# FY2015 Annual Report

## National Program 213—Biorefining

### Introduction

The USDA-ARS National Program for Biorefining (NP213) completed the first year of their new five-year research objectives in 2015. Scientists in NP213 continue to make extraordinary impact in numerous diverse areas of research relating to the conversion of agricultural materials into fuels, value-added co-products, and biobased products. The program's vision and relevance can be found at http://www.ars.usda.gov/research/programs/programs.htm?NP_CODE=213, including the current five-year Action Plan for NP213, which went into effect in early 2014.

The overarching goal of NP 213 is to conduct research that enables new, commercially-viable technologies for the conversion of agricultural materials into fuels, value-added co-products, and biobased products. To achieve this goal, this Action Plan was designed to meet the following criteria:

1. *Maximize the long-term economic impact of ARS biorefining research*
2. *Emphasize ARS' unique capabilities and avoid overlap with research at other institutions.*
3. *Maximize returns to agricultural stakeholders from ARS investment of public funds.*

By developing commercially viable technologies for the production of biobased industrial products, ARS biorefining research increases the demand for agricultural products and therefore benefits both agricultural producers and rural communities.

During FY 2015, 36 full-time scientists working at 4 locations across the U.S. actively engaged in 9 ARS-led and 40 cooperative research projects in NP213. The gross fiscal year 2015 funding for NP213 was $15.4 million.

**The following scientists retired from the ranks in NP213:**

- **Dr. Michael Cotta** of the Bioenergy Research Unit, Peoria, IL.

Dr. Cotta's distinguished record of service is recognized world-wide, and he will be missed in NP213.

**The following scientists in NP 213 received prominent awards in 2015:**

- **Dr. Yaseen Elkasabi,** Wyndmoor, PA received ARS's 2015 Northeast Area Early Career Scientist of the Year Award.
- **Dr. Robert Moreau,** Wyndmoor, PA received the Alton E. Bailey Award from the American Oil Chemists Society.
- **Dr. Gillian Eggleston**, New Orleans, LA received the international Meade Award for best paper at the Sugar Industry Technologists meeting in Osaka, Japan; Best Manufacturing Paper for the American Society of Sugar Cane Technologists; and the Best Poster for the

American Society of Sugar Cane Technologists annual meeting (biochar from sugarcane residues).

The quality and impact of NP 213 research was further evidenced in 2014 by the following:

- 68 refereed journal articles published
- four new patent applications
- seventeen material transfer agreements with stakeholders, and
- ten new invention disclosures.

**In 2015, NP 213 scientists participated in research collaborations with scientists in**: Austria, Brazil, Canada, China, Colombia, Denmark, Japan, Mexico, New Zealand, Nigeria, Pakistan, Spain, Tanzania, and United Kingdom.

# NP 213 Accomplishments for FY2015

This section summarizes significant and high impact research results that address specific components of the FY 2014 – 2019 action plan for NP 213. Each section summarizes accomplishments of individual research projects in NP 213. Many of the programs summarized for FY 2015 include significant domestic and international collaborations with both industry and academia. These collaborations provide extraordinary opportunities to leverage funding and scientific expertise for USDA - ARS research by rapidly disseminating technology, which enhances the impact of ARS research programs.

This National Program is organized into three problem areas:

- Biochemical conversion
- Biodiesel
- Pyrolysis

## Component 1: Biochemical conversion

Napier grass is a warm season grass that has low water and nutrient requirements, can be grown in marginal or uncultivated lands, does not compete with food crops for growing space, and is being developed as a bioenergy crop for production in the southeastern United States. ARS scientists in Peoria, Illinois, and Tifton, Georgia, converted Napier grass into ethanol with an estimated yield of 10,300 liters per hectare. By comparison, a corn field that typically yields 444 bushels a hectare could produce only 4,640 liters per hectare. Napier grass can be grown alongside corn as a "pull" crop, attracting insects away from corn while improving soil fertility and preventing erosion, and can be grown as a high-yielding, non-food feedstock to produce ethanol. This research furthers the feasibility of producing bioenergy crops in the southeastern United States as a feedstock for production of liquid biofuels.

Cellulosic ethanol production from agricultural residues and lignocellulosic materials is a complex procedure involving many factors affecting biocatalyst performance. Yeast strain performances are thus difficult to compare due to varied processes and cellulosic materials used in the fermentation process. ARS scientists in Peoria, Illinois, designed a fundamental technical assessment based on industrial applications to identify practical yeast strains for improved efficiency and cost of cellulosic ethanol production. Applying this method of assessing utility under standard industrial conditions, one yeast strain was confirmed to produce over 40 grams of yeast per liter of cellulosic slurry within 72 hours. This yeast produces the same enzyme needed to break down cellulose into fermentable glucose, thus it requires fewer added enzymes when compared with other strains. This new technology allows for a major enzyme cost reduction and consolidates process efficiencies, providing an estimated savings of about 35 cents a gallon in the selling price of ethanol. Lowering cellulosic ethanol production costs will enhance rural economic development.

The U.S. citrus industry produces nearly 15 billion tons of peel waste annually. Converting citrus peels into value-added products would help eliminate this waste; however, no enzyme system currently exists to convert citrus pectins and other similar polysaccharides into bioproducts. Through genomic mining of bacterial strains, ARS scientists in Albany, California, identified a highly active enzyme called exo-polygalacturonase from *Thermotoga* sp., a non-pathogenic compost microbe that lives at temperatures of 55-90° C. Exo-polygalacturonase converts peel waste into acid that can be used for the development of fine chemicals, such as adipic acid, which is used to make nylon, the base material for the multi-billion dollar sanitary wipe industry. Exo-polygalacturonase is hyperthermostable and in combination with a previously identified hyperthermostable pectin, methylesterase, this dual-enzyme system allows for processing of pectin-rich citrus waste at elevated temperatures. This process would also be useful with the pectin from sugar beet peels, which accounts for 240 billion tons of waste a year. With this enzyme process, the production of nylon is cleaner, faster, and well-suited for the use of non-fossil fuels for a greener footprint. Additionally, the process can easily substitute for the current highly corrosive chemical production process. The technology transfer of this process is under a confidentiality agreement with a commercial manufacturer.

Sweet sorghum is a high energy biomass crop that is regarded as one of the most promising crops for biofuel production. ARS scientists in Peoria, Illinois, have developed a novel process to pretreat sweet sorghum bagasse with hot water to generate fermentable sugars after enzymatic hydrolysis. The pretreatment of sweet sorghum bagasse made the bagasse easily fermentable into acetone-butanol-ethanol with butanol as the major bio-fuel. Heat-pretreated sweet sorghum butanol productivity was found to be between 23 percent and 86 percent higher than when using the glucose fermentation typical when corn is the feedstock. Butanol is an advanced biofuel that packs 30 percent more energy than ethanol on a per gallon basis. The sweet sorghum conversion industry has shown interest in adopting this technology, and lowering cellulosic butanol production costs will enhance rural economic development.

Microbial contamination of extracted sugarcane juice during commercial processing is a serious problem that causes sugar losses and formation of degradation products that interfere with processing. Although commercial biocides used to control microbial growth are currently added to juice in U.S. sugarcane factories, their efficacy has long been questioned. ARS scientists in New Orleans, Louisiana, evaluated three commercial biocides based upon bleach, sodium carbamate, and hops, plus a new treatment: heat. The biocides were studied individually and in combinations at two commercial Louisiana sugar processing facilities, and none of the treatments worked effectively. In contrast, heat applied during the juice clarification process eliminated microbial contamination. This research has prompted numerous U.S. sugar factories to replace costly commercial biocide treatments with heat. A current estimate is that each factory has saved approximately $47,000 per year on average, or about $515,000 per year for the industry in Louisiana alone.

Brewer's yeasts are the preferred organism for industrial ethanol production, but not all yeasts are tolerant to industrial processes. Two yeasts originally isolated from Brazilian fuel ethanol production facilities have been shown to perform well under harsh industrial conditions. These yeasts are extremely efficient at converting glucose to ethanol but they are not able to use xylose, the second most abundant sugar in lignocellulosic biomass. ARS scientists in Peoria, Illinois, have engineered these strains to express all of the proteins required for conversion of xylose to ethanol. One of these two strains was capable of rapid utilization of xylose when it was the only sugar available. The engineered strains consumed all of the xylose and made 70% more ethanol compared to other engineered industrial strains. Complete utilization of all biomass-derived sugars from the feedstock is important to achieve the highest productivity. This new technology is expected to promote the economics of cellulosic ethanol, furthering rural development.

The biorefining industry is still in need of biobased products produced from residual lignin in order to make more efficient use of the feedstock. ARS scientists in Peoria, Illinois examined a variety of laccase enzymes, both commercially prepared and crude extracts, for their ability to modify lignin model compounds. Both mediated and non-mediated laccase-catalyzed reactions were identified that converted lignin compounds into functional chemicals. Interestingly, the products produced by the concerted action of the laccase mediator system on the model substrates are the same as those produced by chemical catalytic approaches. The enzymatic approach affords the opportunity for a biological approach to convert lignin into valuable specialty chemicals that have use in a variety of industrial, consumer, and pharmaceutical applications.

New enzymes from thermophilic bacteria release sugar from biomass.  New enzymes that function under harsh industrial conditions of extreme temperature and pH are needed to help overcome some of the technical barriers to using agricultural residues as sources of fermentable sugars. ARS scientists in Peoria, Illinois collaborated with scientists at a university in South Dakota, to characterize the biomass-degrading enzymes from a novel thermophilic (high temperature) bacterium. Xylanases and xylosidases (enzymes which help break down the xylan component of biomass) were examined, and the enzymes were found to be active over a broad range of pH, and they were very stable at high temperatures. The enzymes from this organism enable the conversion of biomass to sugars for production of valuable fermentation products.

**Component 2:  Biodiesel**

Nearly 1.3 billion tons of plant biomass (lignocellulose) could be harvested each year in the United States in the form of energy crops and forest and agricultural residues, but current biofuel conversion is inefficient.  This biomass could potentially be converted into 30 billion gallons of biodiesel/year (62 percent of current U.S. diesel consumption) using microorganisms called “oily” yeasts.  ARS scientists in Peoria, Illinois, screened numerous yeast strains from ARS’ Culture Collection at Peoria, Illinois, which are capable of producing high lipid concentrations. Applying an advanced two-stage process to manage sugar and nitrogen supplies, the top yeast strains were able to rapidly accumulate 50-65 percent of cell biomass as lipid.  This corresponded to economically harvestable concentrations of lipid with up to 30 grams of oily yeast per liter, even under typical industrial acidic pH conditions. This new technology is expected to advance the economic feasibility of high quality biodiesel and jet fuels from renewable biomass.

Twenty-two oleaginous yeasts were screened for lipid production by AR S scientists in Peoria, Illinois, from the ARS Culture Collection. The set included members of the *Lipomyces* cleave (e.g. family) with a known lipid producing yeast included as control. The yeasts were ranked in terms of the lipid concentration accumulated, intracellular lipid content of yeast (dry weight basis), and rate of lipid production. Each of these strains produced lipids on all the sugars and furthermore exceeded the yield for the control yeast strains. The produced fatty acid profiles of the strains were also analyzed and found favorable for production of biodiesel. The new strains are expected to make the economics of producing renewable fuels more favorable, reducing our dependence on foreign oil while supporting the rural economy and preserving the environment.

**Component 3:  Pyrolysis**

Guayule, a woody desert shrub cultivated in the southwestern United States, will soon be commercialized as a source of natural rubber, organic resins, and possibly a high energy biofuel feedstock.  ARS researchers in Wyndmoor, Pennsylvania, used guayule bagasse, the residue left after rubber extraction, as feedstock in a pyrolysis process that employs a reactive gas environment to formulate a special intermediate bio-oil product that can be used as a hydrocarbon (drop-in) fuel.  This novel guayule bagasse conversion was completed without the use of catalysts, producing a bio-oil with much less oxygen than catalyst-generated bio-oils, but which produces high energy.  This breakthrough has resulted in a patent application with the U.S. Patent and Trademark Office, entitled “Bio-oils and Methods of Producing Bio-oils from Guayule Bagasse and/or Leaves.”

A pyrolysis-based bio-refinery must produce other valuable chemicals alongside fuel in order to financially succeed, similar to the model used in the petroleum industry.  When heated to very high temperatures (>1200 degrees C), petroleum coke reacts to become nearly pure carbon with many useful physical characteristics (termed "calcined petroleum coke," or CPC). Global producers of aluminum and steel rely on CPC for their production, and their demand for better CPC continues to increase, but increasingly, impurities such as sulfur, nickel, and vanadium have interfered with efficient use of CPC. ARS researchers at Wyndmoor, Pennsylvania successfully converted the heaviest fractions of renewable pyrolysis bio-oil, recovered after distillation of the volatiles and free of impurities, into a product similar to CPC but with improved chemical properties. The technology is the subject of a recently-filed provisional patent application (DN: 0126.14) and a manuscript currently under revision (Biomass and Bioenergy, ARIS log#308470). Furthermore an MTA has been established and an MTRA is currently in preparation with Rio Tinto Alcan, a global consumer of calcined coke, who has expressed continued interest in cooperative research and development on said technology with ARS.