National Program 213: Biorefining
National Program Annual Report: FY2016

Introduction

The USDA-ARS National Program for Biorefining (NP213) completed the second year of their new five-year research projects in 2016. Scientists in NP213 continue to make extraordinary impact in numerous diverse areas of research related to conversion of agricultural feedstocks into biofuels (mostly bio-diesel and bio-jet) and high-value co-products.
NP213's Vision & Relevance can be found at http://www.ars.usda.gov/research/programs/programs.htm?NP_CODE=213, which includes 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 biofuels, 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 2016, 36 full-time scientists, 23 postdoctoral research associates, and 22 graduate students working at 4 locations across the U.S. actively engaged in 9 ARS-based projects and 35 ARS-led cooperative research projects in NP213. Total funding for NP213 was $13.5 M in 2016.

Personnel news for NP 213:

The following scientists retired from the ranks in NP213 in 2016:

- Dr. Richard Offeman of the Bioproducts Research Unit, Albany, CA.

The following scientists in NP 213 received prominent awards in 2016:

- **Dr. Nasib Qureshi** of the Bioenergy Research Laboratory, Peoria, IL was elected Fellow of the American Institute of Chemists.
- **Dr. Charles Mullen** of the Sustainable Biofuels and Co-products Research Unit, Wyndmoor, PA received the 2016 American Chemical Society, Energy and Biofuels Award for excellence in publication.
- **Dr. Isabel Lima** of the Commodity Utilization Research Laboratory, New Orleans, LA received the 2016 Advances in Sugar Crop Processing and Conversion conference (ASCPC), Best Paper (publication) Award.
- **Drs. Isabel Lima** and **Gillian Eggleston** of the Commodity Utilization Research Laboratory, New Orleans, LA received two awards for poster presentations: the 2016 American Association of Sugar Cane Technologists, Denver T. Loupe Outstanding Poster Award, and the 2016 ASCPC Best Poster Award.

- **Dr. Badal Saha** of the Bioenergy Research Laboratory, Peoria, IL received the 2016 Charles Porter Award from the Society for Industrial Microbiology and Biotechnology.

The quality and impact of NP 213 research was further evidenced in 2016 by the following:

- 67 refereed journal articles published,
- 4 new patent applications, 4 new patents issued, and a new invention disclosure submitted,
- 1 current cooperative research and development agreement with stakeholders, and
- 22 material transfer agreements with stakeholders.

In 2016, NP 213 scientists participated in research collaborations with scientists in 17 different countries: Australia, Austria, Brazil, Canada, China, Colombia, Denmark, India, Japan, Korea, Mexico, New Zealand, Pakistan, Spain, Tanzania, Thailand, and United Kingdom

NP 213 Accomplishments for FY2016

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 highlights accomplishments of individual research projects in NP 213. Many of these projects entail 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.

NP 213 is organized into three component areas:

- Biochemical conversion
- Biodiesel
- Pyrolysis

Component 1: Biochemical conversion

Conversion of switchgrass and corn stover extracted sugars into single cell oil. ARS researchers in Peoria, Illinois, working with a private company, demonstrated the feasibility of directly converting sugars extracted from plants into lipids, which may be suitable for conversion into either biodiesel or jet fuel. The new method allows the sugars to be extracted without the aid of enzymes, which represent a major cost for producing sugars from plant fibers. This commercial process for producing sugars from fibrous plant feedstocks is suitable for producing a renewable intermediate for biodiesel production, making those feedstocks a more viable option for conversion to biofuels.

Improved industrial yeast strains for producing bio-ethanol from biomass-derived sugars. Industrial yeasts typically perform well under the harsh conditions found in an industrial setting, but not all of them function well in the presence of the inhibiting chemical compounds that are generated when producing sugars from biomass feedstocks. A yeast strain from a Brazilian fuel ethanol production facility was found to tolerate these inhibitors. ARS scientists in Peoria, Illinois, engineered the Brazilian yeast strain to express all of the proteins required for converting xylose to ethanol and then identified a strain with excellent performance. When hydrolyzed switchgrass was used as the sugar source, the modified Brazilian strain produced 30% more ethanol than the parent strain. Complete and efficient utilization of all biomass-derived sugars from any feedstock is important to achieve the highest ethanol production. The use of this new strain is expected to decrease the production cost for any process using biomass-derived sugars, thereby increasing profit.

Production of ethanol from byproducts of coffee processing. Economical production of fuels from agricultural residues requires a microorganism that fully utilizes all available sugars derived from these residues. *Kluyveromyces marxianus* is a yeast that can use a variety of sugars to make ethanol. ARS scientists in Peoria, Illinois, evaluated the ability of mutant strains of *K. marxianus* to make ethanol from inulin, a major polysaccharide derived from coffee processing waste. One strain was capable of converting coffee inulin to ethanol. Application of this strain to produce fuel ethanol will also aid in the disposal of waste products from coffee processing, yielding significant economic and environmental benefits.

Component 2: Biodiesel

Novel cellobiose-fermenting yeast for lower cost cellulosic ethanol production. Cellulolytic enzymes, including beta-glucosidase, are a major cost factor in the conversion of lignocellulose to ethanol. ARS scientists in Peoria, Illinois, have developed a novel yeast strain Clavispora NRRL Y-50464 that is able to produce its own beta-glucosidase in sufficient amounts to convert cellulose to ethanol, potentially eliminating a portion of the enzyme cost. The success of the yeast lies in its unique three-member beta-glucosidase gene family. The resulting enzymes produced not only showed a broad range of substrate specificity, but also demonstrated strong resistance to glucose product inhibition, high ethanol tolerance, and a superior resistance to furan aldehydes commonly present in hydrolysates. Confirmation of its dual functions of cellulolytic and cellobiose-fermenting capability by ARS scientists made Clavispora NRRL Y-50464 a candidate for lower-cost cellulosic ethanol production using simultaneous saccharification and fermentation. In collaboration with scientists in India, cellulosic ethanol production of 36.7 g/L in 36 h from green solvent-pretreated rice straw with a conversion efficiency of 90.1% was observed. This new technology would reduce the production costs by reducing the need for enzymes and the efficiency gained by consolidating the process would result in an estimated savings of ~$0.35/gal in the selling price of ethanol. This technology supports the rural economy and is expected to reduce risks and increase profitability in existing industrial biorefineries which produce ethanol and other products.

Application of lytic enzymes to control contamination of cellulosic ethanol fermentations. Lactic acid bacteria frequently contaminate commercial fuel ethanol fermentations, reducing yields and decreasing profitability of biofuel production. ARS scientists in Peoria, Illinois, and Beltsville, Maryland, have isolated and purified four lytic enzymes that possess antibacterial activity, and tested their ability to mitigate bacterial contamination in cellulosic ethanol production. Treatment with each enzyme reduced the concentration of lactic acid bacteria and increased ethanol yield in experimentally infected fermentations. Lytic enzymes have application in the fuel ethanol industry as an alternative to antibiotics for prevention and control of bacterial contamination.

Technology for the long-term storage of sweet sorghum syrup. Sweet sorghum syrup is vulnerable to surface microbial spoilage during storage because of its rich sugar content, and this represents a major technical challenge to the commercial, large-scale manufacture of biofuels and bioproducts from this feedstock. ARS scientists at New Orleans, Louisiana, with an industrial collaborator at Heckemeyer Mill in Sikeston, Missouri showed that adding only a 1.7 cm layer of inexpensive soybean, canola, or sunflower oil as a surface sealant allowed sweet sorghum syrup to be stored for at least 1 year at ambient temperature without significant spoilage. Heckemeyer Mill is now using this novel technology to store syrup in 7,500 gal tanks.

New methods to characterize quality traits of sweet sorghum. There is currently a dearth of methods to rapidly, simply, and inexpensively measure quality traits in new sweet sorghum feedstocks for the manufacture of biofuels and bioproducts. Breeders need these methods to develop better hybrids and cultivars, and industrialists need them to measure feedstock quality and for grower payment systems. ARS scientists at New Orleans, Louisiana, developed several multivariate statistical methods for breeders to predict the key sweet sorghum genotype traits on-farm. The new methods will replace currently available near-infrared and chromatography techniques with inexpensive fluorescence and light absorption methods, and allow breeders and growers to predict the concentration of sugars or select impurities based on the maximum light absorption/reflectance by a juice/bagasse sample. .The ability to measure quality traits inexpensively will increase sweet sorghum's value as a feedstock.

Component 3: Pyrolysis

The first successful HPLC method to analyze sorghum wax and commercial waxes. ARS researchers in Wyndmoor, Pennsylvania developed a new HPLC (high performance liquid chromatography) method to quantitatively analyze sorghum wax. This new method is valuable because it provides an accurate method to quantify sorghum wax in sorghum oil, distiller's milo oil, and in sorghum grain processing fractions such as bran and distillers dried grains and solubles (DDGS). It will also be very useful for the analysis of commercial waxes such as carnauba wax, candelilla wax, sunflower wax, rice bran wax and beeswax. It is the first successful HPLC method for waxes. Gas chromatography has traditionally been used for analysis of waxes but it has drawbacks and inaccuracies because it uses very high temperatures and the wax components can break down during analysis. This new method employs an evaporative light scattering detector for quantification and LC-mass spectrometry for chemical structural analysis. The new method also has the advantage of using methanol and chloroform as solvents, which are the best solvents to solubilize all of the wax components. It is anticipated that this new method will become widely used for commercial wax analysis.