. C. Saha, A. Yasar, F. A. Ayaz, and N. Yayli. Purification and Characterization of a Highly Thermostable Alpha-L-Arabinofuranosidase from *Geobacillus caldoxylolyticus* TK4. Submitted to Applied and Environmental Microbiology 11/1/06.

Freer, S. N., C. D. Skory, and R. J. Bothast. 1999. Yeast β-glucosidases and the Saccharification of Cellulosic Biomass, pp. 146-166. *In* S. H. Imam, R. V. Greene, and B. R. Zaide (eds.), Biopolymers: Untilizing Nature's Advanced Materials. American Chemical Society, Washington, DC.

Li, X., L. G. Ljungdahl, E. A. Ximenes, H Chen, C. R. Felix, M. A. Cotta, and B. S. Dien. 2004. Properties of a Recombinant β-glucosidase from Polycentric Anaerobic Fungus *Orpinomyces* PC-2 and Its Application for Cellulose Hydrolysis. Applied Biochemistry and Biotechnology. 113-116:233-250.

Saha, B. C. 2000. α-L-Arabinofuranosidase: Biochemistry, Molecular Biology and Application in Biotechnology. Biotechnol. Advances 18:403-423.

Saha, B. C. 2001. Xylanase from a Newly Isolated *Fusarium verticillioides* Capable of Utilizing Corn Fiber Xylan. J. Appl. Microbiol. Biotechnol. 56:762-766.

Saha, B. C. 2002. Production, Purification and Properties of Xylanase from a Newly Isolated *Fusarium proliferatum*. Process Biochem. 37:1279-1284.

Saha, B. C. and R. J. Bothast. Glucose and Cellobiose Tolerant β-Glucosidase from *Candida Peltata*. U.S. Patent #5,747,320.

Saha, B. C. and R. J. Bothast. Thermostable α-L-Arabinofuranosidase from *Aureobasidium pullulans*. U.S. Patent #5,882,905.

Ximenes, E. A., H. Chen, I. A. Kataeva, M. A. Cotta, C. R. Felix, L. G. Ljungdahl, and Xin-Liang Li. A Mannanase, ManA, of the Polycentric Anaerobic Fungus *Orpinomyces* sp. Strain PC-2 has Carbohydrate Binding and Docking Modules. Canadian Journal of Microbiology 51:559-568.

- **Isolated and expressed fungal enzymes for biomass processing**

Isolated plant cell wall hydrolyzing enzymes, including cellulases, hemicellulases, and esterases, with very high specific activities from the anaerobic fungus *Orpinomyces* PC-2. Recombinant DNA technology was used to construct *E. coli* hosts capable of producing these enzymes in large quantities. Many of these enzymes have been patented and have the capability to reduce the processing cost of biomass sugars prior to fermentation. The technology is being investigated for applications by other researchers within and outside of ARS as a novel source for fungal biomass processing enzymes.

Chen, H., X. Li, D. L. Blum, E. A. Ximenes, and L. G. Ljungdahl. *CelF* of *Orpinomyces* PC-2 has an Intron and Encodes a Cellulase (CelF), Containing a Carbohydrate-Binding Module. Appl. Biochem. Biotechnol. 105-108:775-785.

Freer, S. N., R. V. Greene, and R. J. Bothast. 2001. Gene Structure of a Bifunctional Cellulase Gene (celA) Isolated from *Teredinobacter turnerae*. IN: M. E. Himmel, J. O. Baker, and J. N. Saddler (eds.) Glycosyl Hydrolases for Biomass Conversion, ACS Symposium Series 769.

Jordan, D. B., B. S. Dien, X. Li, and M. A. Cotta. 2006. Hemicellulases for Mediating Biomass Saccharification. In: Renewable Energy 2006 Proceedings, Renewable Energy 2006 Organizing Committee, Chiba, Japan, October 9-13, 2006. p. 1036-1041.

Li, X., E. A. Ximenes, H. Chen, M. A. Cotta, and L. G. Ljungdahl. Cloning and Sequencing of Two Highly Homologous Cellulase Genes, *celH* and *celI* from the Anaerobic Fungus *Orpinomyces* Strain PC-2, pp. 638-641. In Ohimya, K., Sakka, K., Karita, S., Kimura, T., Sakka, M., and Onishi, Y. (eds.) Biotechnology of Lignocellulose Degradation and Biomass Utilization. Uni Publishers Co., Tokyo, Japan. 2004. Book Chapter.

Li, X., C. D. Skory, E. A. Ximenes, D. B. Jordan, B. S. Dien, S. R. Hughes, and M. A. Cotta. 2007. Expression of an AT-Rich Xylanase Gene from the Anaerobic Fungus *Orpinomyces* sp. Strain PC-2 in and

Secretion of the Heterologous Enzyme by *Hypocrea jecorina*. Journal of Applied Microbiology and Biotechnology. Accepted 12/4/2006.

- **Expressed a highly active xylanase enzyme in the fungus *Trichoderma reesei* used for producing industrial hydrolyases**
  The xylanase gene originated from an anaerobic fungus and was then engineered to overcome problems with codon bias in the new host so that high yields of the heterologous enzyme could be produced. The enzyme cocktail produced by the modified *T. reesei* should be more efficient at degrading cellulosic biomass into fermentable sugars and may find application for production of livestock feed or the conversion of biomass to ethanol. This is the first time an anaerobic fungal enzyme has been successfully expressed in *T. reesei*. *T. reesei* is used to produce commercial cellulase preparations, but the hydrolytic enzymes produced by anaerobic fungi tend to have much higher specific activities. This achievement should now allow for other important enzymes from anaerobic fungi to be produced by *T. reesei*, thereby, increasing the effectiveness of the cellulases produced by it.

  Chen, H., Hopper, S.L., Li, X.-L., Ljungdahl, L.G., Cerniglia, C.E. Isolation of extremely AT-rich genomic DNA and analysis of genes encoding carbohydrate-degrading enzymes from *Orpinomyces* sp. strain PC-2, (2006) Current Microbiology, 53 (5), pp. 396-400.

  Li, X.-L., Skory, C.D., Ximenes, E.A, Jodan, D.B., Dien, B.S., Hughes, S.R., and Cotta, M.A. 2007. Appl. Microbiol. Biotech. DOI 10.1007/s00253-006-0787-6.

  Li, X.-L., B. S. Dien, M. A. Cotta, Y. V. Wu, and B. C. Saha. Profile of Enzyme Production by *Trichoderma reesei* Grown on Corn Fiber Fractions. Applied Biochemistry and Biotechnology 121-124:321-334.

- **Created genetic libraries for screening for novel biomass processing enzymes**
  Several genomic libraries have been generated, resulting in the identification of genes encoding more than 20 novel xylanolytic enzymes, enzymes critical for pretreatment of straw and woody materials. These genes were subcloned into bacterial expression systems for biochemical characterization and directed evolution experiments. High throughput screening methods using natural substrates are being developed to select active mutants. Enhanced xylan processing activities is highly desirable and necessary when considering woody biomass as a biomass feedstock.

  Saha, B. C. and R. J. Bothast. 1999. Enzymology of Xylan Degradation, pp. 167-194. *In* S. H. Imam, R. V. Greene, and B. R. Zaide (eds.), Biopolymers: Utilizing Nature's Advanced Materials. American Chemical Society, Washington, DC.

- **Developed new corn fiber biochemical treatment processes using fungal preparations**
  Developed custom hydrolytic enzyme preparations – prepared by growing fungi on hot water-treated corn fiber – that convert oligomers of corn fiber into monosaccharides with arabinose, glucose, and xylose yields of 80%, 100%, and 80%, respectively. The results have been published in *Enzyme and Microbial Technology*. The research was also presented to our partners at Purdue University and Aventine Renewable Energy, which is demonstrating Purdue's hot water treatment. Corn fiber conversion for ethanol production or coproduct development is a promising area of research to significantly reduce the overall cost of biomass processing and increase biorefinery profitability.

  Dien, B. S., X.-L. Li, L. B. Iten, D. B. Jordan, N. N. Nichols, P. J. O'Bryan, and M. A. Cotta. 2006. Enzymatic Saccharification of Hot-water Pretreated Corn Fiber for Production of Monosaccharides. Enzyme and Microbial Technology 39:1137-1144.

  Mosier, N. S., R. Hendrickson, M. Brewer, N. Ho, M. Sedlak, R. Dreshel, G. Welch, B. S. Dien, A. Aden, and M. R. Ladisch. 2005. Industrial Scale-up of pH-Controlled Liquid Hot Water Pretreatment of Corn Fiber for Fuel Ethanol Production. Appl. Biochem. Biotechnol. 125:77-97.

- **Successful pilot plant demonstration of engineered bacteria capable of processing multiple, non native sugars**
  Mixtures of different sugars were fermented to ethanol, from a pilot plant demonstration project utilizing corn fiber. The project used an ARS-developed, recombinant *Escherichia coli* strain (*E. coli FBR16*) engineered to co-ferment arabinose, glucose, and xylose sugars. Ethanol yields were 84-97% of the maximum possible based upon initial sugar concentrations. The demonstration project is the outcome of a highly successful collaboration with an academic institution (Purdue University), U.S. D.O.E. laboratory (NREL), a local ethanol producer (Aventine), and ARS (NCAUR) and proves the proof of concept that bacteria can be engineered to processes non native sugars.

  Dien, B. S., N. N. Nichols, and R. J. Bothast. 2001. Recombinant *Escherichia coli* Engineered for Production of L-lactic Acid from Hexose and Pentose Sugars. J. Ind. Microbiol. Biotechnol. 27:259-264.

- **Developed new pretreatment processing solutions for wheat straw with enhanced biomass processing**
  Pretreated wheat straw will undergo complete enzymatic saccharification to fermentable sugars. No common fermentation inhibitors were produced. Both simultaneous hydrolysis and fermentation and simultaneous saccharification and fermentation approaches worked equally well for producing ethanol from peroxide-pretreated wheat straw by use of an ARS-developed ethanologenic recombinant bacterium capable of converting multiple sugars (glucose, xylose, arabinose). The findings will contribute to the development of an integrated bioprocess technology for ethanol production from lignocellulose for significant processing cost gains.

  Saha, B. C. and M. A. Cotta. 2006. Ethanol Production from Alkaline Peroxide Pretreated Enzymatically Saccharified Wheat Straw. Biotechnol. Prog. 22:449-453.

  Saha, B. C. and M. A. Cotta. 2005. Alkaline Peroxide Pretreatment, Enzymatic Saccharification and Fermentation of Wheat Straw to Ethanol. In: Proceedings of the 34th Meeting of the United States - Japan Cooperative Program in Natural Resources (UJNR) Food and Agriculture Panel, October 23-29, 2005, Susono, Shizuoka, Japan. p. 168-172.

- **Developed biological processes to remove fermentation inhibition products**
  Strains of organisms (*Saccharomyces cerevisiae* and *Pichia stipitis*) that have enhanced ability to convert toxic compounds (furfural and 5-hydroxymethylfurfural – HMF) into less toxic compounds have been developed. These improved strains grow normally in the presence of both furfural and HMF and produce normal ethanol yields. These strains, which were developed through directed adaptation, are the subject of intensive genomics studies to derive a genetic blue print of the stress tolerance mechanisms. Such a blueprint will aid further strain enhancement by adaptation and/or genetic engineering techniques and guide fermentation studies to determine process conditions needed to optimize expression of tolerance traits. Furfural and HMF-tolerant strains have potential to be used to detoxify fermentation inhibitors and improve bioethanol production rates, yield, and economics. Nine peer-reviewed scientific journal articles and one invited book chapter have been published from this work, and twenty-five presentations have been given at international meetings. Following our progress, other researchers are now applying similar approaches and techniques to develop more robust yeast for converting biomass to ethanol.

  ERCC (Liu, Z. L. served as a core writing member; co-authors listed in alphabetical order of the last name). Proposed methods for testing and selecting the ERCC external RNA controld. BMC Genomics. 6:150. 2005.

  ERCC (Liu, Z. L. served on workshop planning committee and white paper writing member; co-authors listed in alphabetical order of the last name). The external RNA control consortium: A progress report. Nature Methods 2:731-734. 2005.

Gorsich, S. W., Dien, B. S., Nichols, N. N., Slininger, P. J. Liu, Z. L., and Skory, C. Tolerance to furfural-induced stress is associated with pentose phosphate pathway genes *ZWF1, GND1, RPE1*, and *TKL1* in *Saccharomyces cerevisiae.* Appl. Microbiol. Biotechnol. 71:339-349. 2006.

Liu, Z. L. Genomic adaptation of ethanologenic yeast to biomass conversion inhibitors. Appl. Microbiol. Biotechnol. 73:27-36. 2006.

Liu, Z. L., and Slininger, P .J. Universal external RNA controls for microbial gene expression analysis using microarray and qRT-PCR. J. Microbiol. Methods. doi:10.1016/j.mimet.2006.10.014. 2006.

Liu, Z. L., Slininger, P. J. and Gorsich, S. W. Enhanced biotransformation of furfural and hydroxymethylfurfural by newly developed ethanologenic yeast strains. Appl. Biochem. Biotechnol. 121-124: 451-460. 2005.

Liu, Z. L., Slininger, P. J., Dien, B. S., Berhow, M. A., Kurtzman, C. P. and Gorsich, S. W. Adaptive response of yeasts to furfural and 5-hydroxymethylfurfural and new chemical evidence for HMF conversion to 2,5-bis-hydroxymethylfuran. J. Ind. Microbiol. Biotechnol. 31: 345-352. 2004.

Liu, Z.L. and Slininger, P.J. Development of genetically engineered stress tolerant ethanologenic yeasts using integrated functional genomics for effective biomass conversion to ethanol. In: Collins, K., Duffield, J., Outlaw, J., editors. Agriculture as a Producer and Consumer of Engergy. Wallingford, UK: CAB International, p. 283-294. 2005.

Nichols, N. N., B. S. Dien, G. M. Guisado, and M. J. Lopez. Bioabatement to Remove Inhibitors from Biomass-derived Sugar Hydrolysates. Applied Biochemistry and Biotechnology 121-124:379-390.

Slininger, P. J., Dien, B. S., Gorsich, S. W., Liu, Z. L. Nitrogen source and mineral optimization enhance D-xylose conversion to ethanol by the yeast *Pichia stipitis* NRRL Y-7124. Appl. Microbiol. Biotechnol. 72:1285-1296. 2006.

Song, M., Liu, Z. L. A linear discrete dynamic system model for temporal gene interaction and regulatory network influence in response to bioethanol conversion inhibitor HMF for ethanologenic yeast. Lecture Notes Bioinfomatics. (Accepted) 2006.

- **Creation of glucose resistant ethanolic microorganisms capable of using multiple sugars**
  A series of recombinant ethanol-producing microorganisms, notably *FBR8* & *FBR14*, which use all sugars contained in cellulosic biomass at equal rates despite the presence of glucose have been developed. The work extends our previous patented metabolic engineering efforts to develop new ethanologens for enhanced consolidated biomass processing which may improve efficiency and overall cost. This work has been reported in scientific publications.

Bothast, R. J., N. N. Nichols, and B. S. Dien. 1999. The Future of Fermentative Microorganisms. The Energy Independent 5:8-9.

Bothast, R. J., N. N. Nichols, and B. S. Dien. 1999. Fermentations with New Recombinant Organisms. Biotechnol. Prog. 15:867-875.

Dien, B. S., N. N. Nichols, P. O'Bryan, and R. J. Bothast. 2000. Development of New Ethanologenic *Escherichia coli* Strains for Fermentation of Lignocellulosic Biomass. Appl. Biochem. Biotechnol. 84-86:181-196.

Dien, B. S., L. B. Iten, and R. J. Bothast. 1999. Conversion of Corn Fiber to Ethanol by Recombinant *E. coli* Strain FBR3. J. Ind. Microbiol. Biotechnol. 22:575-581.

Saha, B. C. and R. J. Bothast. 2000. Enzymes in biotechnology, p. 222-235. *In* A. I. Laskin (ed.), Encyclopedia of Microbiology, 2nd ed., Academic Press, Inc., San Diego, CA.

- **Engineered bacteria created to produce bioplastic feedstocks**
  Metabolic engineering was employed to create new strains of bacteria that utilize multiple sugars from agricultural biomass. In addition, other bacterial strains were engineered to efficiently produce

optically pure L-lactic acid for polylactate plastics, materials which are amenable to biodegradation. This work has been reported in the scientific literature.

Bura, R., S. D. Mansfield, J. N. Saddler, and R. J. Bothast. 2002. SO_2-catalyzed Steam Explosion of Corn Fiber for Ethanol Production. Appl. Biochem. Biotechnol. 98-100:59-72.

Dien, B. S., N. N. Nichols, and R. J. Bothast. 2002. Fermentation of Sugar Mixtures Using *Escherichia coli* Catabolite Repression Mutants Engineered for Production of L-lactic Acid. J. Ind. Microbiol. Biotechnol. 29:221-227.

Dien, B. S., R. J. Bothast, L. B. Iten, L. Barrios, and S. R. Eckhoff. 2002. Fate of Bt Protein and Influence of Corn Hybrid on Ethanol Production. Cereal Chemistry 79:582-585.

Hespell, R. B., H. A. Wyckoff, B. S. Dien, and R. J. Bothast. Stabilization of PET Operon Plasmids and Ethanol Production in Bacterial Strains Lacking Lactate Dehydrogenase and Pyruvate Formate Lyase Activities. U.S. Patent #6,280,986.

Nichols, N. N., B. S. Dien, and R. J. Bothast. 2001. Use of Catabolite Repression Mutants for Fermentation of Sugar Mixtures to Ethanol. Appl. Microbiol. Biotechnol. 56:120-125.

Saha, B. C. 2001. Enzymatic Saccharification of Complex Heteroxylan in Corn Fiber. Proc. U.S.-Japan Cooperative Program in Natural Resources (UJNR) Protein Resources Panel 30th Annual Meeting, Tsukuba, Ibaraki, Japan, October 15-19, 2001. pp. 184-190.

Weil, J. R., B. Dien, R. Bothast, R. Hendrickson, N. S. Mosier, and M. R. Ladisch. 2002. Removal of Fermentation Inhibitors Formed during Pretreatment of Biomass by Polymeric Adsorbents. Ind. Eng. Chem. Res. 41:6132-6138.

Wu, Y. V. and R. A. Norton. 2001. Enrichment of Protein, Starch, Fat, and Sterol Ferulates from Corn Fiber by Fine Grinding and Air Classification. Ind. Crops and Products 14:135-138.

- **Novel application of *Lactobacillus plantarum* as biocatalyst for ethanol production**
  Genetic modification of a strain of *Lactobacillus plantarum* to express pyruvate decarboxylase (PDC), an enzyme involved in the production of ethanol. Evaluation of this modified strain in flask fermentations demonstrated that the PDC enzyme was expressed in an active form and that the modified strain produced increased levels of ethanol in comparison to the parent strain. Although significant optimization of the recombinant strain remains to be explored, these results demonstrate that metabolic engineering of lactic acid bacteria is a viable strategy for the development of new biocatalysts for the production of bioethanol. This work has lead to the publication of manuscripts in peer-reviewed journals.

Liu, S. 2006. A simple method to generate chromosomal mutations in *Lactobacillus plantarum* strain TF103 to eliminate undesired fermentation products. Applied Biochemistry and Biotechnology. 129-132:854-863.

Liu, S., Nichols, N.N., Dien, B.S., Cotta, M.A. 2006. Metabolic engineering of a *Lactobacillus plantarum* double LDH knockout strain for enhanced ethanol production. Journal of Industrial Microbiology and Biotechnology. 33(1):1-7.

Liu, S., Dien, B.S., Cotta, M.A. 2005. Functional expression of bacterial *Zymobacter palmae* pyruvate decarboxylase gene in lactic acid bacteria. Current Microbiology. 50:1-6.

Nichols, N. N., B. S. Dien, and R. J. Bothast. 2003. Engineering Lactic Acid Bacteria with Pyruvate Decarboxylase and Alcohol Dehydrogenase Genes for Ethanol Production from *Zymomonas mobilis*. J. Ind. Microbiol. Biotechnol. 30:315-321.

- **Developed cold ethanol displacement for enhanced wheat separation**
  Cold ethanol displacement is a wheat separation process which results in high yields of starch and wheat proteins (glutens) with enhanced properties. The wheat starch could be used to produce

bioethanol or other biobased products such as lightweight concrete, building materials and packaging materials. This work has led to one CRADA, one patent, and several peer reviewed publications

Robertson, G. H., Cao, T. K. and Ong, I. 1999. Wheat gluten swelling and partial solubility with potential impact on starch-from-gluten separation by ethanol washing. Cereal Chem. 76:843-845.

Robertson, G. H., Cao, T. K. and Irving, D. 2000. Effect of morphology of mechanically developed wheat flour and water on starch from gluten separation using cold ethanol displacement. Cereal Chem. 77:439-444.

Robertson, G. H. and Cao, T. K. 2001. Farinograph responses for wheat flour dough fortified with wheat gluten produced by cold-ethanol or water displacement of starch. Cereal Chem. 78:538-542.

Robertson, G. H. and Cao, T. K. 2002. Mixograph responses of gluten and gluten-fortified flour for gluten produced by cold-ethanol or water displacement of starch from wheat flour. Cereal Chem. 79:737-740.

Robertson, G. H. and Cao, T. K. 2003. Effect of processing on functional properties of wheat gluten prepared by cold-ethanol displacement of starch. Cereal Chem. 80:212-217.

Robertson, G. H. and Cao, T. K. 2004. Proteins extracted by water or aqueous ethanol during refining of developed wheat dough to vital wheat gluten and crude starch as determined by capillary-zone electrophoresis (cze). Cereal Chem. 81:673-680.

Robertson, G. H., Gregorski, K. S. and Cao, T. K. 2005. Changes in secondary protein structures during mixing development of high absorption (90%) flour and water mixtures. Cereal Chem. 83:136-142.

Robertson, G. H. and Orts, W. J. 2005. Cold ethanol for biorefining. Pages 1-13 in: 4th Intl. Starch Tech. Conf: Bioproducts. K. Rausch, V. Singh and M. Tumbleson, eds, University of Illinois, Champaign, Ill.

- **Developed processes for dry fractionation of field pea into enriched protein and starch streams and for fermenting the pea starch to ethanol**

  Ethanol yields from pea starch were comparable to that from corn starch, and the enriched protein was similar in protein content to high-protein soy meal, with a well balanced amino acid profile. This is the first work to demonstrate that pea starch can be fermented to ethanol and, importantly, that the process can be accomplished using commercial enzymes and yeast. The Northern Pulse Growers Association, which represents legume producers in the Northern and Midwestern U.S., has offered financial support to test the process at a larger scale. An informational bulletin released by the USDA has been widely reprinted and led to several interviews, including Harper's Magazine, The National Journal, and Forbes Small Business.

  Nichols, N. N., B. S. Dien, Y. V. Wu, and M. A. Cotta. 2005. Ethanol Fermentation of Starch from Field Peas. Cereal Chemistry. 82(5):554-558.

  Wu, Y. V. and N. N. Nichols. 2005. Fine Grinding and Air Classification of Field Pea. Cereal Chemistry 82:341-344.

- **Developed novel biochemical/chemical processing methodology to produce butanol from wheat straw**

  Butanol, which can serve not only as fuel but also as a chemical, can be produced from dilute acid pretreated enzymatically saccharified wheat straw hydrolyzate without using any detoxification step (lime treatment) typically required for dilute acid pretreated substrate. The fermentative bacterium efficiently utilized multiple sugars present in wheat straw hydrolyzate. Butanol was recovered simultaneously by gas stripping during fermentation. This solves the problem of product inhibition and will help to reduce the production cost of butanol significantly as a result of integration of fermentation with recovery. It is anticipated that the developed process would be economical to produce butanol from wheat straw.

  Ezeji, T. C., N. Qureshi, and H. P. Blaschek. 2004. Acetone Butanol Ethanol (ABE) Production from Concentrated Substrate: Reduction in Substrate Inhibition by Fed-Batch Technique and Product Inhibition by Gas Stripping. Appl. Microbiol. Biotechnol. 63:653-658.

Ezeji, T. C., N. Qureshi, and H. P. Blaschek. 2005. Continuous Butanol Fermentation and Feed Starch Retrogradation: Butanol Fermentation Sustainability Using *Clostridium beijerinckii* BA101. Journal of Biotechnology 115:179-187.

Ezeji, T. C., N. Qureshi, and H. P. Blaschek. 2007. Production of Acetone-Butanol-Ethanol (ABE) in a Continuous Flow Bioreactor Using Degermed Corn and *Clostridium beijerinckii*. Process Biochemistry 42:34-39.

Ezeji, T. C., N. Qureshi, and H. P. Blaschek. Bioproduction of Butanol from Biomass: From Genes to Bioreactors. Current Opinion in Biotechnology. Accepted 2-21-07.

Ezeji, T. C., N. Qureshi, and H. P. Blaschek. Production of Acetone Butanol (AB) From Liquefied Corn Starch, a Commercial Substrate, Using *Clostridium beijernickii* Coupled with Product Recovery by Gas Stripping. Submitted to J. Industrial Microbiology and Biotechnology 8/30/2005.

Ezeji, T. C., N. Qureshi, and H. P. Blaschek. 2004. Butanol Fermentation Research: Upstream and Downstream Manipulations. The Chemical Record 4:305-314.

Ezeji, T. C., P. M. Karcher, N. Qureshi, and H. P. Blaschek. 2005. Improving Performance of a Gas Stripping-based Recovery System to Remove Butanol from *Clostridium beijerinckii* Fermentation. Bioprocess and Biosystems Engineering 27:207-214.

Ezeji, T., N. Qureshi, and H. P. Blaschek. Butanol Production from Agricultural Residues: Impact of Degradation Products on *Clostridium beijerinckii* Growth and Butanol Fermentation. Biotechnology and Bioengineering. Accepted 1/29/07.

Ezeji, T., N. Qureshi, P. Karcher, and H. P. Blaschek. 2006. Production of Butanol from Corn. In: Minteer, S., editor. Alcoholic Fuels. Boca Raton, FL: Taylor & Francis Group. p. 99-122.

Karcher, P. M., T. C. Ezeji, N. Qureshi, and H. P. Blaschek. 2005. Microbial Production of Butanol: Product Recovery by Extraction. In: Satyanarayana, T., Johri, B.N., editors. Microbial Diversity: Current Perspectives and Potential Applications. New Delhi:I.K. International. p. 865-880.

Qureshi, N. and H. P. Blaschek. 2006. Butanol Production from Agricultural Biomass. In: Shetty, K., Paliyath, G., Pometto, A., Levin, R.E., editors. Food Biotechnology. Boca Raton, FL:Taylor & Francis. p. 525-549.

Qureshi, N. and I. S. Maddox. Production of Butanol from Concentrated Lactose/Whey Permeate Using *Clostridium acetobutylicum* and Removal by Perstraction. Submitted to the Journal of Applied Biochemistry and Biotechnology 4/25/03.

Qureshi, N. and L. S. Maddox. Reduction in Butanol Inhibition by Perstraction: Utilization of Concentrated Lactose/Whey Permeate by *Clostridium acetobutylicum* to Enhance Butanol Fermentation Economics. Transactions of the Institution of Chemical Engineers 83(C1):43-52.

Qureshi, N., B. C. Saha, and M. A. Cotta. Butanol Production from Wheat Straw by Simultaneous Saccharification and Fermentation Using *Clostridium beijerinckii*: Part II-Fed-batch Fermentation. Submitted to Biomass and Bioenergy 2/8/07.

Qureshi, N., B. C. Saha, R. E. Hector, S. R. Hughes, and M. A. Cotta. Butanol Production from Wheat Straw by Simultaneous Saccharification and Fermentation Using *Clostridium beijerinckii*: Part I - Batch Fermentation. Submitted to Biomass and Bioenergy 12/11/06.

Qureshi, N., L. L. Lai, and H. P. Blaschek. 2004. Scale-up of a High Productivity Continuous Biofilm Reactor to Produce Butanol by Adsorbed Cells of *Clostridium beijerinckii*. Food and Bioproducts Processing 82(C2):164-173.

Qureshi, N., P. Karcher, M. Cotta, and H. P. Blaschek. 2004. High-Productivity Continuous Biofilm Reactor for Butanol Production: Effect of Acetate, Butyrate, and Corn Steep Liquor on Bioreactor Performance. Applied Biochemistry and Biotechnology 113-116:713-721.

Qureshi, N., S. Hughes, I. S. Maddox, and M. A. Cotta. 2005. Energy-Efficient Recovery of Butanol from Model Solutions and Fermentation Broth by Adsorption. Bioprocess and Biosystems Engineering 27:215-222.

Qureshi, N., T. C. Ezeji, J. Ebener, B. Dien, M. A. Cotta, and H. P. Blaschek. Butanol Production by *Clostridium beijerinckii*: Part I. Use of Acid and Enzyme Hydrolyzed Corn Fiber. Submitted to Bioresource Technology 11/8/06.

Qureshi, N., X. Li, S. Hughes, B. Saha, and M. Cotta. 2006. Butanol Production from Corn Fiber Xylan Using *Clostridium acetobutylicum*. Biotechnol. Prog. 22:673-680.

Saha, B. C. and R. J. Bothast. 1999. Production of 2,3-Butanediol by Newly Isolated *Enterobacter cloacae*. Appl. Microbiol. Biotechnol. 52:321-326.

- **High throughput proteomic workcell built and successfully demonstrated**
A plasmid-based functional proteomic workcell for high-throughput assembly, optimization, and modification of gene libraries and microbial strains has been constructed. Mechanical hardware was integrated and controlling software developed to provide a robotic platform that picks colonies, cultures bacteria, prepares plasmid DNA, performs in vitro transcription/translation, and assays enzyme activity. This workcell will significantly decrease the time intensive labor involved and speed discovery of ethanologenic yeast capable of fermenting xylose via high-throughput screening strategies. The workcell and its supporting technology have broad application in the field of laboratory automation, and are being commercially developed by our specific cooperator, Hudson Controls, Inc. The work has lead to the publication of two manuscripts in peer-reviewed journals.

Hughes, S.R., Riedmuller, S.B., Mertens, J.A., Li, X., Bischoff, K.M., Qureshi, N., Cotta, M.A., Farrelly, P.J. 2006. High-throughput screening of cellulase F mutants from multiplexed plasmid sets using an automated plate assay on a functional proteomic robotic workcell. Proteome Science. 4:10.

Hughes, S.R., Riedmuller, S., Mertens, J.A., Li, X., Bischoff, K.M., Cotta, M.A., Farrelly, P. 2005. Development of a liquid handler component for a plasmid-based functional proteomic robotic workcell. Journal of the Association for Laboratory Automation. 10(5):287-300.

- **Developed environmentally sensitive pretreatment of lignocellulose biomass**
Ferulic acid esterase pretreatment is potentially a non-corrosive, environmentally-friendly method to replace chemical (e.g., dilute sulfuric acid) pretreatment for lignocellulose to ethanol. This method takes advantage of the particular aromatic-carbohydrate linkages in grasses, specifically phenolic acid esterified to sugars. In laboratory studies, this enzymatic pretreatment before cellulase treatment for saccharification has showed a significant enhancement in release of sugars for fermentation over those released by cellulase alone. Further, ferulic acid is released in significantly higher concentrations. Ferulic acid is a particularly important compound that can be used to make vanillin (a food flavoring ingredient), antioxidant in foods, anti-aging and UV absorbent ingredient in creams and ointments, and an antimicrobial agent. Therefore, pretreatment of grasses with ferulic acid esterases offers to potential impact of increased sugar for ethanol and ferulic acid as a high-value co-product with many industrial applications.

Akin, D.E., Morrison, W.H. III, Rigsby, L.L., Barton, F.E. II, Himmelsbach, D.S., and Hicks, K.B. Corn stover fractions and bioenergy: chemical composition, structure, and response to enzyme pretreatment. Applied Biochemistry and Biotechnology 129-132: 104-116. 2006.

Anderson, W.F., Peterson, J., Akin, D.E., and Morrison, W.H. III. Enzyme pretreatment of grass lignocellulose for potential high-value co-products and an improved fermentable substrate. Appl. Biochem. Biotechnol. 121-124:303-310. 2005.

- **Developed solvent extraction methods for economic separation of ethanol and water**
Solvents with especially good performance in the separation of ethanol and water for use in solvent extraction from fermentation broths or in combination with membrane processes as alternatives to

energy inefficient distillation have been identified. Optimization of this technology could significantly impact the cost of ethanol production and may lead to development of continuous glucose fermentation. This research led to one CRADA, one patent application, and 4 publications.

Offeman, R.D., Stephenson, S.K., Robertson, G.H., Orts, W.J. Solvent extraction of ethanol from aqueous solutions. I. Screening methodology for solvents (2005) Industrial and Engineering Chemistry Research, 44 (17), pp. 6789-6796.

Offeman, R.D., Stephenson, S.K., Robertson, G.H., Orts, W.J. Solvent extraction of ethanol from aqueous solutions. II. Linear, branched, and ring-containing alcohol solvents (2005) Industrial and Engineering Chemistry Research, 44 (17), pp. 6797-6803

Offeman, R.D., Stephenson, S.K., Robertson, G.H., Orts, W.J. Solvent extraction of ethanol from aqueous solutions using biobased oils, alcohols, and esters (2006) JAOCS, Journal of the American Oil Chemists' Society, 83 (2), pp. 153-157.

Stephenson, S.K., Offeman, R.D., Robertson, G.H., Orts, W.J. Ethanol and water capacities of alcohols: A molecular dynamics study (2006) Chemical Engineering Science, 61 (17), pp. 5834-5840.

- **Completed quantitative surveys of ethanol facilities to identify contaminating microorganisms**
Quantitative surveys of bacterial populations in commercial corn-based ethanol facilities were performed. *Lactobacillus* species were the most abundant isolates, averaging 51%, 38%, and 77% of total isolates from a wet mill and two dry grind facilities, respectively. Although populations varied over time, individual facilities tended to exhibit characteristic bacterial profiles, suggesting the occurrence of persistent endemic infections. Results will be of interest to ethanol producers and researchers attempting to develop intervention strategies that control bacterial contamination. Under a Material Transfer Agreement, strains isolated from this work are being used by the recipients to test the effectiveness of new antibacterial agents. The work has lead to the publication of two manuscripts in peer-reviewed journals.

Skinner, K.A., Nicholls, N., Leathers, T.D. 2007. Biofilm Formation by Bacterial Contaminants of Fuel Ethanol Production. Biotechnology Letters. In press.

Skinner, K.A., Leathers, T.D. 2004. Bacterial contaminants of fuel ethanol production. Journal of Industrial Microbiology and Biotechnology. 31:401-408.

- **Demonstrated benefits of anhydrous ammonia pretreatment to steep corn kernels**
Showed that brief exposure of corn kernels to anhydrous ammonia could reduce the time required to steep (soften) corn kernels, lower the cost of fuel ethanol production, and may also improve coproduct recovery in wet-pre-fractionation processes such as the quick germ process. This innovative process was recognized by U.S. Patent 6,592,921 issued July 15, 2003: "Method of Removing the Hull from Corn Kernels " by F. Taylor and V. Singh. The process allows corn kernels to be more easily separated into component parts before ethanol production so that only fermentable components go into the fermentor, and non-fermentable products like corn germ and fiber can be used to make value-added coproducts. This process attracted attention of one of the major corn wet millers in the U.S. and resulted in the development of a CRADA with that organization to further develop ammoniation of corn kernels for improving the dry grind corn-to-ethanol process.

Corn-Milling Pretreatment with Anhydrous Ammonia, F. Taylor, J.C. Craig, Jr., M.J. Kurantz and V. Singh. Applied Biochemistry and Biotechnology (2003) 104:141-148

- **Created and made available corn grain to ethanol cost and production models**
Developed detailed technical (process and cost) models for the production of fuel ethanol from corn and made them available free of charge to stakeholders and partners on a number of different computer software platforms. These interactive models contain the numerous processing operations involved in the production of this important biofuel. In 2006 the models have been made available to

the USDA Office of the Chief Economist, U.S. Environmental Protection Agency, the National Corn to Ethanol Research Center, the Nebraska Ethanol Board, ADM, DuPont, Batelle Institute, Monsanto, Genencor , Novozymes, Booze Allen & Hamilton, Chevron, Cornel University, Lafayette College, Purdue University, Southern Illinois University, the Wharton School at the University of Pennsylvania, the University of Minnesota, the University of Illinois, Washington University, the University of Nebraska and more than one hundred other requestors. Access to this model has helped the recipients understand the costs, energy, inputs and outputs of the ethanol process and to guide research efforts to improve the process. They have used it for modeling, design and business decisions, and thus helped to improve the cost-effectiveness of fuel ethanol production from corn.

Modeling the Process and Costs of Fuel Ethanol Production by the Corn Dry-Grind Process. J.R. Kwiatkowski, A.J. McAloon, F. Taylor and D.B. Johnston. Industrial Crops and Products (2006) 23:288-296.

- **Developed new methods for dehulling barley for starch extraction and coproduct generation**
New milling methods were developed for dehulling barley and producing value added fractions for foods and fuel ethanol feedstock. This process removes the hard, abrasive hull on most barley varieties and fractionates the grain based on particle size and particle density. If this process proves to be economically feasible, it could lower the cost of fuel ethanol from barley by providing high-starch feedstock needed for high yields of ethanol, fiber and nutrient rich coproducts for nutritious foods, and hulls that could be used in a co-located fluidized bed gasifier or combustor, that could produce much of the process steam needed to run the ethanol plant. Because of the promise of this technology, a CRADA with one of the largest milling equipment manufacturers in the world is now being developed.

High-ß-Glucan Barley Fractions Milled with Experimental Mills. R.A. Flores, K.B. Hicks, D.W. Eustace and J.G. Phillips. Cereal Chemistry (2005) 82(6):727-733.

Accomplishments without listed peer review publications

- **Developed new azeotropic separation technology for ethanol purification**
A novel pervaporation membrane separation system for (a) removing ethanol from fermentation broths or (b) removing water from ethanol (azeotropic separation) has been developed and is patent pending. Researchers are working with industrial partners to test this process and related technology for ethanol "drying" before application in transportation fuel. This technology could enhance azeotropic separation of water from ethanol as an alternative to molecular sieve chromatography, thus obtaining neat ethanol at reduced costs.

- **Identified improved furulic acid esterases , enzymes that hydrolyze the linkages between lignin and hemicellulose.**
Isolated, cloned and expressed genes for esterase production and are applying directed evolution to improve enzyme activity and properties. This work is performed in collaboration with a corporate partner. Furulic acid esterases have been tested on rice and wheat straw and shown to improve digestibility, with fiber digestibility studies underway. Lignin is a complex macromolecule and difficult to separate from biomass material prior to cellulose hydrolysis, and therefore this research stands to impact biomass processing by reducing the processing steps and cost of lignin separation.

- **Completed pilot scale testing for pretreatment of straws for biofuel production in a co-mingled waste facility**
A pilot scale demonstration was built to addresses the need for flexible processing of lignocellulosic material via creation of the "athletic biorefinery"; whereby, biomass from a wide array of feedstocks is converted to ethanol within the same plant throughout all seasons. The ability for a biorefinery to

utilize multiple feedstocks will significantly impact the economics of biomass processing by eliminating the need to build single feedstock specific biomass processing plants. A corporate partner, CR^3, Reno, Nevada, is building a biorefinery facility based on this technology in Central California.

- **Developed quantitative analytical procedures to monitor lignin composition**
  Applied chemical analyses to quantify the change in lignin and change in lignin "quality" as a function of genetic modifications of both corn and switchgrass. Tested levels of all major cell wall components in several transgenic plant species and correlated this information with biofuels yield. Modifying and understanding lignin quality and concentration is critical economic driver for eventual application of biomass for biofuels.

- **Performed basic research on *Clostridium thermocellum* for cellulose processing**
  Four genes involved in cellulose degradation by *Clostridium thermocellum*, an obligate anaerobe that grows at relatively high temperatures, were shown to be regulated in a manner that varied in response to the conditions under which the bacterium is grown. This research provides new information on the regulation of cellulose degradation by an organism that shows particular promise for converting cellulosic biomass to both ethanol and a polymer having desirable properties as a wood adhesive.

- **Engineered yeast microorganisms to process starch**
  Isolated, cloned, and expressed the genes for raw starch-degrading enzymes in *Saccharomyces cerevisiae*, and through directed evolution, created enzyme mutants with enhanced activities. The recombinant enzymes synergistically hydrolyzed raw starch, and the genes were integrated into yeast chromosomes for cell-based saccharification and fermentation. Successful development of the enzyme system will enable the conversion of starch to glucose at low temperatures (cold hydrolysis), which will improve energy efficiency and reduce the cost of ethanol production. The research has resulted in one ongoing CRADA, one filed patent, and 18 peer-reviewed publications.

- **Developed genomic methods to identify and clone biomass processing enzymes from complex microbial sources**
  Isolated and characterized over two dozen novel genes for biomass-degrading enzymes by screening complex genomes of mixed environmental microbial sources. Several of these genes have been cloned and expressed, and the enzymes purified and characterized for unique properties, such as cold active, bi-functional, high catalytic efficiency, and desired substrate specificity. Successful development of a complete set of novel enzymes to collectively and synergistically hydrolyze major cell wall polymers will increase efficiency and extensibility of biomass degradation. This research has resulted in one ongoing CRADA, two patent disclosures, and 7 peer-reviewed publications.

- **New methods for barley starch processing developed**
  Key enzymes have been discovered that boost fuel ethanol production from barley. New feedstocks for fuel ethanol production are needed. Currently 95% of fuel ethanol is made from corn and continued growth of the ethanol market could create shortages of corn for food and feed uses. Barley could be a potential alternative feedstock if problems with its high viscosity and low ethanol yields could be solved. Our research has found a new combination of enzymes (β-glucanases and β-glucosidases) that has the potential to solve problems of viscosity and increase ethanol yields another 2-4%. The key discovery was the use of β-glucosidase enzymes that are currently missing from commercial enzymes for barley conversion to ethanol. Development of new commercial enzyme preparations using this new knowledge will greatly benefit the small, but growing barley-to-fuel ethanol industry, which will allow building of ethanol plants outside the corn belt, in the barley belts of the East Coast, Northwest, and Upper Midwest and produce an additional 1-2 billion gallons of fuel ethanol for the U.S. market. Because of the significance of this work, one of the largest enzyme

companies in the world entered into a CRADA with our labs to fully develop new commercial processes for making ethanol from fuel ethanol.

Topic: Coproduct Development

Goal: To develop higher-valued new coproducts or improve the existing ones from the non-starch portion of the grain, resulting in a reduced net cost of the starch used for producing ethanol.

Summary: Numerous coproducts and bioproducts can be derived from both starch and cellulosic biomass processing. These products include enriched protein and fat products for livestock feed, high value corn oil, phytosterols, and other valuable products such as sweeteners, adhesives, and environmentally sensitive materials. Coproducts represent an important component to the successful commercialization of the biomass to ethanol industry. Coproducts are a potentially high revenue stream which may significantly and positively impact the economics of both corn grain and cellulose processing.

Significant Accomplishments & Impact:

- **Developed simple, inexpensive methods to obtain enriched protein, starch, and fat from corn fiber, and other biomaterials**
  A corn fiber gum (Zeagen) patent (U.S. 6,147,206) was co-developed with a major food company and a corn fiber gum product is very close to being commercialized. Seven other major companies are currently expressing interest in developing and using the technology if co-development can be arranged. Corn fiber oil is a high value dietary supplement that has been shown to lower cholesterol levels. A corn fiber oil patent (U.S. 5,843,499) has been licensed by two separate companies, but both encountered obstacles during development. The corn fiber oil patent is now available for licensing. Our numerous publications on corn fiber oil and its unique functional lipid components have indirectly benefited the development of corn kernel oil (its composition is a combination of corn germ oil and corn fiber oil). We have learned about a company that is planning to produce a corn kernel oil product in the near future. Due to our major patents, publications, consultation, and presentations from this research area, interest in phytosterols and corn fiber arabinoxylan is evident all around the world. Every major corn and soybean processor in the United States has contacted our scientists and has their own phytosterol products on the market. One major company used our technology to gain DOE funding for a corn fiber biorefinery in their wet mills. A major announcement of this project is forthcoming. Corn fiber is only worth $.02 per pound and corn fiber oil is worth >$8/pound; corn fiber gum could be worth from $2-4/pound. This is real "value added".

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Mellon, J.E., and Moreau, R.A., Inhibition of aflatoxin biosynthesis in *Aspergillius flavus* by diferuloylputrescine and p-coumaroylputrescine, J. Agric. Food Chem. 52:6660-6663 (2004).

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Moreau, R.A., and Hicks, K.B., A reinvestigation of the effect of heat pretreatment of corn fiber on the levels of extractable tocopherols and tocotrienols, J Ag Fd Chem. 54:8093-8102, 2006.

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Moreau, R.A., Hicks, K.B., and Norton, R.A. Phytosterols and phytostanols lower cholesterol. International News on Fats Oils and Related Materials (INFORM) 10:572-577. 1999.

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- **Developed a new biochemical process to extract >90% of corn oil from distillers' dried grains**
An aqueous enzymatic process to extract greater than 90% of the corn oil from oven dried corn germ produced by wet milling has been developed. This technology could replace the current process that uses hexane, a toxic and flammable organic solvent, for extracting corn oil. Because of the explosive expansion in the fuel ethanol industry and the desire to maximize coproduct profits by co-producing corn germ and corn oil at "new generation" dry-grind ethanol plants, there has been much interest in our Aqueous Enzymatic Oil Extraction (AEOE) project. The potential of this method is due to the fact that an AEOE process could be set up inexpensively to extract oil from corn germ right at the ethanol plant so that the plant could sell crude corn oil at $.30/lb rather than dry mill germ at only $.05-.10 per pound. Without AEOE technology, one would have to use expellers or hexane extraction, both very expensive technologies that may be out of the reach of most corn dry grind ethanol plants. We have been invited to give presentations on our AEOE project at symposia at two international meetings and to write a chapter of an upcoming ACS book. Because the project is relatively new, it is not yet possible to predict long term impact but based upon interest in the technology and preliminary results, the future is promising.

  Moreau, R.A., D.B. Johnston, M.J. Powell, and K.B. Hicks, A Comparison of Commercial Enzymes for the Aqueous Enzymatic Extraction of Corn Oil from Corn Germ, J. Am. Oil. Chem. Soc. 81; 1071-1075, 2004.

  Moreau, R.A., D.B. Johnston, and K.B. Hicks, The Influence of Moisture Content and Cooking on the Screw Pressing and Pre-Pressing of Corn Oil from Corn Germ, JAOCS 82:851-854, 2005.

  Singh, V., Moreau, R.A., Hicks, K.B., and Eckhoff, S.R. Effect of alternative milling techniques on the yield and composition of corn germ oil and corn fiber oil. Cereal Chem. 78:46-49. 2001.

- **Developed and optimized processes to produce 'quick fiber' oil containing high value products**
Process parameters to produce 'quick fiber' oil with high levels of phytosterols have been optimized. ARS scientists discovered that high levels of phytosterols were only present in the "quick corn fiber oil" when SO_2 was added during the steeping of the corn. If corn fiber oil is commercialized in the future, then quick fiber may become an economical feedstock for corn fiber oil.
Publications:

  Dien, B.S., Nagle, N., Hicks, K.B., Singh, V., Moreau, R.A., Tucker, M.P., Nichols, N.N., Johnston, D.B. Cotta, M.A., Nguyen, Q., and Bothast, R.J., Fermentation of "quick fiber" produced from a modified corn-milling process into ethanol and recovery of corn fiber oil, Appl. Biochem. Biotechnol. 113-116:937-949, 2004.

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  Singh, V., Moreau, R.A., Hicks, K.B., Belyea, R.L., and Staff, C.H. Removal of fiber from distillers dried grains with solubles (DDGS) to increase value. Trans. A.S.A.E. 45:389-392. 2002.

- **Identified physiological location of phytosterols in corn fiber**
Discovered that almost all of the phytosterols in corn fiber are in the aleurone cells (about one third by weight) of the fiber and developed a process to purify the aleurone cells and obtain corn fiber oil from them. It was demonstrated that this new process could lower the cost of producing corn fiber oil and could make it an economically viable coproduct from ethanol production. After the discovery that

most of the phytosterols in corn fiber are in the aleurone layer, ARS scientists developed and patented a process to fractionate corn fiber into an aleurone-enriched fraction. Although this processing step produces a feedstock with higher levels of phytosterols, the improvement is a small one and the improved economics of corn fiber oil production are also modest. There has been commercial interest in this proprietary technology but currently it is less costly to obtain phytosterols from other sources.

Moreau, R.A., Singh, V., Nunez, A., Hicks, K.B. Phytosterols in the aleurone layer of corn kernels. London, Portland Press, Biochem. Soc. Trans. 28:803-806. 2000.

Singh, V., Moreau, R.A., and Cooke, P.H. Effect of corn milling practices on the fate of aleurone layer cells and their unique phytosterols. Cereal Chem. 78:436-441. 2001.

Singh, V. and Moreau, R.A. Enrichment of Oil in Corn Fiber by Size Reduction and Floatation of Aleurone Cells. Cereal Chem. 80:123-125. 2003.

Singh, V. and Moreau, R.A., Methods of preparing corn fiber oil and of recovering corn aleurone cells from corn fiber, U.S. patent 7,115,295, issued October 3, 2006.

- **Performed basic research towards identifying corn genotypes with higher levels of phytosterols**
Extracted oil from multiple aleurone lines and found that some had higher levels of phytosterols than common yellow dent corn. These findings could lead to development of corn genotypes suitable for economic extraction of corn fiber oil and that, as a result, would reduce the net cost of ethanol production. Most corn cultivars and hybrids have an aleurone layer that consists of one layer of aleurone cells. However, some cultivars and hybrids have multiple aleurone layers. Because of their earlier discovery that most of the phytosterols in corn fiber are in the aleurone layer, ARS scientists measured the levels of phytosterols in several "multiple aleurone" corn cell lines and confirmed that the levels of phytosterols were higher. This information may be valuable to corn breeders who could potentially breed a high phytosterol hybrid by breeding genes for multiple aleurone layers.

Moreau, R.A., Singh, V., and Hicks, K.B. A comparison of the levels of oil and phytosterols in the seeds of germplasm accessions of corn, teosinte, and Job's tears. J. Agric. Food. Chem. 49:3793-3795. 2001.

Singh, V., Moreau, R.A., Haken, A.E., Eckhoff, S.R., and Hicks, K.B. Hybrid variability and effect of growth location on corn fiber yields and corn fiber oil composition. Cereal Chem. 77:692-695. 2000

- **Developed and optimized processes for continuous extraction of zein**
Verified that continuous extraction of zein, a major corn protein, is feasible. Also, developed a technology for removing the solids from a zein isolate by passing the ethanol extract over a layer of water and allowing the solids to settle. These technologies could significantly lower the cost of zein and allow its more abundant use in biobased adhesives, coatings, and films. It was demonstrated that a zein product with a cost of approximately one dollar per pound could be produced by extracting whole ground corn. All commercial zein is produced by extracting corn gluten meal (a 60% protein coproduct of corn wet milling, which is commonly used as premium animal feed) but we showed that the composition of zein was different when it was obtained from whole ground corn and this zein product may have advantages for some applications. Many companies expressed interest in our zein research. We recently learned that a U.S. company is planning to produce a new zein product and their process will obtain zein by the extraction of whole ground corn.

Dickey, L.C. and N. Parris, Improving particle separation from an ethanol extract to water: Settling dependence on fine particle content, Industrial Crops and Products 21:379-385, 2005.

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Dickey, L.C., A.J. McAloon, J.C. Craig and N. Parris, Estimating the cost of extracting cereal protein with ethanol, Industrial Crops and Products 10:137-143, 1999.

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Parris, N., Dickey, L.C., Wiles, J.L., Moreau, R.A., and Cooke, P.H. Enzymatic hydrolysis, grease permeation, and water barrier properties of zein isolate coated paper. J. Agric. Food Chem 48:890-894. 2000.

Parris, N., Douds, D.D., Dickey, L.C., Moreau, R.A., and Phillips, J., Effect of zein films on growth of tomato plants and evaporative water loss, Hort. Science 39:1324-1326, 2004.

- **Oil from hulless barley found to contain beneficial coproducts**

Oil extracted from nonfermentable portions of hulless barley kernels is high in phytosterols, tocopherols and tocotrienols, all of which have beneficial dietary attributes. Profits from this coproduct could improve the economics and establish hulless barley as a viable energy crop. Previous researchers have reported that barley oil contains high levels of tocotrienols, a group of vitamin E isomers which are receiving much attention because they can lower serum cholesterol (by inhibiting its biosynthesis, similar to the mechanism of statins) and inhibit the growth of several cancer cell lines. Because barley kernels only contain about 2% oil, it would probably not be economically feasible to obtain barley oil by extracting oil from the kernels. However, ARS scientists have demonstrated that a novel barley oil, with very high levels of tocotrienols can be obtained by extracting the abraded fines fraction obtained by pearling or scarification of hulled or hulless barley. The levels of tocotrienols in this "barley fines oil" are the highest that have ever been reported in any natural oil, even higher than in palm oil and rice bran oil, which were previously thought to have the highest levels of tocotrienols (and are currently the commercial sources of tocotrienols). Because barley pearling fines and barley scarification fines contains high levels of oil (8-10%) it would probably be economically feasible to use them as a feedstock to obtain a high-tocotrienol barley fines oil. An indication of the level of interest in this project is the fact that ARS researchers were invited to give a presentation about this research in 2006 at a symposium at the annual AOCS meeting. Producing a nutraceutical oil from barley could be part of an overall barley biorefinery that could also produce health-promoting beta-glucans and much-need fuel ethanol. Another ARS researcher was asked to speak on this at the 2007 AACC meeting. The full impact of this discovery will be known in the future.

Lampi, A-M., Moreau, R.A., Piironen, V., and Hicks, K.B., Pearling barley and rye to produce phytosterol-rich fractions, Lipids 39:783-787, 2004.

Moreau, R.A., Flores, R., and K.B. Hicks, The composition of functional lipids in hulled and hulless barley, in fractions obtained by scarifiction, and in barley oil, Cereal Chem. 84:1-5, 2007

- **Production of bioactive peptides from protease treated corn germ**
  Treating corn germ with certain protease enzymes such as thermolysin or flavourzyme produces peptides that could act as physiological modulators in the human body. One such effect might lower blood pressure since these peptides inhibit angiotensin I-converting enzyme. The ability to produce valuable nutraceuticals from the proteins in corn would improve the long-term business stability of corn-ethanol producers. In addition to lowering blood pressure in humans and farm animals, bioactive peptides that inhibit angiotensin I-converting enzyme also have many other biological properties (antibiotic, antifungal, diuretic, treating congestive heart failure, etc.). A fairly simple treatment of corn germ by common proteases has been demonstrated to produce bioactive peptides; these changes may likely increase its feed and food value. Because the project is a new one its long term impact is not yet known, but the technology may prove to be an economical alternative production scheme for bioactive peptides.

  Parris, N., L.Dickey, and R. Moreau, Characterization of proteins in wet milled corn germ and dry milled corn germ, J Ag Fd Chem 54: 4868-4872, 2006.

- **Developed methods for enzymatic milling (e-milling) to recover high value coproducts from dry grind ethanol processing**
  The concept of enzymatic milling to the dry grind ethanol process for the purpose of recovering additional high value coproducts (germ and pericarp fiber) has been adapted and optimized. Bench scale and pilot scale procedures for fractionation have been developed and evaluated. This process has been patented (Johnston, D.B. and Singh, V. (2005) "Processes for Recovery of Corn Germ and Optionally Corn Coarse Fiber (Pericarp)" U.S. Patent # 6,899,910.). Information regarding this process has been shared at meetings and with engineering design firms specializing in ethanol production. As ethanol margins decrease under high corn costs and lower gasoline prices, wet pre-fractionation processes like these will become essential to ethanol plant profitability. Currently one of the most successful milling equipment manufacturers in the world is negotiating a CRADA for development of this and other ERRC technology. As part of this study, ERRC researchers did the first compositional and economic analysis of corn germ that can be produced as a coproduct from ethanol plants using a number of proposed processes. The study, which was published in 2005 (paper 2, below), was given a "Best Paper Award" at the 2005 AOCS meeting.

  Singh, V, Johnston, D.B., Naidu, K., Rausch, K.D., Belyea, R.L. and. Tumbleson, M.E. (2005) "Comparison of Modified Dry Grind Corn Processes for Fermentation Characteristics and DDGS Composition". Cereal Chemistry 82(2): 187-190.

  Johnston, D.B., McAloon, A.J., Moreau, R.A., Hicks, K.B. and Singh, V. (2005) "Composition and Economic Comparison of Germ Fractions Derived from Modified Processing Technologies". JOACS 82(8): 603-608.

  Dien, B.S., Cotta, M.A., Johnston, D.B., Hicks, K.B., and Singh, V. (2005) "Hydrolysis and Fermentation of Pericarp and Endosperm Fiber Recovered from Enzymatic Corn Dry Grind Process". Cereal Chemistry 82(5): 616-620.

  Wang, P., V. Singh, L. Xu, K. D. Rausch, M. E. Tumbleson, and D. B. Johnston. (2005). Comparison of enzymatic (E-Mill) and conventional dry-grind corn processes using a granular starch hydrolyzing enzyme. Cereal Chemistry 82(6): 734-738.

- **Production of high value sweetener by recombinant bacteria**
  A recombinant bacterium that produced xylitol, a low calorie anticariogenic sweetener, from xylose with very high productivity from a mixture of xylose and arabinose without producing any arabitol from arabinose, has been developed. Constructed two recombinant bacterial strains that converted arabinose to xylitol using different conversion routes. The production of xylitol by fermentation,

using hemicellulosic biomass such as agricultural residues, is attractive for reducing the processing cost of biomass conversion to ethanol.

Leathers, T. D. and B. S. Dien. 2000. Xylitol Production from Corn Fibre Hydrolysates by a Two-stage Fermentation Process. Process Biochem. 35:765-769.

Saha, B. C. and R. J. Bothast. 1999. Production of Xylitol by *Candida peltata*. J. Ind. Microbiol. Biotechnol. 22:633-636.

- **Evaluation of Distillers' Dried Grains (DDGs) physical properties**
  Physical and chemical properties of typical corn-based DDGs streams were quantified, including flowability properties. DDGS flowability appears to primarily depend on moisture content, fat content, storage time, and storage pressure. DDGS are the principle coproduct of corn grain ethanol processing and represent an alternative protein, fat and nutrient source for livestock. Considering the significant expansion of the corn grain to ethanol industry, DDGS 2007 production is estimated to surpass 17 million tons. Research into the physical and chemical properties of DDGS is essential to facilitate DDGS use as a practical, economical, and nutritional corn meal substitute.

Rosentrater, K. A. 2006. Some physical properties of distillers dried grains with solubles (DDGS). Applied Engineering in Agriculture 22(4): 589-595.

Rosentrater, K. A. and K. Muthukumarappan. 2006. Corn ethanol coproducts: generation, properties, and future prospects. International Sugar Journa*l* 108 (November): 1295

- **Developed methods to utilize DDGs as aquaculture feed**
  Laboratory-scale extrusion processing techniques for corn-based DDGS in order to produce aquaculture feeds for Nile Tilapia has been developed. DDGS appears to be a promising protein alternative compared to fish meal, but the lack of starch poses challenges with pellet durability and cohesion.

Chevanan, N., K. A. Rosentrater, and K. Muthukumarappan. 2007. Effects of Processing Conditions on Single Screw Extrusion of Feed Ingredients Containing DDGS. Cereal Chemistry (in review)

Chevanan, N., K. Muthukumarappan, and K. A. Rosentrater. 2007. Effect of Die Dimensions on Extrusion Processing Parameters and Properties of DDGS-Based Aquaculture Feeds. Cereal Chemistry (in review).

- **Incorporated corn-based DDGs into plastic composites**
  DDGs appear to be a promising biodegradable filler, but inclusion can lead to decreased mechanical strength. Through research, other DDGs based products could be developed to add increased value to the product and corn grain to ethanol industry.

Tatara, R. A., S. Suraparaju, and K. A. Rosentrater. 2007. Compression molding of phenolic resin/corn-based DDGS blends. Journal of Polymers and the Environment (in press).

Accomplishments without listed peer review publications

- **Developed processes to extract valuable coproducts and lignin from flax shive waste**
  Methods for delignification of flax shives, identification of aromatic co-products, and subsequent fermentation of residue to ethanol (preliminary study) are under development. Impact: flax shive, the lignified waste material left over after processing flax fiber, is a major part of the flax stem. In laboratory trials, lignins and aromatics, which are diverse, have been extracted and identified. The residue after extraction of aromatics is mostly cellulose, which has been saccharified with cellulose and subsequently fermented to ethanol. Therefore, from an already collected waste product at the processing plant of several hundreds of tons of material, extracted aromatics could provide

antioxidants and antimicrobial agents and a carbohydrate for fermentation to ethanol. Currently, the value of shive (animal bedding, burning) is about $ 0.05 / ton.

- **Potential biobased adhesive identified from cellulosic processing**
  Discovered that residues of consolidated bioprocessing of cellulosic materials to ethanol by the bacteria *Ruminococcus albus* or *Clostridium thermocellum* yields a fermentation residue that displays novel adhesive properties, and that may be useful as a bio-based adhesive or co-adhesive for preparation of plywood panels (patent pending; 3 publications). This technology may provide a value added coproduct of biomass processing which could enhance biomass processing economics.

Component II: Biodiesel

Vegetable oils and animal fats and their derivatives (biodiesel) are attractive as alternative fuels, extenders, and additives for compression ignition (diesel) engines. Opportunities for biodiesel include off-road markets such as underground mines, marine applications, mass transit (subways, trains), and stationary power generation. However, research is needed to improve cold start-up and operability, to identify and reduce harmful exhaust emissions (e.g., nitrogen oxides), to develop a rapid and low-cost fuel quality test, and to reduce feedstock and processing costs. [Note that the work reported from ERRC is largely from CWU 1935-41000-066, which is coded to NP306 and initially minority-coded to NP307. Work on the fermentation of glycerol emanates from ERRC CWU 1935-41000-067, which is and has always been coded entirely to NP306.]

Topic: Combustion and exhaust emissions

Goal: Develop technology to meet exhaust emissions regulations.

Summary: Although there are new challenges to the earlier findings that biodiesel combustion leads to increased emissions of nitrogen oxides (NO_x), those findings correlated the amount of NO_x emissions to the amount of unsaturation in the biodiesel molecules; emissions from soy biodiesel were shown to be higher than those from the more saturated biodiesel derived from animal fat. ERRC researchers speculated that the presence of small amounts of antioxidants in the fuel might suppress the amount of NO_x emissions. New insights regarding exhaust emissions generated by a new-technology engine operating on biodiesel were obtained. New cetane test results showed the suitability of a new method for cetane testing. Additives for reducing NOx exhaust emissions were identified.

Significant Accomplishments & Impact:

- **Determined additives can be effective to reduce NOx biodiesel emissions**
  Several potentially effective additives that reduce NOx emissions from biodiesel fuels have been identified. NOx exhaust emissions are the only kind of regulated exhaust emissions species that are problematic for biodiesel. This work is of significance for older technology diesel engines not meeting new exhaust emissions regulations.

  G. Knothe, M.O. Bagby, T.W. Ryan, III, Cetane Numbers of Fatty Compounds: Influence of Compound Structure and of Various Potential Cetane Improvers, SAE Techn. Paper Series No. 971681, published in SP-1274.

- **ARS scientists developed NOx biodiesel reducing additives and demonstrated effectiveness in collaboration with Department of Energy**
  ERRC researchers developed NOx-reducing fuel additives (oil-soluble antioxidants) for biodiesel, demonstrating their effectiveness in an on-site emissions-instrumented diesel engine at ERRC and in collaboration with DOE-National Renewable Energy Lab (NREL, CO) engine emissions team. Reducing NOx emissions is an essential researchable goal and necessary for EPA standards.

  Hess, M. A.; Haas, M. J.; Foglia, T. A.; and Marmer, W. N. Effect of antioxidant addition on NO_x emissions from biodiesel. Energy and Fuels, 19(4):1749-1754, 2005.

  Hess, M. A., Haas, M. J., and Foglia T. A. Reformulating Biodiesel to Reduce NO_x Emissions. Proceedings of the 231st American Chemical Society National Meeting, American Chemical Society, Atlanta GA, March 2006.

  Hess, Haas, Foglia. Reformulating Biodiesel to Reduce NO_x Emissions. Submitted to Fuel Processing Technology. In review. (2007)

- **Comparison of biodiesel and diesel performance in a modern engine**
  The first tests of biodiesel versus petrodiesel and components of both fuels in a modern technology engine were conducted. Biodiesel generated particulate matter emissions nearly at the low level of new exhaust emissions regulations. This may affect the technology level of exhaust emissions treatment for engines running on biodiesel suggesting biodiesel emissions can be decreased through engine improvements.

  G. Knothe, C.A. Sharp, T.W. Ryan III, Exhaust Emissions of Biodiesel, Petrodiesel, Neat Methyl Esters, and Alkanes in a New Technology Engine, Energy Fuels 20 (1), 403-408 (2006).

- **Developed new analytical methods to determined cetance number of biodiesel**
  The cetane number is an indicator of the ignition quality of a diesel fuel. The usual method for its determination is rather cumbersome, requiring a so-called cetane engine and large amounts of fuel. A new method utilizing a so-called Ignition Quality Tester was applied to biodiesel and its components, showing that it is well-suited. This procedure simplifies cetane testing for all applications, facilitating routine determinations also for commercial applications potentially encouraging further commercialization of biodiesel fuel.

  G. Knothe, A.C. Matheaus, T.W. Ryan III, Cetane Numbers of Branched and Straight-Chain Fatty Esters Determined in an Ignition Quality Tester, Fuel 82, 971-975 (2003).

- **Determined blending petroleum diesel with biodiesel rendered from animal fats had lower NOx emissions**
  Found that, when blended at 20% with petroleum diesel, biodiesel from animal fats had lower nitrous oxide emissions than did biodiesel from soybean oil. The lubricity and oxidative stability were also better for biodiesel from animal fats; however, the cold temperature properties were poorer. ERRC researchers produced biodiesel from underutilized low-cost animal-derived lipids, including tallow, lard, chicken fat and restaurant grease. Characterized resulting fuel and conducted small-scale engine testing on site. Formed a CRADA with NCAUR, the rendering industry technical group Fats and Proteins Research Foundation, and the University of Illinois to further explore emissions properties at the latter location's engine test lab. Animal fats are now used in Europe and the North America (Canada and portions of the U.S.). A recent study at Kansas State Univ. (Nelson et al.) showed the economic viability of such conversions. Results of ongoing collaboration between ERRC and NCAUR on how biodiesel properties relate to the amount of unsaturation in the biodiesel molecule has enticed the National Renewable Energy Laboratory, DOE, Golden, CO, to undertake heavy duty engine testing of these fuels.

Foglia, T. A., Nelson, L. A., and Marmer, W. N., "Production of Biodiesel, Lubricants and Fuel and Lubricant Additives" U.S. Patent 5,713,965, February 3. 1998.

Abigor, R.D. [NIFOR], Uaidia, P.O. [NIFOR], Foglia, T.A., Haas, M.J., Jones, K.C., Okpefa, E. [NIFOR], Obibuzor, J.U. [NIFOR], and Bafor, M.E. [NIFOR], Lipase-catalyzed production of biodiesel fuel from some Nigerian lauric oils, Biochem. Soc. Trans., 28:979-801, 2000.

Foglia, T. A., Jones, K. C., Haas, M. J., and Scott, K. M., Technologies supporting the adoption of biodiesel as an alternative fuel. In "Proceedings of the 49th Oilseed Conference: Surviving in a Changing Global Economy" held at New Orleans, LA Sponsored by AOCS, NCPA & ARS. Published by AOCS Press, Champaign, IL. (2000).

Foglia, T. A., Nelson, L. A., Dunn, R. O. [N], and Marmer, W. N., Low-Temperature properties of alkyl esters of tallow and grease. J. Am. Oil Chem. Soc. 74: 951-955. 1997.

Foglia, T. A., Nelson, L. A., Marmer, W. N., Knothe, G. [N], Dunn, R. O. [N], and Bagby, M. O. [N], Improving the low-temperature properties of vegetable oils and fats for use as biodiesel. Vol.1, pp. 121-125. In Proceedings of the World Conference on Oilseed and Edible Oil Processing, Koseoglu, S. S., and Rhee, K. C. (eds.), AOCS Press, Champaign, IL. 1997.
Goering and Briggs: Univ. of Illinois

Moser, [N] Haas, Wikler, [N] Jackson, [N] Erhan, [N] List [N]. Evaluation of Partially Hydrogenated Soybean Oil Methyl Esters as Biodiesel. European J. Lipid Science and Technol. 109, p. 17-24, 2007.

Wu W. H., Foglia, T. A., Marmer, W. N., and Phillips, J. G., Optimizing production of ethyl esters of grease using 95% ethanol by response surface methodology. J. Am. Oil Chem. Soc., 76:517-521, 1999.

Wu, W-H, Foglia, T. A., Marmer, W. N., Dunn, R. O. [N], Goering, C. E., and Briggs, T. E., Low-temperature property and engine performance evaluation of ethyl and isopropyl esters of tallow and grease. J. Am. Oil Chem. Soc. 75:1173-1178. 1998.

Wyatt, V. T., Hess, M. A., Dunn, R. O. [N], Foglia, T. A., Haas, M. J., and Marmer W. N. Fuel properties and nitrogen oxide emission levels of biodiesel produced from animal fats. J. Am. Oil Chem. Soc., 82:585-591, 2005.
[N] = NCAUR collaborator.
[NIFOR] = NIFOR, Nigeria Inst. for Oil Palm Res.

Topic: Fuel quality testing and on-line process control

Goal: Develop technology for rapid, easy-to-use process control and for fuel quality analysis.

Summary: The rapidly expanding market for biodiesel demands the strict monitoring of fuel quality during its production and storage. The consequence of substandard fuel, as experienced in Minnesota during the winter of 2005-2006, resulted in a severe drop in public confidence for the use of this alternative fuel. The tools available for quality monitoring have been limited and complex. The targeted species for such monitoring--free glycerol and total glycerides, blend levels (% biodiesel in petrodiesel), and cold temperature properties, for example—have increased as EPA requirements become stricter (e.g., sulfur) and on-the-road experience brings to light new fuel issues as a result of minor contaminants in the fuel (e.g., constituents found in clogged fuel filters). Various analytical methods such as near-infrared spectroscopy and high-performance liquid chromatography were adapted to analyzing biodiesel in form of the finished product as well as monitoring its production. These methods present alternatives to the slow and cumbersome existing chromatographic method. Physical properties of biodiesel were evaluated and it was found that minor components of biodiesel play a significant role in imparting lubricity to low-level blends of biodiesel with petrodiesel. The effect of structure of fatty compounds on

viscosity was determined, showing that some feedstocks may yield biodiesel beyond specifications in standards if these specifications are tightened.

Significant Accomplishments & Impact:

- **Analytical methods to assess biodiesel quality developed and demonstrated**
  A rapid and easy-to-use near-infrared spectroscopy analytical method for assessing biodiesel quality in terms of conversion of vegetable oil to biodiesel has been developed. This method, which requires less than a minute, is an alternative to the relatively slow gas chromatography method now used and has potential for development into an on-line production monitoring system. The method was expanded to monitoring the transesterification reaction which produces biodiesel and also to the determination of blend levels of biodiesel with petrodiesel.

  G. Knothe, Rapid Monitoring of Transesterification and Assessing Biodiesel Fuel Quality by Near-Infrared Spectroscopy Using a Fiber-Optic Probe, J. Am. Oil Chem. Soc. 76, 795-800 (1999)

  G. Knothe, Monitoring a Progressing Transesterification Reaction by Fiber-Optic Near-Infrared Spectroscopy with Correlation to ^{1}H Nuclear Magnetic Resonance Spectroscopy, J. Am. Oil Chem. Soc. 77, 489-493 (2000)

  G. Knothe, Determining the Blend Level of Mixtures of Biodiesel with Conventional Diesel Fuel by Fiber-Optic Near-Infrared Spectroscopy and ^{1}H Nuclear Magnetic Resonance Spectroscopy, J. Am. Oil Chem. Soc. 78, 1025-1028 (2001)

  G. Knothe, Analyzing Biodiesel: Standards and Other Methods, J. Am. Oil Chem. Soc. 83 (10), 823-833 (2006)

- **Developed methods to quickly determine fatty acid composition of biodiesel**
  A rapid and reliable spectroscopic method for determining the fatty acid composition of biodiesel fuel has been developed. Results with this new method were in good agreement with measurements made with the chromatographic method normally used which is not suitable for all kinds of samples. Thus this method was applied to oxidized biodiesel with new insights on the composition of this material.

  G. Knothe, J.A. Kenar, Determination of the fatty acid profile by 1H NMR spectroscopy, Eur. J. Lipid Sci. Technol., 106, 88-96 (2004)

  G. Knothe, Analysis of Oxidized Biodiesel by ^{1}H-NMR and Effect of Contact Area with Air., Eur. J. Lipid Sci. Technol. 108 (6), 493-500 (2006).

- **ARS analytical methods for biodiesel blend and residue oil level transferred to private sector**
  ERRC researchers developed rapid methods for measuring biodiesel blend levels that also can be used to measure residual oil levels in biodiesel fuels and blend levels in biodiesel/petrodiesel blends. The Technical Director of the National Biodiesel Board (who is also the Head of the ASTM Committee on Biodiesel Quality) is using this information following a visit to ERRC to discuss the methods, and to distribute them to potential field users for evaluation.

  Foglia, T. A., Jones, K. C., Nuñez, A., Phillips, J. G., and Mittelbach, M^{KFU}. Comparison of chromatographic methods for the determination of bound glycerol in biodiesel. Chromatographia 60(5/6):305-311, 2004.
  ^{KFU} Karl Franzens Univ., Graz, Austria

  Foglia, T. A., Jones, K. C., and Phillips, J. G. Determination of biodiesel and triacylglycerols in diesel fuel by LC. *[blend level determination]* Chromatographia, 62(3/4):115-119, 2005.

- **Determined typical biodiesel contaminants are excellent engine lubricators**
  Found that common biodiesel contaminants, such as free fatty acids and monoglycerides, possess better lubricity than neat alkyl esters and are largely responsible for the superior lubricity of low-level biodiesel blends versus neat petrodiesel. This work impacts the use of such materials as lubricity additives and also the level of blending of biodiesel with petrodiesel.

  G. Knothe, K.R. Steidley, Lubricity of Components of Biodiesel and Petrodiesel, The Origin of Biodiesel Lubricity, Energy & Fuels 19 (3), 1192-1200 (2005).

- **Determined the suitability of different biodiesel feedstocks regarding viscosity specifications**
  The influence of compound structure on the viscosity of fatty esters, the major components of biodiesel components, was determined. This work demonstrates the suitability of different biodiesel feedstocks in light of viscosity specifications in biodiesel standards. Viscosity is also a significant property any biodiesel fuel optimized for fatty acid composition will need to meet in order to facilitate commercialization.

  G. Knothe, K.R. Steidley, Kinematic Viscosity of Biodiesel Fuel Components, Influence of Compound Structure and Comparison to Petrodiesel Fuel Components, Fuel 84 (9), 1059-1065 (2005)

  G. Knothe, Dependence of Biodiesel Fuel Properties on the Structure of Fatty Acid Alkyl Esters, Fuel Proc. Technol. 86, 1059-1070 (2005).

Accomplishments without listed peer review publications

- **Successfully identified source of problem for stakeholders regarding biodiesel fuel filter fouling**
  Additionally, ERRC researchers were asked by stakeholders to determine the cause for premature fuel filter fouling of biodiesel-blended fuels. The clogged filters were extracted and the species most likely the cause for this problem were identified. This information allowed the industry to identify the causes of engine failure and producers to modify their technologies to generate reliably high quality fuels. Among requesters of assistance have been multiple private sector cooperators and such public sector entities as the Pennsylvania Turnpike Commission and the Minnesota Agricultural Utilization Research Institute (AURI).

Topic: Reduce cost of feedstocks

Goal: Develop conversion technology for production of biodiesel from low-cost, non-conventional, agriculturally derived feedstocks.

Summary: Refined oils are relatively expensive feedstocks for biodiesel. Cheaper feedstocks, such as tallow, greases, soapstock, and lipid-bearing materials (e.g., intact soy flakes, DDGs, meat & bone meal) are abundant, but require alternative processes (e.g., *in situ* conversion; enzymatic or other pretreatment steps) for efficient conversion to biodiesel. Accomplishments in this area of ARS research are reported below.

Significant Accomplishments & Impact:

- **Developed robust technical cost models for biodiesel production processes**
  ERRC researchers and its cost engineering team developed detailed models to estimate the capital and process costs of biodiesel production from refined vegetable oil, from vegetable oil soap stock, and from whole soybeans. The models incorporate the chemistry, engineering, capital, feedstock, and operating costs to present the public with a realistic picture of the economic feasibility to produce the fuel. The models have been distributed to more than 60 requestors, mainly from the private sector, in

the first year. They are being used not only to do economic feasibility studies for potential producers, but also as illustrative tools in professional lectures, as guides by college students, and as college teaching tools, especially in engineering departments.

Foglia, T. A., Haas, M. J., McAloon, A., and Marmer, W. N. Cost modeling of biodiesel production: Effect of feedstock and processing. Proceedings of the United States-Japan Cooperative Program in Natural Resources, Protein Resources Panel, 33rd Annual Meeting, Honolulu, HI, pp 50-54, 2004.

Haas, M.J., McAloon, A.J., Yee, W.C., Foglia, T.A. 2006. A process model to estimate biodiesel production costs. Bioresource Technology. 97:671-678.

- **Developed novel methodology to produce biodiesel from waste soapstock**
  In collaboration with Cargill (CRADA) and Runyon Industries (Memphis, TN), ERRC researchers developed new methods for the production of biodiesel from soapstock, an underutilized byproduct of edible oil refining. Conducted process optimization, quality validation testing, a patent filing, and--with university collaboration (Colorado School of Mines) -- heavy duty engine testing. Multiple requests for information on the process have been received.

  Haas, M. J., Bloomer, S.[C], and Scott, K. M. Simple, High-Efficiency Synthesis of Fatty Acid Methyl Esters from Soapstock. J. Am. Oil Chem. Soc. (2000) 77(4):373-379 [C] Cargill

  Haas, M. J., Bloomer, S.[C], and Scott, K. M. Process for the Production of Fatty Acid Alkyl Esters U.S. Patent No. 6,399,800. June 4, 2002.[C] Cargill

  Haas, M. J., Scott, K. M., Alleman, T. L. [NREL], and McCormick, R. L. [NREL]. Engine Performance of Biodiesel Fuel Prepared from Soybean Soapstock: A High Quality Renewable Fuel Produced from a Waste Feedstock Energy & Fuels (2001) 15(5):1207-1212[NREL] NREL, DOE

- **New method for biodiesel production from acid oil was adopted by private industry**
  Developed and patented an efficient method to produce biodiesel from acid oil, a coproduct of edible oil refining that sells for approximately half the price of refined oils. This yielded efficient conversion routes (a) from soapstock to acid oil and (b) from acid oil to biodiesel. Numerous inquires have been fielded regarding this technology. The first conversion (a) was adopted by Runyon Industries, resulting in the sale of greater than 1 million pounds of product to date.

  Haas, M. J., Michalski, P. J., Runyon, S.[R], Nuñez, A., and Scott, K. M. Production of FAME from Acid Oil, a By-Product of Vegetable Oil Refining. J. Am. Oil Chem. Soc. (2003) 80(1):97-102
  [R] Runyon Industries, Memphis

  Haas, M. J., Scott, K. M., Michalski, P. J., and Runyon, S[R]. Lipid Rich Compositions, Production of Lipid Rich Compositions, Production of Fatty Acid Alkyl Esters from Heterogeneous Lipid Mixtures *[soapstock and acid oil]* U.S. Patent No. 6,855,838, February 15, 2005
  [R] Runyon Industries, Memphis

- **Developed and demonstrated *in situ* production of biodiesel in oilseeds**
  Developed and optimized a process to produce biodiesel directly in oilseeds, termed in-situ transesterification. In cooperation with Ohio State University researchers, the scientists tested the suitability of the spent meal resulting from the process as an animal feed, which is crucial to the economic viability of the process. Young chickens accepted the oil-depleted meal in their diets, gained weight normally, and showed no signs of toxicity. Findings indicate that this process could reduce the cost of producing biodiesel while eliminating the need for EPA-regulated organic solvents currently used to extract oil from oilseeds. A private investor (PA) has indicated his intention of incorporating the technology for in situ transesterification, and a research partnership with ERRC, into a bioenergy facility that he intends to construct on the campus of the Pennsylvania State University. The potential inherent in this new technology caused ERRC researchers to be invited to

describe it before the Governing Board of the National Biodiesel Board (NBB). At annual research planning meetings sponsored by NBB, in situ transesterification has lately been the sole new biodiesel production technology discussed.

Haas, M. J., Scott, K. M., Marmer, W.N., and Foglia, T. A. In situ alkaline transesterification: An effective method for the production of fatty acid esters from vegetable oils *[direct conversion from soy flakes]*. J. Am. Oil Chem. Soc., 81: 83-89, 2004.

Haas, M. J., and Foglia, T. A. Alternative feedstocks and technologies for biodiesel production, in The Biodiesel Handbook, Knothe, G, Krahl, J., and Van Gerpen, J., (Eds.). AOCS Press, Champaign, IL pp. 42-61, 2005. (Invited Book Chapter)

Haas, M.J., and Scott, K.M., Moisture Removal Substantially Improves the Efficiency of in situ Biodiesel Production from Soybeans. J. Am. Oil Chem. Soc., 84:197-204, (2007).

Haas, M. J., Scott, K., Foglia, T. A., and Marmer, W. N.., The General Applicability of in situ Transesterification for the Production of Fatty Acid Esters from a Variety of Feedstocks *[soy flakes, DDGS, meat & bone meal]*. Submitted to J. Am. Oil Chem. Soc., in review. (2007)

ARS News Release: New Method to Produce Biodiesel.
http://www.ars.usda.gov/News/docs.htm?docid=12006

- **Developed an immobilized enzyme bioreactor for biodiesel production**
  Processes that use renewable catalyst for biodiesel production have been developed. ERRC researchers examined the use of enzymes, both free and immobilized, as catalysts for the production of biodiesel from oils, fats, and other rendered materials such as restaurant grease. The progress demonstrated led to a request by Novozymes Inc. (Denmark) to employ their lipases in producing an immobilized enzyme bioreactor for biodiesel production from high free fatty acid fats and oils. The bioreactor method has advantages of less byproducts and less energy use.

  Haas, M. J., Piazza, G. J., and Foglia, T. A., Enzymatic Approaches to the Production of Biodiesel Fuels. In Kuo, T. M. and Gardner, H.W., (Eds.) Lipid Biotechnology, Marcel Dekker, Inc, New York, 2002. (Invited Book Chapter)

  Hsu A-F., Jones, K. C., Foglia, T. A., and Marmer, W. N. Optimization of alkyl ester production from grease using a phyllosilicate sol-gel immobilized lipase. Biotechnology Letters, 25:1713-1716, 2003.

  Hsu, A-F, Jones, K. C., Foglia, T. A., and Marmer, W. N. Continuous production of ethyl esters of grease using an immobilized lipase. J. Am. Oil Chem. Soc., 81:749-752 2004.

  Hsu, A-F, Jones, K., Marmer, W. N., and Foglia, T. A., Production of alkyl esters from tallow and grease using lipase immobilized in a phyllosilicate sol-gel. J. Am. Oil Chem. Soc., 78:585-588, 2001

  Hsu, A-F., Jones, K. C., Foglia, T. A., and Marmer, W. N. Immobilized lipase-catalyzed production of alkyl esters of restaurant grease as biodiesel. Biotechnol. Appl. Biochem., 36:181-186, 2002.

  Lee, J-H [C], DiCiccio, R. J., Hsu, A-F, Lee, K-T [C], and Foglia, T. A. Production of alkyl esters by a phyllosilicate sol-gel immobilized lipase from *Pseudomonas cepacia*. Food Sci. Biotechnol., 12:342-345, 2003.

  Lee, K-T. [C], Foglia, T. A., and Chang, K-S [C]. Production of alkyl ester as biodiesel from fractionated lard and restaurant grease. J. Am. Oil Chem. Soc., 79:191-195, 2002.[C] Chungnam Univ., Korea

Accomplishments without listed peer review publications

- **Established successful partnership with Russian lab to study biodiesel production**
  An international collaboration with Russian research to aid their plans to produce biodiesel using immobilized acid catalysts was established. ERRC and NCAUR researchers were invited to establish collaboration between ARS and GOSNIOKhT, a Russian research facility, to plan research on

biodiesel production. The ARS Former Soviet Union (FSU) Program promotes collaborative research projects between ARS and FSU institutes. It is a component of a program, funded by the USA (by the Dept. of State) and our allies, that engages former Soviet weapons scientists in peaceful, agriculturally related research. ERRC and NCAUR researchers together designed a research project with GOSNIOKhT, the first project on biodiesel in Russia; the project was ultimately funded by the Canadian government.

- ***In situ* transesterification model developed to estimate cost of biodiesel production**
  Constructed a quantitative computer model and performed an economic analysis to estimate the cost of biodiesel production via in-situ transesterification. This analysis showed that the new method had substantially higher predicted process costs than did traditional biodiesel production methods but also identified the cause of the increased costs. Through subsequent research, the researchers have successfully modified the original protocol and reduced the cost of the high-cost operation. (Manuscript and CRADA in preparation)

- **Methods under development with private industry to eliminate hexane use in biodiesel processing**
  The attractive potential abilities of this method to eliminate hexane use in oil extraction, and to reduce biodiesel production cost, have led to requests to ERRC researchers for collaborative investigation of this method from the private and public sectors (ADM, Super Soy Feeds [WI], Pulsewave Technologies [CO], Custom Extruding [MN], Crown Iron Works [MN], and Minnesota Ag. Utilization Research Inst.). Collaboration is underway with ADM to develop a continuous process. This work has high potential to significantly reduce the cost of biodiesel processing through environmentally friendly chemistry. The potential of this method to provide a new type of animal diet component (lipid-depleted spent meal exiting the process) has led to collaborations among ERRC researchers, the ARS Fish Culture Experiment Station (ID), the Ohio State University Poultry Dept., and R. L. Stroup Inc. (OH). Soy feedstock was provided by Cargill and Perdue Farms.

- **Developed novel processes to convert waste stream oil to biodiesel and partnered with private industry for demonstration**
  The technology for the conversion of waste-stream lipids (such as trap grease) to biodiesel has been developed and is in early stage commercialization. ERRC researchers teamed with Philadelphia Fry-O-Diesel (PA) in the successful development of technology and pilot and production facilities for the synthesis of specification grade biodiesel from restaurant trap grease. Pilot technology is in validation phase at this time. This work expands the range of potential biodiesel feedstocks and moves the technology toward commercialization. The work is of significant importance as it represents the first success of producing biodiesel from trap grease.

  http://www.npr.org/templates/story/story.php?storyId=6585629

- **800 pound butter sculpture converted to biodiesel to demonstrate technology to public**
  ERRC researchers conceived, facilitated, planned, and are presently participating with Philadelphia Fry-O-Diesel Co. on the conversion to biodiesel of an 800-pound keynote butter sculpture produced for and displayed at the 2007 Pennsylvania Farm Show. This dovetailed nicely with the Food and Renewable Fuels theme of that Show, and has received local and national press attention. The study was coordinated with and approved by Pennsylvania Secretary of Agriculture D. C. Wolff and ERRC's Center Director.

Topic: Cold flow properties

Goal: Develop fundamental understanding of cold flow phenomena and develop technology to reduce cold flow operability problems.

Summary: Results from this research emphasize improving the cold flow properties and performance of biodiesel and its blends with conventional diesel fuel and jet fuel. Cold flow properties of biodiesel are significantly influenced by chemical composition of the fatty acid alkyl esters, presence of cold flow improver additives and trace quantities of contaminants. Automated light scattering and differential scanning calorimetry (DSC) methods were developed to analyze crystal formation and phase transitions associated with low-temperature phenomena. New cold flow improver additives were synthesized from oleochemical sources and tested. Model mixtures of fatty acid alkyl esters were analyzed to develop a thermodynamic model for predicting cold flow property behavior.

Significant Accomplishments & Impact:

- **Investigated effects of biodiesel blending with aviation fuel**
  The impact of blending biodiesel with jet fuel on cold flow properties, oxidative stability and other factors relevant to performance in aviation applications was determined. Results showed that cold flow properties of such blends were significantly improved when biodiesel pre-treated with additives or winterized (partially crystallized and filtered to remove solids) prior to blending. This research also led to formal participation in an ongoing reimbursable cooperative agreement with Alternative Aviation Fuels LLC (Ryebrook, NY), collaboration under a material transfer agreement with Proctor and Gamble plus many related informal collaborations.

  R.O. Dunn, Biojet: An Alternative Jet Fuel Formulated with Methyl Soyate (Biodiesel), Proc. 93rd Annual Meeting of the ASAE, July 2000

  R.O. Dunn, Alternative Jet Fuels from Vegetable Oils, Trans. ASAE 44, 1751-1757 (2001)

  R.O. Dunn, Biodiesel in the Wild Blue Yonder, Inform 14:36-37 (2003).

- **Developing analytical procedures to enhance biodiesel cold flow properties**
  Earlier research on winterization (partially crystallization and filtration) process for improving the cold flow properties of biodiesel was extended. ARS scientists and collaborating with De Smet (Edegem, Belgium) on lab-scale continuous winterization and Alternative Aviation Fuels LLC on pilot-scale continuous winterization of biodiesel. A database of screened organic solvents suitable to assist in partial crystallization of high-melting components in biodiesel has been developed.

  M.J. Delafontaine, C.R. Krishna, R.O. Dunn, M. Hubert, Fractional Crystallization of Fatty Acid Methyl Esters, Final Report for NYSERDA PON 737 Stage 1 Project No. 8208, 2005.

- **Developed automated analytical methods to rapidly measure cold flow properties of biodiesel and its blends with conventional diesel and jet fuels**
  These methods based on light scattering detection and differential scanning calorimetry were developed to analyze formation of solid crystals and phase transitions associated with low-temperature phase transitions. These methods proved to be more accurate than conventional manually based methods.

  R.O. Dunn, Thermal Analysis of Alternative Diesel Fuels from Vegetable Oils, J. Am. Oil Chem. Soc. 76, 109-115 (1999)

R.O. Dunn, abstract for paper on updated results presented orally at American Oil Chemists' Society meeting, May 2005.

- **Developed and tested low temperature additives for biodiesel**
  A number of biobased low temperature operability additive candidates were developed from oleochemical sources. These branched synthetic adducts have improved low temperature fluidity in comparison to biodiesel fuel. As such, they may impart more favorable cold weather operability to biodiesel fuel at additive levels by disrupting macrocrystalline formation at reduced temperatures thereby increasing cold start properties.

  B.R.. Moser, S.Z. Erhan, Synthesis and Evaluation of a Series of α-Hydroxy Ethers Derived from Isopropyl Oleate, J. Am. Oil Chem. Soc. 83, 959-963 (2006).

- **Developed blending and processing decisions for improved biodiesel cold flow properties**
  Demonstrated that mixing biodiesel made from soybean oil converted with methanol with biodiesel made from soybean oil converted with another alcohol improves cold flow properties. Results showed that though such mixtures yield lower cloud and pour points, such mixtures were slightly more expensive.

  R.O. Dunn, abstract for paper presented orally at Pacifichem conference, December 2005.

- **Created models to predict physical properties of biodiesel**
  A thermodynamic model to predict the cloud point and other cold flow properties of biodiesel has been developed. The model is based on freezing point depression theory and requires measurement of parameters associated with crystallization of different chemical compounds in biodiesel. The model may be useful for screening cold flow additives and determining the effects of contaminants (compounds that are not fatty acid alkyl esters) on cold flow properties of biodiesel.

  R.O. Dunn, abstract for paper presented at American Oil Chemists' Society meeting, May 2006; Manuscript in preparation.

Topic: Storage stability

Goal: Identify factors that reduce oxidative stability of biodiesel and develop technology that improves storage stability.

Summary: This research emphasizes analysis of relative resistance to oxidation and development of oxidation inhibitors (antioxidants) and other approaches to improve the relative resistance of biodiesel. Various factors affecting the oxidative stability such as compound structure, concentration, extraneous materials and temperature were investigated. Biodiesel from many sources and its blends with diesel and jet fuels were analyzed. Analytical methods were developed based on pressurized-differential scanning calorimetry (P-DSC) and oil stability index (OSI). Kinetics were studied to develop rapid and accurate methods to evaluate relative resistance to oxidation of biodiesel under real world storage conditions. Approaches such as synergistic antioxidants were investigated for improving oxidative stability. New indices related to oxidative stability were developed.

Significant Accomplishments & Impact:

- **Developed rapid analytical techniques for assess oxidation limitation in biodiesel**
  A rapid method for screening relative resistance to oxidation of biodiesel from various sources and experimental conditions has been developed. This method was based on analysis of oxidation temperature from non-isothermal pressurized-differential scanning calorimetry heating scans analyzed in static-mode (zero purge flow). Results were correlated with isothermal (constant temperature) oil stability index analyses. Effects of oxidation inhibitors (antioxidants) were also analyzed.

  R.O. Dunn, Analysis of Oxidative Stability of Methyl Soyate by Pressurized-Differential Scanning Calorimetry, Trans. ASAE 43, 1203-1208 (2000).

- **Evaluated effects of oxidation on other biodiesel fuel properties**
  Effects of oxidation on several fuel properties of biodiesel and diesel/biodiesel blends have been determined. Studies were conducted on biodiesel samples from five separate sources and differing levels of abuse during storage following acquisition. Results generally confirmed the concerns voiced by producers and marketers that care should be extended during storage and handling of biodiesel to prevent degradation affects on fuel quality and combustion characteristics.

  R.O. Dunn, Effect of Oxidation Under Accelerated Conditions on Fuel Properties of Methyl Soyate (Biodiesel), J. Am. Oil Chem. Soc. 79, 915-920 (2002).

- **Determined effects of biodiesel composition on fuel storage**
  The influence of compound structure and concentration of individual compounds on oxidative stability of biodiesel was determined as was the effect of trace metals. This information is essential for assessing storage of biodiesel as well as the corresponding effect of novel fatty acid compositions of biodiesel.

  G. Knothe, R.O. Dunn, Dependence of Oil Stability Index of Fatty Compounds on Their Structure and Concentration and Presence of Metals, J. Am. Oil Chem. Soc. 80, 1021-1026 (2003).

- **Developed new analytical techniques to replace less precise methodology**
  The iodine value, a measure of total fat unsaturation, has been commonly used to correlate with various biodiesel properties, including oxidative stability. It was shown that the iodine value does not necessarily correlate well with such properties. Rather, new indices, termed allylic and bis-allylic position equivalents, were developed which correlate better with observed properties. This work stands to significantly impact the analytical procedures needed to qualify and qualify biodiesel stability, an absolute necessity in terms of wide spread adoption as a liquid fuel.

  G. Knothe, Structure Indices in FA Chemistry. How Relevant is the Iodine Value?, J. Am. Oil. Chem. Soc. 79, 847-854 (2002).

- **Evaluated the effects of blending biodiesel and aviation fuel on fuel properties**
  Demonstrated oil stability index of aviation fuels blended with up to 30 percent biodiesel in jet fuel by volume decreases with increasing temperature. Increasing blend ratio (biodiesel volume percent) also reduces resistance to oxidation. This work established maximum analysis temperatures for biodiesel/jet fuel blends and for neat (100 percent) biodiesel derived from soybean oil.

  R.O. Dunn, G. Knothe, Oxidative Stability of Biodiesel in Blends with Jet Fuel by Analysis of Oil Stability Index, J. Am. Oil Chem. Soc. 80, 1047-1048 (2003).

- **Evaluated mixtures of antioxidants for synergistic stability effects on biodiesel by rapid screening**
  Identified binary mixtures of oxidation inhibitors (antioxidants) exhibiting synergistic effects when used to improve oxidative stability of biodiesel. Biodiesel from soybean oil treated with binary antioxidants was analyzed for relative resistance to oxidation at constant temperature. Combining two antioxidants improved stability to a degree that exceeded either antioxidant acting individually. A rapid and accurate method for screening oxidation inhibitors (antioxidants) for capacity to improve the relative resistance to oxidation of biodiesel has been developed. Results from non-isothermal pressurized-differential scanning calorimetry scans were shown to be applicable to treatment of biodiesel from any feedstock (soybean oil, rapeseed oil and tallow) by both natural and synthetic antioxidants.

  R.O. Dunn, abstract for paper presented orally at 95th American Oil Chemists' Society Meeting, May 2004.

  R.O. Dunn, Effect of Antioxidants on the Oxidative Stability of Methyl Soyate (Biodiesel), Fuel Processing Technol. 86, 1071-1085 (2005).

- **Determined isothermal stability analyses as the most reliable measure of oxidative resistance**
  Demonstrated that isothermal (constant temperature) oil stability index analyses was most suitable for measuring relative resistance to oxidation of biodiesel from various sources. Results were mostly compared with respect to a standard control material (methyl oleate) by normalize data with response factors.

  R.O. Dunn, Oxidative Stability of Soybean Oil Fatty Acid Methyl Esters by Oil Stability Index, J. Am. Oil Chem. Soc. 82, 381-387 (2005).

- **Developed rapid and accurate methods for determining relative resistance to oxidation of biodiesel under various experimental conditions**
  Results from thermogravimetric analysis, conventional (ambient pressure) differential scanning calorimetry, pressurized-differential scanning calorimetry in static (zero purge flow) and dynamic (flow-through gas purge) modes, and isothermal oil stability index analyses were correlated and compared.

  R.O. Dunn, Oxidative Stability of Biodiesel by Dynamic Mode Pressurized-Differential Scanning Calorimetry, Trans. ASABE 49, 1633-1641 (2006).

- **Measured reaction kinetics of biodiesel oxidation under various conditions**
  Developed rapid and accurate method for measuring activation energy and other parameters associated with oxidation degradation reaction kinetics of biodiesel under variable experimental conditions. Parameters measured by non-isothermal pressurized-differential scanning calorimetry heating scans under accelerated conditions were then employed to predict the relative resistance to oxidation of biodiesel under conditions such as those encountered during long-term storage. Results demonstrated that pressurized-differential scanning calorimetry analyses yield results in significantly less time than many industry standard test methods. This work stands to impact decision making regarding biodiesel storage conditions for enhanced stability. Demonstrated oil stability index of biodiesel from various feedstocks and sources decreases with increasing temperature. Relative resistance to oxidation as indicated by larger oil stability index values also varied significantly with respect to feedstock. Results were also used to infer activation energies for oxidation of biodiesel under various conditions.

  R.O. Dunn, Oxidation Kinetics of Biodiesel by Non-Isothermal Pressurized-Differential Scanning Calorimetry, Proceedings of the 34th Annual NATAS Conference, August 2006.

R.O. Dunn, abstract for paper presented orally at 94th American Oil Chemists' Society meeting, May 2003; Manuscript submitted for publication in journal 'Energy Fuels'

Topic: Biodiesel Coproducts

Significant Accomplishments & Impact:

- **Developed copolymers containing glycerol as a feedstock for biodegradable coatings**
  Co-polymers of glycerol with citric acid, lactic acid, and starch prepared and tested as biodegradable coatings. Agricultural uses for these materials include formulation with straw for weed barriers and hydroseeding or hydromulch. A field trial was completed for the weed barrier application last season and the hydromulching tests are planned locally after the frost free date in cooperation with Summit Seed (Manteno, IL). Other applications of these polymers are anticipated. This technology could significantly impact the cost of biodiesel processing by developing commercialization opportunities for glycerol products.

  Exploring Glycerin Uses, Biofuels J., Vol. 4(2):12-16 (2006).

- **Utilization of glycerol as a feedstock for production of bioplastics**
  ERRC researchers are applying crude biodiesel glycerol as a carbon and energy source in the microbial production of poly(hydroxyalkanoate) polymers, which can be used to create biodegradable plastics. And, with a private-sector CRADA partner, biosurfactants (sophorose and rhamnolipids) are being investigated. This technology possesses enormous potential to develop biodegradable products for a variety of applications while favorably impacting biodiesel production economics.

  Ashby, R. D., Solaiman, D. K. Y., and Foglia, T. A. Bacterial poly (hydroxyalkanoate) polymer production from the biodiesel co-product stream. J. Polym. Environ. 12:105-112, 2004.

  Ashby, R. D., Nuñez, A., Solaiman, D. K. Y., and Foglia, T. A. Sophorolipid *[a biodegradable natural surfactant]* biosynthesis from a biodiesel coproduct stream. J. Am. Oil Chem. Soc., 82:625-630, 2005.

  R.D. Ashby, D.K.Y. Solaiman and T.A. Foglia, Synthesis of Short-/Medium-Chain-Length Poly (hydroxyalkanoate) Blends by Mixed Culture Fermentation of Glycerol. Biomacromolecules (2005) 6:2106-2112

- **Created polymer feedstocks from glycerol and dicarboxylic acids**
  ERRC researchers are producing polymer precursors to synthesize hyperbranched polymers from glycerol and dicarboxylic acids. This work is under development but stands to potentially impact the chemical polymer industry.

  Wyatt, V. T., Nuñez, A., Foglia, T. A., and Marmer, W. N. Acid-catalyzed synthesis of hyperbranched poly (glycerol-diacid) oligomers. J. Am. Oil Chem. Soc., 83:1033-1039, 2006.

Accomplishments without listed peer review publications

- **Developed processes for direct conversion of fats and oils to energy**
  The potential for incorporating inexpensive fats and oils directly (i.e., not converted to biodiesel) as burner fuel or as feedstock for thermal conversion to diesel-like fuel has been established. In collaboration with private sector companies (Clean Burn Technologies [PA] and Laughing Stock Farms [ME]), ERRC researchers are investigating intact triglycerides, particularly used cooking oils, as burner fuels. ERRC researchers have also collaborated with Changing World Technologies (CWT; PA) to demonstrate the feasibility of producing biofuel from soybean soapstock (residue from soy oil

refining) using CWT's thermal conversion technology for transforming organic materials into liquid and gaseous fuels and carbon black. CWT's process offers an alternative to ERRC's successful technology for conversion of soapstock to biodiesel. This work will potentially enable the direct utilization of fats and oils to energy with significant benefits for one site energy production.

- **Created glycerol based polymers for potential new uses**
  Finding new, value-added uses for glycerol will help ensure favorable production economics for biodiesel manufacturers. ARS has synthesized linear and hyperbranched polymers from glycerol. Glycerol-based hyperbranched polymers represent a new class of water-soluble polymers that could open up new markets for glycerol. Working with Rohm & Haas Co. under a USDA/DOE Green Chemistry Program grant, ERRC researchers are investigating the incorporation of biodiesel glycerol into new adhesive and elastomer products. This work stands to make significant contributions to biodiesel processing economics as well as new uses for biodiesel glycerol as a green product.

- **Developed potential for new glycerol based cosmetics**
  Cosmetic formulations prepared with glycerol are high-value products. Glycerol was combined with cinnamic acid to prepare a UV absorbing material that shows lower transdermal rates greater than fatty compounds with greater solubility. Liposomes prepared from glycerol and jojoba oil offer the possibility of delivering antioxidants or other bioactive ingredients in topical cosmeceutical formulations. Validation of these formulations is planned and may represent yet another commercial outlet for biodiesel coproducts.

Component III: Energy Alternatives for Rural Practices

Topic: Energy to provide reliable, safe drinking water for livestock and humans, and adequate basic electrical service

Goal: Develop and evaluate autonomous wind and solar powered water pumping systems for irrigation, livestock, and farmstead water, and develop electric generation systems for rural and remote areas.

Summary: Autonomous water pumping systems are needed to supply livestock water in semiarid areas where precipitation is sufficient for forage production, but is not adequate for watering livestock. Renewable energy systems are well-suited for this application because they are independent of environmentally hazardous, expensive distribution systems. Remote renewable energy systems are needed to supply additional electric power to rural areas that are under-supplied by overloaded rural electric distribution systems. On-farm electric generating systems powered by a combination of wind, solar, and biofuels will be developed.

Several autonomous wind and solar powered water pumping systems were developed and tested during the tenure of this action plan. Pump designs were changed to better match the variable energy from the wind and sun. Conversion from centrifugal type pumps to positive displacement helical designs have allowed for use of smaller wind turbines and fewer solar panels. Advances in control technology have also improved performance and reliability of these remote water pumping systems. This research team has worked hand-in-hand with manufacturers and this has allowed these new concepts to be transferred to current products. Jointly working with engineers from the Department of Energy, this research team has developed new technology for improved

rotor blade testing and control applications for wind-hybrid electric generating systems for remote villages.

Significant Accomplishments & Impact:

- **Designed and demonstrated reliability of wind-electric water pumping for livestock**
  A wind-electric water pumping system was designed and found to operate maintenance free for over 3 years while supplying water from a 280 foot well for 75 beef cattle. The findings show that this new wind-electric water pumping system can be as reliable as utility powered systems. Several wind turbine manufacturers used the ARS control logic for their wind-powered water pumping systems.

  Clark, R. N. and Vick, B. D. 2005. Livestock water pumping with renewable energy systems. In Agriculture as a Producer and Consumer of Energy, CABI Publishing, UK.

  Vick, B.D., Clark, R.N., and Ling, S. 1999. One and a half years of field testing a wind-electric system for watering cattle in the Texas panhandle. AWEA Windpower '99 Proceedings, June 20-24, Burlington, VT, 10 pp.

- **Successfully designed alternative pump system for low wind regime operation and facilitated commercialization by private sector**
  A wind pumping system for groundwater to operate in low wind regimes by using a helical positive-displacement type pump was developed. This system used a smaller wind turbine but still was able to meet the water demands by pumping water at a slower rate over longer periods of time. Two international pump companies are marketing this type of system using wind turbines manufactured by a United States wind company. Several hundred of these systems have been sold in the southwestern United States and are now in operation.

  Nelson, V. Clark, R. N. and Foster, R. 2004. Wind Water Pumping. Published as a CD by West Texas A&M University Bookstore, Canyon, TX.

  Vick, B.D. and Clark, R.N. 2005 Performance and acoustic analysis of a small wind turbine used with a helical pump for livestock watering. Windpower 2005 Proceedings, May 15-18, Denver, CO, 11 pp.

- **Designed and demonstrated the reliability of solar powered water pump system up to 100 ft**
  Demonstrated that 100-Watt DC solar-powered, one gallon per minute diaphragm-type water pumping systems operated for six years when pumping water from a depth of 90 feet. The system pumped water sufficient for 25 head of cattle. When comparing fixed panel solar systems with those having passive solar tracking, it was also found that the additional water flow obtained by use of passive solar tracking did not warrant purchase of the tracking equipment. This research shows that solar-powered diaphragm-type water pumping systems are reliable for supplying livestock needs as long as the pumping depth does not exceed 100 feet.

  Vick, B.D. and Clark, R.N. 2002. Solar-PV Water Pumping with Fixed and Passive Tracking Panels. ASES Solar 2002: Sunrise on the Reliable Energy Economy, Jun. 15-19, Reno, NV, 6 pp

- **Evaluated use of solar power for a helical water pump sufficient for large cattle herds**
  Solar photovoltaic (PV) water pumping field studies to evaluate the use of helical pumps for watering livestock were conducted. A helical pump powered by either 320 or 640 Watts of PV modules was evaluated to determine the optimum power requirements for pumping depths from 50 meters (164 ft) to 100 meters (328 ft). This pumping system did not show a significant change in performance during 3 years of operation. Because of the capability to pump water over a wide range of pumping

depths, these solar powered helical pump systems now appear to be the preferred stand-alone water pumping system for large herds (75 to 160 cattle). This work validated proof of concept and spurred commercialization and adoption. These pumps are sold by at least two international companies and are available world wide.

Vick, B.D. and Clark, R.N. 2005. Water Pumping Performance of a Solar-PV Powered Helical Pump. ISEC/ASES Solar 2005: 2005 Solar World Congress: Bringing Water to the World, Aug. 6-12, Orlando, FL, pp. 6

- **Assessed the ability of wind powered water pumping for irrigation of crops**
  Wind power usage for irrigation did not match the periods of significant crop water use unless the producer was growing mostly winter wheat. Also a producer needed to be in a location that allowed for net energy billing of the electricity to receive a profitable return for the excess energy generated during the non-irrigation periods. This analysis led to searching for other rural energy users that better matched the available wind resources. Recent analyses have suggested that dairies, feed yards, and rural schools are better matches to the wind resource than irrigation because of the relatively short pumping time. Methods of performing these analyses have been provided to electric cooperatives and wind turbine manufacturers. Several rural schools have purchased wind machines in the last year.

Vick, B. D. and Clark, R. N. 2000. Wind-powered drip irrigation systems for fruit trees. ASAE Paper No. 004030. American Society of Agricultural and Biological Engineers, St. Joseph, MI.

- **Conducted and provide essential and influential analysis of turbine rotor and tower design for peak wind energy capture**
  Wind turbine rotor and tower designs have been oversized because of the inability to capture peak wind loads caused by peak winds. Long-term continuous data sets were not feasible because of limited data acquisition systems. Working with Sandia National Laboratories, data acquisition hardware and software were developed to acquire continuous, long-term data from an operational wind turbine. This data acquisition allowed for the simultaneous collection of stress loads from the rotating rotor, incoming wind speed, and power output. Sampling rates were at 30 times per second with over 40 gauges recorded. Continuous data sets of over 1000 hours provide the type of long-term data needed to improve wind turbine designs. Data reduction and analysis was and is being completed by several universities and other federal and state agencies. The capabilities of this system and methodology are currently used to atmospheric test the new advanced wind turbine blades developed from carbon fiber for performance and turbine structural loads. Commercial applications of this data acquisition system are currently being used in many research applications and control systems.

Jones P. L., Sutherland H. J., Neal B. A. 2001. LIST/BMI Turbines Instrumentation and Infrastructure. Sandia Report SAND2001-1642, June 2001

Jones P. L., Sutherland H. J., Zayas J. R., Sterns A. J., Neal B. A. 2004. Update of the Long-Term Inflow and Structural Test Program. ASME/AIAA Wind Energy Symposium, Jan. 2004

Zayas J. R., Jones P. L., Holman A. J. 2005. CX-100 and TX-100 Blade Field Tests. Sandia Report SAND2005-7454, Dec. 2005

- **Demonstrated significant efficiency gains for a wind hybrid diesel system for off grid electric power generation**
  ARS engineers designed and installed wind-hybrid diesel controls for remote electric power generation in cooperation with the Department of Energy's National Renewable Energy Laboratory (DOE/NREL) and contractors Encorp and Northern Power Systems. These controls were designed to allow for high penetration of wind power on a diesel electric grid whereby the diesel generators could be completely shut down during periods of high winds for maximum fuel savings. An average fuel

savings of 22% was measured during almost 1000 hours of operation and all control transitions were smooth and stable. The system was successfully operated in a wind-only generation mode providing stable electric power. The fuel efficiency increased from 2.63 kWh per liter for diesel only to 4.01 kWh per liter with hybrid system without storage and to 10.09 kWh per liter with battery storage. The potential run time in wind only was increased from 2% without storage to 26% when the battery storage was added.

Clark, R. N. 2002. Hybrid electric power generation with wind and diesel. ASAE Paper No. 024186. American Society of Agricultural and Biological Engineers, St Joseph, MI.

Clark, R. N. 2002. Wind/diesel power generation with short-term storage. 2002 Wind-Diesel Workshop, Anchorage, AK, September 23-24, 2002. Posted at www.eren.doe.gov/windpoweringamerica/.

Topic: Renewable Hydrogen and Energy from Crop Residues, Energy Crops, and Low-Valued Ethanol Byproducts

Goal: Develop improved processes to convert low valued crop-related biomass, byproducts and energy crops being developed in the ARS energy crop program into renewable hydrogen or liquid fuels.

Summary: Thermochemical methods for conversion to energy, oil, and/or synthesis gas are being improved or developed. These technologies are attractive to on site/on farm energy production for dedicated energy crops, waste agriculture or woody materials, or biomass sources not amenable to mechanical/biochemical processing.

Significant Accomplishments & Impact:

- **Developed analytical procedures to identify products of flash pyrolysis from various biomass feedstocks**
  Developed analytical pyrolysis methods (PY-GC/MS) to identify the variety of compounds formed during flash pyrolysis of biomass substrates such as corn residues, grain byproducts, and energy crops that are being studied at ARS feedstock development centers. These studies help predict the yields of the major products of primary gasification reactions as functions of temperature and heating conditions. This work is critical for determining the thermochemical potential (potential to generate fuels and chemicals) for energy crops in development and can be used to guide breeding efforts toward superior energy crop cultivars. It also is used to determine the potential for converting grain residues, such as barley hulls, into bio oil, syn gas, and value added char-based products.

  Boateng, A.A., P.H. Cooke and K.B. Hicks "Microstructure Development of Chars Derived from High-Temperature Pyrolysis of Barley (*Hordeum vulgare L.*) Hulls," Fuel, 86: 735-742 (2007).

  Boateng, A.A., K.B. Hicks, R.A. Flores and A. Gutsol "Pyrolysis of hull-enriched byproducts from scarification of barley (*Hordeum vulgare L.*)," J. Analy. & Appl. Pyrolysis, 78: 95-103 (2007).

- **Determined the effects of plant maturity on thermochemical conversion efficiency and product formation**
  There is a significant effect of plant physiological stages of maturity (at harvest) on the thermochemical conversion of warm- and cool-seasoned grasses and legume (alfalfa) into syngas and pyrolysis products. This work guides energy crop growers and purchasers on growing and harvesting practices that will yield feedstock with maximum potential for conversion to liquid and gaseous fuels via thermo-chemical processes.

  Paul R. Adler, Matt A. Sanderson, Akwasi A. Boateng, and Paul J. Weimer, and Hans J-G. Jung "Biomass yield and biofuel quality of switchgrass harvested in fall or spring," Agronomy Journal, 98: 1518-1525 (2006)

Boateng, A.A., H. J-G Jung and P. Adler "Pyrolysis of energy crops including alfalfa stems, reed canarygrass, and eastern gamagrass," Fuel, 85: 2450-2457 (2006)

Boateng, A.A., K.B. Hicks and K.P. Vogel "Pyrolysis of Switchgrass (Panicum virgatum) Harvested at Several Stages of Maturity," J. Analy. & Appl Pyrolysis, 75: 55-64 (2006).

- **Determined plant genotype is not a parameter that will influence thermochemical product formation or yields**
  There is no significant effect of genotype on the yields of thermochemical conversion products of bermudagrass when used as energy crop. This work is the first report of conversion of this crop into renewable fuels via thermo-chemical processes. Because the work is so new, the impact is yet to be seen, but it is an important contribution nonetheless to the general understanding of biomass thermochemical fundamental knowledge. Published 1 peer-reviewed journal article on the effect of two bermudagrass genotypes on syngas yield and quality.

  Boateng, A.A., W.F. Anderson and J.G. Phillips "Bermudagrass for bio-fuels: Effect of two genotypes on pyrolysis product yield," Energy & Fuels. Published on Web (12/29/06).

- **Designed and demonstrated a pilot scale fluidized bed thermochemical reactor (designed for on farm use)**
  Developed a pilot-scale fluidized bed thermochemical reactor for the production of pyrolysis liquids (bio-oil) and successfully produced bio-oil from switchgrass. This is the first reactor of its type in ARS and because of the unique facility and stature of researchers at ERRC, the site has been selected by ThermalNet- the European Network for biomass pyrolysis, gasification and combustion - as one of only 6 International sites to participate in a multi-location study on the production of bio-oil from plant lignin.

  Boateng, A.A., D.E. Daugaard, N. Goldberg, and K.B. Hicks "Pilot-Scale Fluidized-Bed Pyrolysis of Switchgrass for Bio-Oil Production," J. IEChemRes. (Completed proofs, 2/23/2007).

- **Developed technology for on farm conversion of straw and switchgrass into synthetic gas**
  ARS scientists assisted in development of on-farm thermochemical systems for the conversion of straw and switchgrass into syngas for the production of heat and power and demonstrated operation of the units. Technology adopted by a grass seed farm in the Pacific Northwest and at a CRP land in Western Pennsylvania, accomplishment featured in Chemical Engineering Progress, an AIChE monthly magazine and in the Philadelphia Inquirer. This represents the first on-farm gasifier for switchgrass on the East Coast and the first straw gasifier of its type in the Pacific Northwest.

  Boateng, A.A., G.M. Banowetz, J.J. Steiner, T.F. Barton, D.G. Taylor, K.B. Hicks, H. El-Nashaar and V.K. Sethi "Gasification of Kentucky bluegrass (*Poa pratensis L.*) straw in a farm-scale reactor," Biomass & Bioenergy, 31:153-161 (2007).

  Banowetz, G.M., A.A. Boateng, J.J. Steiner, M.W. George, V. Sethi, and H. El-Nashaar. "Potential for on-farm thermochemical conversion of straw to bioenergy in the pacific northwest," J. Biomass & Bioenergy. (In Review, 2005).

  Matt A. Sanderson, Paul R. Adler, Akwasi Boateng, Michael D. Casler, and Guatam Sarath "Switchgrass for biomass feedstock in the U.S" Canadian Journal of Plant Science – Special issue (Accepted 9/9/2006)

Component IV: Energy Crops

Topic: Energy crop production
Goal: Develop biomass plants and production systems that maximize the environmental benefits of biomass crop production.

Summary: Development of dedicated energy crops via traditional breeding and genetic engineering present profound opportunities for introducing and improving the suitability of biomass for increase mass and efficient conversion. This research includes identification of genes involved in cell wall biosynthesis, and the manipulation of these genes at the genomic or cellular level for cell wall digestibility and decreased lignin content. Research in this area also includes the assessment and evaluation of various crops for suitability as a function of ecoregion, genetic variation, and production economics.

Significant Accomplishments & Impact:

- **Survey of biomass feedstocks; Digestibility as a function of plant type and maturity**
  Switchgrass, canary reed grass, and alfalfa stems were evaluated for biochemical conversion.. Cultivars were each harvested at 2-3 maturities and analyzed for complete chemical composition; include soluble, storage and structure carbohydrate contents. Samples were pretreated and studied for sugar yields following treatment with commercial cellulase preparation. This was the first study to compare a legume and cool and warm season grasses for potential ethanol yields and susceptibility to chemical pretreatment. Important and novel conclusions from this study include: digestibility following pretreatment varied by plant type and harvest maturity and the presence of soluble sugars (especially fructose) needs to be considered when developing a pretreatment scheme. Results were published and presented at national meetings. This work also formed the basis for further studies within ARS for developing specific cultivars suitable for biochemical conversion to ethanol.

  Dien, B. S., H. G. Jung, K. P. Vogel, M. D. Casler, J. F. S. Lamb, L. Iten, R. B. Mitchell, and G. Sarath. 2006. Chemical Composition and Response to Dilute-Acid Pretreatment and Enzymatic Saccharification of Alfalfa, Reed Canarygrass, and Switchgrass. Biomass & Bioenergy 30:880-891.

- **Identified important gene in cell wall biosynthesis**
  Identified and characterized a gene, UDP-sugar pyrophosphorylase (USP), which plays an important role in cell wall biosynthesis in plants. The USP gene, found to be widely expressed in plant tissues, was cloned and the properties of the gene product were determined. The isolation of the USP gene and new knowledge learned about the protein product will allow cell walls of alfalfa plants to be modified to improve the value of this crop as a bioenergy feedstock.

  Litterer, L.A., J.A. Schnurr, K.L. Plaisance, K.K. Storey, J.W. Gronwald, and D.A. Somers. 2006. Characterization and expression of Arabidopsis UDP-sugar pyrophosphorylase. Plant Physiol. Biochem. 44:171-180

  Schnurr, J.A., K.K. Storey, H-J.G. Jung, D.A. Somers, and J.W. Gronwald. 2006. UDP-sugar pyrophosphorylase is essential for pollen development in Arabidopsis. Planta 224:520-532

  Litterer, L.A., K.L. Plaisance, J.A. Schnurr, K.K. Storey, H-J.G. Jung, J.W. Gronwald, and D.A. Somers. 2006. Biosynthesis of UDP-glucuronic acid in developing soybean embryos: possible role of UDP-sugar pyrophosphorylase. Physiol. Plant. 128:200-211.

- **Developed lower lignin content sorghum.**
  Developed and released sorghum genetic stocks near-isogenic for individual and stacked genes associated with lower lignin content due to reduced cinnamyl-alcohol dehydrogenase (CAD) and O-methyltransferase (OMT) activity. These genetic stocks are currently being used to support basic research on conversion to ethanol, and in applied breeding programs developing commercial hybrids. Forty distributions of the genetic stocks have been made to public sector and private sector breeders and researchers in the U.S., Argentina, Australia, Bolivia, Canada, France, India, and Japan. One of the genetic stocks was released as a cultivar, 'Atlas bmr-12' seed is being increased for commercial sale. (5 refereed publications and issuance on one Plant Variety Protection Certificate)

  Pederson, J.F., Funnell, D.L., Toy, J.J., Oliver, A.L., Grant, R.J. Crop Science, Vol. 46, Jan.-Feb., 2006, p 478.

- **Biomass-management system was developed for alfalfa to reduce harvest costs and increase yield**
  Compared to a typical hay-management system, alfalfa is seeded less densely to allow individual plants to grow larger and harvested at a later maturity stage (full flower or green bud vs. pre-flower) under the biomass-management system. This biomass-management system increased biomass yield by 40%, with equivalent leaf yield (a valuable livestock protein feed supplement) and greater stem yield for bioenergy. Alfalfa cultivation and production is a lower energy intensity process by nature of the plants recruitment and support of symbiotic nitrogen fixing microorganisms. These plot-trial results are now being evaluated under field-scale conditions.

  Lamb, J.F.S., C.C. Sheaffer, and D.A. Samac. 2003. Population density and harvest maturity effects on leaf and stem yield in alfalfa. Agron. J. 95:635-641

- **A non-lodging, biomass-type of alfalfa was developed to provide high yields of alfalfa leaves and stems when grown under a biomass management system.**
  This biomass-type alfalfa yielded more biomass and doubled potential ethanol compared with standard hay-type alfalfa varieties. Improved germplasm with greater disease and insect resistance, and winter hardiness has been developed through selective breeding in biomass-alfalfa lines. At least two commercial alfalfa seed companies have adopted the biomass ideotype and released commercial varieties with reduced susceptibility to lodging.

  Lamb, J.F.S., H.G. Jung, C. C. Sheaffer, and D.A. Samac. 2007 Alfalfa Leaf Protein and Stem Cell Wall Polysaccharide Yields under Hay and Biomass Management Systems. Crop Sci. (Accepted 1/22/07)

- **Demonstrated modeling approach to minimize feedstock transportation.**
  The low density and large volume typical of lignocellulosic biomass increases the cost of transportation and likely will result in conversion facilities depending on small fuelsheds to achieve highest net energy yields. A fuelshed modeling approach was demonstrated that predicts crop yields by soil type to evaluate placement of biomass plantings to minimize transportation distance and maximize net energy yield within a fuelshed.

  Russelle, M. P., A. S. Birr, and D. G. Tiffany. 2006. Estimated net energy yields of biomass in a fuelshed. ASA-CSSA-SSSA Annual Meetings. Abstracts 167-2 (CDROM)

- **Developed potential coproducts of alfalfa produced for bioenergy.**
  Because energy is a relatively low-value commodity, biomass crops that can yield a valuable by-product would improve the profitability of a bioenergy system. Previously it was demonstrated that leaves could be separated from alfalfa hay and that these leaves are a good supplemental protein feedstuff for dairy and beef cattle The Minnesota Valley Alfalfa Cooperative (http://www.mnvap.com) sells an alfalfa leaf meal product that was developed in cooperation

between ARS, the University of Minnesota, and the cooperative. More recently alfalfa was bio-engineered to product a biodegradable plastic precursor in its leaves. Commercialization of this technology awaits a more efficient extraction procedure for the bioplastic and greater production of the bioplastic by the plants.

DiCostanzo, A., C.M. Zehnder, J.M. Akayezu, M.A. Jorgensen, J.M. Cassady, D.M. Allen D. G. Standorf, J.G. Linn, H. Jung, L.J. Smith, G.C. Lamb, D. Johnson, H. Chester-Jones, and G. Robinson. 1999. Use of alfalfa leaf meal in ruminant diets. Proc. 60th Minn. Nutr. Conf., p. 64-75.

Saruul, P., Sreinc, F., Somers, D.A., and Samac, D.A. 2002. Production of a biodegradable plastic polymer, poly-α-hydroxybutyrate, in transgenic alfalfa (*Medicago sativa* L.). Crop Sci. 42:919-927

- **Alfalfa found to require harsh pretreatment to release sugars.**
In collaboration with other ARS units, it was shown that alfalfa stems require harsher pretreatment conditions than reed canarygrass and switchgrass to achieve similar efficiencies of glucose release through a dilute acid/high temperature pretreatment and enzymatic saccharification conversion process. This is an essential research evaluation step to identify the relative conversion efficiency of alfalfa as a biomass feedstock.

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

- ***Medicago truncatula* found to be good model plant for legume research**
It was demonstrated that *M. truncatula* is a good model of stem tissue and cell wall development in alfalfa. This result provides confidence that genetic information garnered from *M. truncatula* about gene expression related to cell wall biosynthesis will be directly applicable to improving alfalfa stem traits for bioenergy production.

Schnurr, J.A., H.G. Jung, and D.A. Samac. 2007. A comparative study of alfalfa and *Medicago truncatula* stem traits: morphology, chemical composition, and ruminal digestibility. Crop Sci. (accepted 2/23/07)

- **Determined genetic sequences of *Brachypodium*, a model for bioenergy plants**
The first public gene sequences for *Brachypodium*, a grass plant being adopted for all grass research and designated by DOE as the preferred model system for all bioenergy related feedstock research in plants. ARS scientists are leading a national effort to sequence the entire *Brachypodium* genome and apply this knowledge to the development of bioenergy grasses.

Huo, N., Gu, Y., Lazo, G., Vogel, J., Coleman-Derr, D., Luo, M., Thilmony, R., Garvin, D., and Anderson, O. (2006) Construction and characterization of two BAC libraries from Brachypodium distachyon, a new model for grass genomics. Genome 49:1099-1108

Tobias CM, Hayden DM, Twigg P, Sarath G (2006) Genic microsatellite markers derived from EST sequences of switchgrass (*Panicum virgatum* L.). Molecular Ecology Notes 6: 185-187.

Tobias CM, Twigg P, Hayden DM, Vogel KP, Mitchell RM, Lazo GR, Chow EK, Sarath G (2005) Analysis of expressed sequence tags and the identification of associated short tandem repeats in switchgrass. Theor. and Appl. Genet. 111: 956-964.

Vogel, J., Gu, Y., Twigg, P., Lazo, G., Laudencia-Chingcuanco, D., Hayden, D., Donze, T., Vivian, L., Stamova, B., and Coleman-Derr, D. (2006) EST sequencing and phylogenetic analysis of the model grass *Brachypodium distachyon*. Theor. and Appl. Genet. 113:186–195

- **Developed method to transform *Brachypodium***
  A high-efficiency *Agrobacterium*-mediated transformation method for *Brachypodium* has been developed. This method is essential to utilize *Brachypodium* as a model grass to identify all the grass genes that impact feedstock quality.

  Vogel, J., Garvin, D., Leong, O., and Hayden, D (2006) *Agrobacterium*-mediated transformation and inbred line development in the model grass *Brachypodium distachyon*. Plant Cell Tiss. and Organ Cult. 84:199-211. (This method has been requested by many researchers.)

- **Assessed average biomass yield from conservation grasslands**
  Determined from a survey of conservation grasslands conducted in the Northeastern United States that these grasslands produced an average biomass yield of about 3 tons/acre across sites and years, ranging from 0.24 tons/acre on reclaimed mine land sites to 6.47 tons/acre on good cropland. These results were used by the Pennsylvania Department of Agriculture and The Pennsylvania State University as part of a Pennsylvania state biomass resource assessment.

  Adler, P.R., Sanderson, M.A., and Goslee, S.C. 2005. Management and composition of conservation lands in the Northeastern United States, p. 187-200.

  In Thomas G. Barnes and Linda R. Kiesel (ed.) Proceedings of the Fourth Eastern Native Grass Symposium. The University of Kentucky Department of Forestry, Lexington, KY, October 3-6, 2004.

- **Evaluated seasonal variations of switchgrass on thermochemical properties**
  Research determined that delaying switchgrass harvest from fall to spring will reduced biomass yield, but increased biofuel quality for thermochemical conversion. Ernst Conservation Seeds and NRCS personal are using this information in the development of industry recommendations for management of switchgrass and other warm season grass fields for combustion of densified biomass in Pennsylvania.

  Adler, P.R., M.A. Sanderson, A.A. Boateng, P.J. Weimer, and H.G. Jung. 2006. Biomass yield and biofuel quality of switchgrass harvested in fall or spring. Agron. J. 98:1518–1525

- **Conducted a greenhouse-gas life cycle assessment of bioenergy crops grown in the Northeastern USA**
  This assessment will provide better estimates of the impact of bioenergy crops on the U.S. Greenhouse Gas Inventory. These results will be included in the USDA Greenhouse Gas inventory section describing mitigation options.

  Adler, P.R., S.J. Del Grosso, and W.J. Parton. 2007. Life cycle assessment of net greenhouse gas flux for bioenergy cropping systems. Ecol. Appl. (in press, April).

Accomplishments without listed peer review publications

- **Identified and characterized a maize mutant with reduced lignin in plant leaves**
  The ideal biofuel crop will produce a large amount of biomass and have reduced lignin for ease of saccharification. In collaboration with DuPont scientists, ARS scientists isolated the gene behind the Corngrass mutation in maize. Corngrass maize plants have many more tillers that are juvenile in cell wall properties. Corngrass leaves have increased sugars and reduced lignin. Corngrass encodes a microRNA, which allows us to precisely know the targets of this gene. Manipulation of the target genes will allow us to take educated steps toward improved biofuel crops. The isolation of the Corngrass gene and analysis of the target genes is now accepted for publication in *Nature Genetics*.

- **Developed a high resolution, rapid analytical procedure for sugar glycosidic linkage determination**
  Gel-state proton NMR was used to analyze for the ratio of the 1-4 to 1-6 linkages in the starch of rice and corn. This has been accomplished in both in the whole flour and isolated starch. It provides a better calibration for the more rapid methods of analysis such as NIR or Raman spectroscopy. It avoids the errors associated with traditional attribute analysis of grains by wet chemical techniques.

- **Developed tools for the rapid assessment of barley traits suitable for ethanol production**
  Calibrations have been developed for the analysis of barley, for use a source of ethanol, by NIR spectroscopy. Equations have been developed for moisture, starch, β-glucan, protein, oil, and ash. This provides a rapid assessment of barley by type, variety and growing location that will provide the best ethanol yields.

- **Developed spectroscopic methods for assessment of switchgrass biomass properties**
  High-resolution NIR spectroscopy has been used to develop improved calibrations for the evaluation of switch grass as a biomass material. The analysis for neutral detergent fiber, acid detergent lignin, fat and total carbon were improved using high-resolution Fourier-transform NIR spectroscopy.

- **Developed spectroscopic methods for immediate analysis of lignin to carbohydrate ratio**
  The selective detection of lignin in anatomical parts of corn stover was accomplished using attenuated total reflection Fourier-transform (ATR/FT-IR) in the mid-infrared region. This provides immediate evaluation of the relative lignin versus carbohydrate contents of biomass material. Raman spectroscopy has also been used to provide similar analyses.

- **Evaluated the effects of genotype on plant mineral composition with downstream effects on conversion to energy**
  Genotypic differences effect the accumulation of minerals that impact the suitability of grasses as feedstock for thermochemical conversion to energy. Mineral accumulation was quantified in multiple populations of switchgrass, native grasses, and cultivated cool-season grasses grown at contrasting locations. These results suggest that new varieties of grasses with improved characteristics for use as biofuel feedstock can be developed. This research led to submission of two peer-reviewed publications.

- **Identified procedure to identify optimal switchgrass germplasm for ecoregion specific production and conservation**
  Found that phenotypic variability among prairie remnant populations was closely associated with both the USDA hardiness zone (defined by minimum cold temperatures) and ecoregion (defined by historic native vegetation) from which a population was collected. This is the first experimental and quantitative data to support the hypothesis that switchgrass populations have spatial structure at the landscape level, information that will be useful in identifying optimal germplasm for use in breeding for conservation and production. This research has resulted in the development of Plant Adaptation Regions for use of switchgrass as a biomass energy crop.

- **Developed establishment criteria for use of switchgrass for use as a biomass energy crop**
  Field scale trials in the Northern Great Plains states of Nebraska, South Dakota, and North Dakota were used to determine threshold or minimal switchgrass stands for switchgrass when grown as a biomass energy crop. Results from the three-state evaluation area indicate that an establishment year stand frequency levels of 40% or greater as determined by a frequency grid can be considered an establishment year stand threshold for successful establishment and subsequent post-establishment year biomass yields for switchgrass grown as a bioenergy crop in the Northern Great Plains. An

establishment year stand frequency of 25% would be adequate for a switchgrass conservation planting for which no harvests would be planned for several years. Additional research demonstrated that the herbicide combination of atrazine + quinclorac resulted in acceptable first year stands and high biomass yields, and is an excellent herbicide combination for establishing switchgrass for biomass production in the Great Plains. Other broad-leaf herbicides can be substituted for atrazine. This research provided essential management information for the deployment of switchgrass as a biomass energy crop.

- **Switchgrass increases soil carbon storage**.
  Soil carbon (C) stocks were evaluated under established switchgrass stands and nearby cultivated cropland throughout the northern Great Plains and northern Cornbelt. Soil organic C was greater in switchgrass stands than cultivated cropland at soil depths of 0 to 0.05, 0.3 to 0.6, and 0.6 to 0.9 m, and especially so at the deeper soil depths where treatment differences were 7.74 and 4.35 Mg C ha^{-1} for the 0.3 to 0.6 and 0.6 to 0.9 m depths, respectively. Consequently, switchgrass appears to be effective at storing soil organic C not just near the soil surface, but also at depths below 0.3 m where C is less susceptible to loss. Two switchgrass cultivars were evaluated for C sequestration and partitioning among plant components. Root biomass was found to account for >80% of total biomass, and crown tissue contained approximately 50% of total biomass C. Soil organic C was found to increase at a rate of 1.01 kg C m^{-2} yr^{-1} over the 0 to 0.9 m depth, suggesting switchgrass plantings in the northern Great Plains have potential for storing a significant quantity of soil C. This research provides baseline information on the environmental benefits for biomass energy crops in terms of net carbon balance. (More detail see ARS Mandan).

- **Determined amount of available straw for biomass in Northwest**
  ARS scientists quantified the amount of total straw produced on a county-by-county basis in Idaho, Oregon and Washington and calculated the amount of straw, also on a county-by-county basis, that should be returned to the soil for conservation purposes. This accomplishment permitted calculation of the amount of straw available in the region for potential use as biofuel feedstock after conservation requirements have been met. These data provided the basis for preparation and submission of a peer-reviewed manuscript.

- **Determined the economic production cost feasibility of perennial herbaceous energy crops**
  Switchgrass was planted and managed as a biomass energy crop for five years in fields on 10 farms in Nebraska and South and North Dakota with cooperating farmers during the period 2000 to 2005 to determine economic costs of production. Production cost averaged $33/ton plus $17/ton for land rent. If establishment costs were pro-rated over nine production years, cost was reduced an additional $6/t. Experienced farmers produced switchgrass for less than $40/t including land costs which would result in a feedstock cost of $0.50 per gallon of ethanol. Primary production factors that affected feedstock costs were stand establishment which affected first and second year yields, fertilization and harvest management which affected post-establishment yields and available precipitation which affected both establishment and yields. All factors except for precipitation can be significantly improved by management. The study demonstrated that additional improvements in biomass yield and in reduction of biomass costs per ton can be achieved with improved cultivars, seed quality, and management practices. This research was conducted in cooperation with a University of Nebraska agricultural economist. Impact: The U.S. Department of Energy discontinued funding research on perennial biomass energy crops in 2002. The data from this study along with data on potential yield improvements via breeding was provided to the U.S. Department of Energy (DOE) and USDA in a series of pre-publication briefings from March 2005 through December 2005. As a result, beginning in 2006, research emphasis and funding was directed toward perennial cellulosic energy crops such as switchgrass.

- **Quantified the net energy balances of perennial herbaceous energy crops.**
  Biomass yields and farmer inputs for the ten farms in the on-farm economic trials were used to determine the net energy balance of switchgrass grown as a biomass energy crop. All previous estimates have been based on small plot data or estimates. Net energy averaged 43.5 GJ ha^{-1} y^{-1}. Switchgrass produced 540% more renewable energy than nonrenewable energy consumed. Switchgrass managed for high yield had equal or greater net energy than did low input restored prairies and can produce 350% more liquid fuel per hectare. Farms on which the largest biomass yields were obtained also had greater net energy values. Impact: This large scale study clearly demonstrated that perennial herbaceous energy crops are net energy positive and improvements in genetics and management will enhance both total and net energy yields and production economics. (Manuscript in review).

- **Genetically modified plants for improved feedstock properties and production**
  The feasibility of using hybrid cultivars to increase switchgrass biomass yield for use as a bioenergy feedstock has been successfully demonstrated. F1 hybrid populations of the cultivars Kanlow × Summer produced 24% higher biomass yield than Kanlow and 50% greater yields than Summer in sward plots in eastern Nebraska in 2002 and 2003. The F1 hybrid populations produced 20 Mg/ha of biomass during years with below average precipitation. The findings show the potential for increasing biomass yields of switchgrass by the development of hybrid cultivars. The data from research in addition to the economics data was provided to the U.S. Department of Energy (DOE) and USDA in a series of pre-publication briefings from March 2005 through December 2005. As a result, beginning in 2006, major shifts in research emphasis and funding occurred with an emphases being placed on perennial cellulosic energy crops such as switchgrass. In addition, several genetics companies are now investing in research to developed switchgrass for use in biomass crop production systems.

- **Released switchgrass germplasm.**
  Switchgrass germplasm was released for use in breeding switchgrass cultivars adapted to biomass energy production systems in the upper Midwest and Great Lakes Region. This technology is the first release of a switchgrass cultivar specifically for biomass energy production systems and thus represents a significant historical accomplishment.

- **Characterization and analysis of the switchgrass genome**
  The first public switchgrass gene sequences are being characterized. ARS scientists are leading a national effort to sequence and determine the function of most of the switchgrass genes, mapping the switchgrass genome, and accelerating efforts to breed better varieties of switchgrass and other perennial, herbaceous crops.

- **Developing energy cane for increased biomass yields**
  Identified energy cane varieties for further testing that can produce biomass yields that are significantly higher than conventional sugarcane varieties.

- **Determined gene sequence information important for breeding energy crops**
  Gene sequences for *Brachypodium*, a model grass plant, and switchgrass are being sequenced and published. ARS scientists are also leading a national effort to sequence the entire genome for *Brachypodium* as well as most of the genes in switchgrass. The sequence data generated will accelerate efforts to breed better varieties of perennial, herbaceous energy crops such as switchgrass by serving as the ultimate genomic reference library.

- **Characterized sources of genetic variation for biomass quality in switchgrass**
  Multiple generations of breeding for improved forage digestibility as measured by an artificial rumen digestion procedure known as in vitro dry matter digestibility (IVDMD) produced switchgrass populations that differ significantly in digestibility and lignin, cellulose, and hemicelluose concentration of leaf, sheath and stem tissue. In the stems of switchgrass, changes in lignin appear to involve two primary mechanisms: (1) the loss or decrease in cortical fibers and (2) changes in secondary cell wall deposition. These results indicate that plant anatomy can be significantly impacted by a simple selection tool and demonstrate that genes that control these functions can be used to genetically modify biomass composition to genetically improve conversion efficiency to liquid fuels. Impact: As a result of this and previous research on genetically modifying forage plant composition, major new DOE and ARS research initiatives have been funded to genetically modify biomass plant cell walls.

- **Demonstrated that lignin concentration affects plant fitness.**
  Genetically modifying the lignin concentration of perennial grasses was found to affect plant fitness as measured by winter survival. This showed the importance of monitoring cell wall changes that occur when perennials are modified by use of either conventional or molecular genetic technologies. As a result of this research, all public agencies and private companies developing improved biomass crops for use in biomass energy production systems are aware that some genetic modifications of plant cell walls can adversely affect fitness. Fitness is now a mandatory evaluation criterion for perennial biomass energy crops.

- **Developed basic molecular genetic information for the genetic mapping & modification of switchgrass for biomass energy.**
  Switchgrass is a North American prairie grass that is being developed into a perennial biomass energy crop. Expressed Sequence Tags (ESTs) were generated from leaf, stem, crown, and callus tissue of tetraploid switchgrass plants and were used to create a gene inventory of 7810 unique gene clusters in cooperative research by ARS scientists at Lincoln, NE and Albany, CA and a Univ. of Nebraska scientist at Kearney, NE. The sequences have been deposited in GenBank. Similarity searches in genetic databases indicated that 79% of these clusters were similar to other genes and 61% of the sequences were tentatively identified for their function. A total of 334 gene clusters contained short tandem repeats which can be developed into micro-satellite markers. As a result of this research, a major DOE funded project on developing a complete set of EST's for switchgrass is now in progress and is being led by ARS laboratories at Albany, CA and Lincoln, NE.

- **Modified the genetics of switchgrass for enhance energy crop properties**
  The genetics of switchgrass, an herbaceous energy crop, have been altered by *Agrobacterium* mediated transformation. Plants have been regenerated to contain sequences designed to decrease the activity of lignin biosynthetic enzymes. Lignin is a complex, cross linked macromolecule responsible for plant structure and integrity. Therefore, methodology designed to decrease lignin production may provide material that is more readily converted to ethanol by existing technologies. This technology is under development but represents a viable researchable area with the promise of significantly reducing switchgrass biomass processing to ethanol.

Topic: Feedstock logistics
Goal: Develop technology for biomass handling, storage, and transportation systems.

Summary: USDA ARS research activities under Feedstock Logistics is focused on research to assess the effects of harvest time and procedure, on site biomass pretreatment, and alternative uses on biomass handling and effects on storage and transport.

Significant Accomplishments & Impact:

- Showed that harvesting corn stover at higher moisture level (~40 percent moisture) and storing the stover in plastic film under non-ensiling conditions economized field operations, increased the rate and efficiency of harvesting, and reduced storage losses of dry matter to under 5 percent. These results represent the first data on corn stover harvesting economics for biomass.

- Found that burning cotton byproduct (COBY) pellets made from gin trash and COBY/Guayule pellets in commercial pellet stoves gave acceptable exhaust gas emissions and had heating values equivalent to other pellet stove fuels suggesting this material could be useful as an on farm energy source.

Topic: Crop residues
Goal: Determine the amount of crop residue that can be removed without deleterious effects.

Summary: In 2002, U.S. Department of Energy (DOE) discontinued research on perennial biomass energy crops and began a heavy focus of crop residue of energy crops with the assumption that crop residues such as corn stover are an abundant and inexpensive source of biomass that can be removed from fields to produce bioenergy. Assumptions include that with minimum or no-tillage farming methods, there will be no deleterious production or environmental effects. A long-term field study in eastern Nebraska, USA, that was established to compare carbon sequestration of switchgrass managed as a biomass energy crop versus no-till corn under non-irrigation in the western Corn Belt was modified in 2000 to evaluate the effects of stover harvested for biomass energy. In the first five years (2001-2005), removal of half the available stover significantly reduced corn yields. During that same time period, the potential ethanol yield for switchgrass was equal or greater than the potential total ethanol yield of corn grain and harvested stover fertilized at the same optimum nitrogen rate. The data from this research was provided to the U.S. Department of Energy (DOE) and USDA in a series of pre-publication briefings from March 2005 through December 2005. As a result of this research and other related research, the cellulosic industry and DOE are now aware of the importance of crop residue (biomass) in providing carbon to the soil system and the critical need to replenish soil carbon through biomass inputs. Assessments of sustainable production and harvest practices cannot be limited to evaluation of erosion control. Initial estimates of source carbon needed to maintain soil functionality were published and production practices to maximize sustainable biomass harvest are being developed and evaluated.

Significant Accomplishments & Impact:

- **Assessed effects of corn stover removal on soil productivity and sustainability**
  A long-term experiment was established in 1998 in eastern Nebraska to quantify carbon sequestration of switchgrass grown and managed as a biomass energy crop in comparison to rain-fed, no-till corn.

Because of U.S. Department of Energy interest in corn stover as a biomass energy source, corn plots were split in 2000 and half of the stover was mechanically harvested each year from one-half of each corn plot. Results showed that corn grain yields were significantly reduced where corn stover was removed. In addition, potential ethanol yields from switchgrass biomass were found to be significantly greater than potential ethanol yields from corn, including that from both grain and stover. These findings show there is a need to more carefully determine the potential consequences of corn stover removal before the practice is recommended as a way to provide feedstock for biorefineries. These results generated awareness and acceptance within U.S. DOE and the cellulosic ethanol industry of the critical importance of crop residues (corn stover, wheat straw, etc.) in supplying plant nutrients and carbon to the soil ecosystem and that sustainable production practices must be defined in terms of soil carbon dynamics as well as soil erosion control. Research and policy is needed to develop and encourage use of sustainable feedstock production and harvest practices.

Fales, S., J. Richard Hess, and W.W. Wilhelm. Ethanol production from cellulosic biomass: A scientific assessment (CAST Commentary). Commentary QTA 2007-x. CAST, Ames, Iowa. (submitted March 2, 2007).

Johnson, J., D. Reicosky, R. Allmaras, D. Archer, and W.W. Wilhelm. 2006. A matter of balance: Conservation and renewable energy. J. Soil Water Cons 61:120A-125A.

Wilhelm, W.W., J.M.F. Johnson, J.L. Hatfield, W.B. Voorhees, and D.R. Linden. 2004. Crop and soil productivity response to corn residue removal: A literature review. Agronomy Journal 96:1-17.

- **Assessed and experimentally verified precise height of corn stover to be removed for optimal conversion to ethanol**
  Determined that harvesting corn (and the stover with a proto-type one-pass harvester) at the normal combining height, approximately 16 inches high, provided the best mix of material for producing ethanol and retaining stover in the soil to control erosion and supply nutrients and carbon to the soil. Harvesting the lower portion of the corn plant increased the water content and reduced the quality of the stover for producing ethanol and exacerbated sustainability concerns. Nutrients removed with the stover were valued at \$23.21 ac^{-1} or \$10.22 ton^{-1} of biomass (2006 dollars). This information is critical for DOE and equipment manufacturer engineers, research scientists, investors, and others developing processes and products for renewable plant biomass.

Hoskinson, R.L., D.L. Karlen, S.J. Birrell, C.W. Radtke, and W.W. Wilhelm. 2007. Engineering, nutrient removal, and feedstock conversion evaluations of four corn stover harvest scenarios. Biomass and Bioenergy 31:126-136.

- **Quantified minimum corn stover biomass input for maintenance of soil productivity**
  Provided the first estimates of crop biomass inputs needed to maintain soil organic carbon (SOC) for a limited array of crop management practices through use of estimates of total source carbon inputs, harvest index, and the national yield database. Calculated corn stover inputs to maintain SOC ranged from 5.3 Mg ha^{-1} for continuous corn with no tillage to 12.5 Mg ha^{-1} for a corn-soybean rotation with moldboard plow as the primary tillage practice. These data represent the first demonstration of qualitative analysis on minimal corn stover requirements for maintenance of soil productivity and sustainability. This work has and continues to rectify the misperception that corn stover residue is an agricultural waste material with little appreciation for soil maintenance.

Johnson, J.M.F., R.R. Allmaras, and D.C. Reicosky. 2006. Estimating source carbon from crop residues, roots and rhizodeposits using the national grain-yield database. Agronomy Journal 98:622-636.

Wilts, A.R., D.C. Reicosky, R.R. Allmaras and C.E. Clapp. 2004. Long-term corn residue effects: Harvest alternatives, soil carbon turnover, and root-derived carbon. Soil Sci. Soc. Am. J. 68:1342-1351.

- **Assessed effects of ligneous byproduct application on soil productivity**
  Determined that soil application of the ligneous by-product remaining after ethanol production from crop biomass at a rate equivalent to 11.23 Mg ha^{-1} increased humic acid content (an indicator of soil organic carbon content) and aggregate stability (an indicator of the ability of soil to resist erosion). Removing the corn residue from the field may result in greater rates of erosion and loss of soil carbon. Returning the ligneous by-product to soil may reduce the negative effects of removing corn residue. This information is important to the USDA, DOE, and cellulosic ethanol industry in determining the feasibility of using corn residue for ethanol production.

  Johnson, J.M.F., D. Reicosky, B. Sharratt, M. Lindstrom, W. Voorhees, and L. Carpenter-Boggs. 2004. Characterization of soil amended with the by-product of corn stover fermentation. Soil Science Society of America Journal 68:139-147.

- **Determined positive effects of rotating a winter cover crop into summer cropping system for biomass production, and soil protection**
  ARS scientists demonstrated that a cool season winter cover crop, when added to a summer cropping system, can use untapped resource (solar radiation, water, and nutrients) to produce more than 4 Mg ha^{-1} of additional biomass while providing erosion and water quality protection without affecting food production capacity. This report provides scientific rationale for a potential path forward for the farming community to provide the cellulosic ethanol industry with a sustainable feedstock while maintaining feed, food, and environmental service capacity.

  Baker, J.M., W.W. Wilhelm, and T.J. Griffis. 2007. Towards environmentally sustainable co-production of food and biofuel in the U.S. Maize Belt. Agric. Ecosyst. Environ. xxx:xxx-xxx. (submitted January 27, 2007).

- **Conducted long term experiments to address management practices on SOC**
  Results from long-term experiments showed that annual tillage tends to decreased soil organic carbon (SOC), regardless of residue management practice. Further crop residue removal reduces grain yield and generally reduced SOC. SOC is increased only under no tillage management with nitrogen supplementation and retention of agricultural residues. These published results are essential for the proper management of biomass production systems in order to help ensure sustainable soil productivity by highlighting negative effects of tillage and residue removal.

  Clapp, C.E., R.R. Allmaras, M.F. Layese, D.L. Linden, and R.H. Dowdy. 2000. Soil organic carbon and 13C abundance as related to tillage, crop residue, and nitrogen fertilization under continuous corn management in Minnesota. Soil and Tillage Research 55:127-142.

  Linden, D.L., C.E. Clapp, and R.H. Dowdy. 2000. Long-term corn grain and stover yields as a function of tillage and residue removal in east central Minnesota. Soil and Tillage Research 56:167-174.

  Pikul, J.L. Jr., S. Osborne, M. Ellsbury, and W. Riedell. 2007. Particulate organic matter and water stable aggregation of soil under contrasting management. Soil Sci. Soc. Am. J. 71: (in press).

  Reicosky, D.C., S.D. Evans, C.A. Cambardella, R.R. Allmaras, A.R. Wilts and D.R. Huggins. 2002. Continuous corn with moldboard tillage: Residue and fertility effects on soil carbon. J. Soil Water Conserv. 57:277-284.

  Varvel, G.E., K.P. Vogel, R.B. Mitchell, R.F. Follett, and J.M Kimble. 2007. Bioenergy from Crop Residues: A Cautionary Report. Biomass & Bioenergy. 31: (Submitted Nov 30, 2006).

Summary

This report in intended to represent the research activities of ARS National Program 307 and provide program reviewers with an unbiased, objective list of accomplishments relevant to Bioenergy and Energy Alternatives. This report should be evaluated on the basis of the program's Action Plan.

The accomplishments listed in this report illustrate the responsiveness of the program to customer and stakeholder needs and concerns. NP 307 leadership has demonstrated a commitment to address the research needs as defined in the Action Plan. These accomplishments are a reflection of the high standard of scientific achievement and remarkable talent of ARS scientists. While much of this work has provided basic science information, a significant proportion of these efforts have resulted in (pre)commercialization of products and technologies transferred to the private sector. The quality and performance of these technologies are clearly represented by the high impact publications and industrial collaborations listed in this report. Further, these technologies have had substantial impact on the ethanol industry, and the program continues to make seminal contributions in the areas of sustainable feedstock design and production, conversion science, and residue management.

Research in the field of bioenergy encompasses a broad range of scientific disciplines and requires the integration of technical resources across many ARS national programs. NP 307 therefore is not a stand alone program, but rather seeks to facilitate the research necessary to achieve the goals set forth by not only the program and agency, but the Department and Nation as well.

NP 307 has established a strong research foundation by addressing the needs of customers and stakeholders, and is poised to continue in this role as a leader in the field to help commercialize biofuels and bioproducts.

Appendix A: Current Research Projects Contributing to ARS Bioenergy & Energy Alternatives Research

Research Projects Associated with National Program 307

Note: C=contributing to NP307

Location and Principal Investigator	NP Code	Project Number and Title
R.L. Fireovid Beltsville, MD	307	0500-00076-001-00D National Corn To Ethanol Research Pilot Plant
M. Sanderson University Park, PA	307 (C) 205	1902-21000-006-00D Biodiversity Management In Northeastern Grazing Lands
T. Foglia Wyndmoor, PA	306 307 (C)	1935-41000-066-00D Production Of Value-Added Lipids, Biofuels, And Biobased Products From Fats And Oils
R. Moreau Wyndmoor, PA	307 306 (C)	1935-41000-069-00D Aqueous Enzymatic Extraction Of Corn Oil And Value-Added Products From Corn Germ Produced In New Generation Dry-Grind Ethanol Processes
D. Johnston Wyndmoor, PA	306 307 (C)	1935-41000-070-00D Enzyme-Based Technologies For Milling Grains And Producing Biobased Products And Fuels
K. Hicks Wyndmoor, PA	307 306 (C)	1935-41000-072-00D Economic Competitiveness Of Renewable Fuels Derived From Grains And Related Biomass
B. Dien Peoria, IL	307 306 (C)	3620-41000-118-00D Industrially Robust Enzymes And Microorganisms For Production Of Sugars And Ethanol From Agricultural Biomass
K. Bischoff Peoria, IL	307 306 (C)	3620-41000-121-00D Microbial Catalysts To Produce Fuel Ethanol And Value Added Products

B. Saha Peoria, IL	307 306 (C)	3620-41000-122-00D Cost-Effective Bioprocess Technologies For Production Of Biofuels From Lignocellulosic Biomass
P. Slininger Peoria, IL	307 306 (C)	3620-41000-123-00D Genomics And Engineering Of Stress-Tolerant Microbes For Lower Cost Production Of Biofuels And Bioproducts
S. Erhan Peoria, IL	307 306 (C)	3620-41000-124-00D Improving The Performance Of Alternative Fuels And Co-Products From Vegetable Oils
D. Samac St. Paul, MN	205 307 (C)	3640-21410-002-00D Forages For New Uses, Enhanced Quality, & Stress Tolerance Through Genomics And Genetic Improvement
P. Weimer Madison, WI	306 307 (C)	3655-41000-004-00D Value-Added Products From Forages And Biomass Energy Crops
O. Anderson Albany, NY	307	5325-21000-013-00D Biotechnological Enhancement Of Energy Crops
D. Wong Albany, NY	307 306 (C)	5325-41000-046-00D Evolutionary Enzyme Design For Improved Biorefining Of Crops And Residues
G. Robertson Albany, NY	306 307 (C)	5325-41000-047-00 Technologies Enabling Enhanced Product Quality, Product Opportunities, And Energy Efficiency In Grain Biorefining Systems
S. Griffith Corvallis, OR	207 307 (C) 201 (C)	5358-21410-002-00D Integrating Production And Conservation Practices To Maintain Grass Seed Farm Profits

K. Vogel Lincoln, NE	205 307 (C)	5440-21000-021-00D Improved Plants And Production Practices For Grasslands And Biomass Crops In The Mid-Continental USA
K. Rosentrater Brookings, SD	307 306 (C)	5447-41000-002-00D Fiber Extrusion To Improve Use And Production Of Ethanol Byproducts
N. Clark Bushland, TX	307 201 (C)	6209-13610-006-00D Remote Water Pumping And Electric Power Generation With Renewable Energy
B. Venuto El Reno, OK	205 204 (C) 307 (C)	6218-21410-002-00D Develop And Manage Forage Resources For A Sustainable Agriculture
S. Lingle New Orleans, LA	302 307 (C)	6435-21000-013-00D Biochemical And Genetic Basis Of Sucrose Partitioning In Sugarcane Stalks For Sucrose And Biofuel Production
J. Wilson Tifton, GA	205 307 (C)	6602-21410-004-00D Genetic Enhancement And Management Of Warm-Season Grass Species For Forage And Alternative Uses

Appendix B: Timeline of Events and Activities Associated With ARS Bioenergy & Energy Alternatives National Program

April 15, 1999
Initial Customer Workshop, George Washington Carver Center, Beltsville, Maryland
ftp://ftp.nps.ars.usda.gov/NRSAS/NP%20307%20Assessment/1999NP307MeetingMaterials.pdf
The workshop was attended by 28 external customers and 15 ARS scientists. The post-workshop evaluations revealed that attendees overwhelmingly thought that the objectives were clearly defined, the background information provided was useful or very useful, and they had adequate opportunity to provide input. The five-year National Program Action Plan was prepared using input from the workshop.

Nov. 13-15, 2001
Funding Increase and Bioenergy Planning and Coordination Workshop, Laurel, Maryland
ftp://ftp.nps.ars.usda.gov/NRSAS/NP%20307%20Assessment/BioenergyPlanningandCoordination.pdf
To effectively use the new and ongoing funds appropriated for energy research, a strategic plan, "Technology for a Sustainable Energy System," was developed and an implementation and coordination workshop was held. At the workshop, ARS scientists and engineers planned research to be conducted and the National Program Action Plan was amended to its current form.

2002
Congress appropriated an additional $9.52 million dollars for ARS to expand efforts to develop technology that will improve ethanol and biodiesel production and to initiate a program to develop plant species and management practices for sustainable production of energy crops.

July 23-24, 2002
Ethanol Component Workshop, Beltsville, Maryland
ftp://ftp.nps.ars.usda.gov/NRSAS/NP%20307%20Assessment/EthanolComponentWorkshop.pdf
Sept. 18-19, 2002
Biodiesel Component Workshop, Beltsville, Maryland
ftp://ftp.nps.ars.usda.gov/NRSAS/NP%20307%20Assessment/BiodieselComponentWorkshop.pdf
Dec. 3-5, 2002
Energy Crops Component Workshop, Peoria, Illinois
ftp://ftp.nps.ars.usda.gov/NRSAS/NP%20307%20Assessment/EnergyCropsComponentWorkshop.pdf

ARS NPS hosted three component workshops to more precisely discuss current state of research activities and coordinate future planning.

May, 2004
National Program 307 projects external peer review by Office of Scientific Quality Review.

Nov. 29-Dec. 1, 2006
NP 307 Planning and Coordination Meeting (Internal Scientist Workshop), Beltsville, Maryland
ftp://ftp.nps.ars.usda.gov/NRSAS/NP%20307%20Assessment/NP307_213_PCM_DEC2006.pdf
The workshop was held to take stock in the program's accomplishments, enhance communication and coordination amongst locations, and refocus due attention on a program poised for significant growth. The meeting was attended by 75 ARS scientists (both working within NP 307 and in support of bioenergy goals), along with several National Program Staff

and interagency personnel. ARS locations presented research activities and results under two broad thematic areas, *Feedstock Research and Development, and Other Bioenergy Projects* and *Conversion Technology, Biodiesel and Biorefining*. These presentations, which can be downloaded from http://www.ars.usda.gov/research/programs/programs.htm?np_code=307&docid=14536, represent a complete picture of ARS NP 307, and clearly illustrate the significant contributions being made to the Nation's bioenergy goals.