National Program 213 Biorefining
National Program Annual Report: FY2017

Introduction

The USDA-ARS National Program for Biorefining (NP213) in 2017 completed the third year of their new five-year research objectives for the various research Projects. 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.

National Program 213: Biorefining, Vision & Relevance can be found at
http://www.ars.usda.gov/research/programs/programs.htm?NP_CODE=213

The current five-year Action Plan for NP213 went into effect in early 2014. The overarching goal of NP213 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, the 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 2017, 36 full-time scientists working at 4 locations across the U.S. actively engaged in 9 ARS-funded projects and 35 ARS-led cooperative research projects in NP213. ARS hosted five graduate students and 3 postdoctoral research associates in NP213 locations. The fiscal year 2017 funding for NP213 was $16 million.

The following scientists joined the ranks of NP213 in 2017:

- **Dr. Ryan Stoklosa** was hired as a Research Chemical Engineer in the Sustainable Biofuels and Co-Products Research Laboratory, Wyndmoor PA.

The following scientists left NP213 in 2017:

- The National Center for Agricultural Utilization Research, Peoria, IL lost two of its scientists in 2017: **Dr. Terry Whitehead** retired, and **Dr. Kenneth Bischoff** passed away.

The following scientists in NP 213 received prominent awards in 2017:

- **Dr. Gillian Eggleston** of the Commodity Utilization Research Laboratory, New Orleans LA, received the following awards in 2017: the Excellence in Technology Transfer 2017 Award from the Federal Laboratory Consortium, Southeast Region, for "Mannitol measurement to detect deterioration in sugarcane and sugar beet processing and

distilling"; the Denver T. Loupe Best Presentation Award from the American Association of Sugar Cane Technologists, for "First year in operating a mechanical de-trasher system at a sugarcane factory in Louisiana"; and Best Paper from Zuckerindustrie, for "Use of activated carbon to remove undesirable residual amylase from refinery streams."
- **Dr. H.N. Cheng** of the Commodity Utilization Research Laboratory, New Orleans LA, was appointed an Associate Editor of the International Journal of Polymer Analysis and Characterization.
- **Dr. Sophie Uchimiya** of the Commodity Utilization Research Laboratory, New Orleans LA, was appointed Associate Editor of the Journal of Environmental Quality, and received an Outstanding Associate Editor Award from JEQ in 2017.

The quality and impact of NP 213 research was further evidenced in 2017 by the following:
- 57 refereed journal articles published,
- two new patents issued, nine new patent applications submitted, and nine new invention disclosures submitted,
- two new cooperative research and development agreements with stakeholders, and
- five new material transfer agreements with stakeholders.

In 2017, NP 213 scientists participated in international research collaborations with scientists in Brazil, Colombia, and Denmark.

Selected NP 213 Accomplishments for FY2017

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.

This National Program is organized into three component (problem) areas:
- Biochemical conversion
- Biodiesel
- Pyrolysis

Biochemical Conversion

An 'enzyme-ladder' system of different linked enzymes efficiently converts crop waste into green chemicals. Leftover crop material like cornstalks, straw and sugarcane bagasse (what's left after the sugar is extracted) are abundant feedstocks that can supplement the world's fuel and chemical needs; however biorefineries must use many different enzymes to convert plant sugars into commercially viable products. ARS scientists in Albany, California, developed a new way for enzymes to work more efficiently by mounting them on a large, multi-enzyme ladder. This artificial enzyme-ladder, called a Rosettazyme, combines up to eighteen different critical enzymes necessary to release sugars from lignocellulose. An enzyme-ladder with a different combination of enzymes is then used to further convert the released sugars into aldaric acids, which are building blocks needed to manufacture nylon plastics. Four different types of enzymes were used to develop these enzyme-ladders, and the resulting “ladders” had 71 percent greater activity than the same combination of non-linked enzymes in a biorefining system. This system will allow more efficient conversion of lignocellulosic feedstocks to commercially useful products.

‘Liberty’ switchgrass produces high yields of bio-ethanol. An established field of ‘Liberty’ switchgrass grown on marginal, non-food cropping land in central Wisconsin in 2014 and 2015 produced 4,960 liters/hectare when it was processed for bioethanol. By comparison, corn grown on nearby “good” land—yielding 200 bu/acre—produced 5,300 liters/hectare of bioethanol. ‘Liberty,’ the newest ARS-released cultivar bred for biomass production of bioethanol far exceeded two existing popular switchgrass cultivars (summer and kanlow). This is a rare example of a field-to-fermentation integrated study and the first using ‘Liberty.’ The ability to produce 94% as much bioethanol as can be produced in a corn plot on a plot of land not suitable for food production establishes ‘Liberty’ switchgrass as a viable industrial crop. Its use in bioethanol production will be critical to a lowered risk for U.S. farmers and ethanol processors considering the production of advanced biofuels in the northern U.S.

New bioconversion-yeast strain with improved robustness to breakdown biomass. Yarrowia yeast strains are critical for the bioconversion of lignocellulosic biomass into diverse lipids converted into biodiesel; food/ healthcare applications; organic acids; and recently, as proteinaceous feed supplements for the animal and aquaculture industries. ARS scientists in Peoria, Illinois, screened 45 isolates of the Yarrowia clade from the ARS microbial Culture Collection. Isolates were evaluated in harsh dilute-acid pretreated switchgrass hydrolyzate media for growth robustness, breadth of sugar metabolism, and lipid production. The top producing isolate accumulated more than 3-fold greater lipid concentrations than a control native Yarrowia strain commonly used in commercial bioconversion systems. This more robust Yarrowina islotae will now be used to advance research on the conversion of biomass into lipid biofuels and other bioproducts by improving the genetic diversity available and thereby reducing the risk bioconversion companies face venturing into synthetic biology applications.

Optimizing novel antibacterial oil production from renewable biomass. Liamocins are novel antibacterial oils that do not cause antibacterial resistance. They are produced by the fungus Aureobasidium pullulans, during bioconversion that have specific control of Streptococcus spp., and other pathogens which cause important infections of swine and dairy cows. A. pullulans produces liamocins when grown on a variety of food-grade sugars and polyols; but ARS scientists in Peoria, Illinois discovered that agricultural biomass wheat straw is a promising, abundant, low-cost agricultural substrate. Twenty-seven strains of A. pullulans were surveyed for production of liamocins from wheat straw and sucrose. Liamocins yields were highest from cultures grown on sucrose than wheat straw. However, when supplementary enzymes were added to the fermentation, liamocin production on wheat straw was equivalent to that on sucrose. An unexpected discovery found that liamocins produced from wheat straw were under-acetylated, which means they produced more liamocin species with the highest activity against Streptococcus. Production of liamocins from low-cost sustainable substrates is appropriate for bulk agricultural applications, such as in dairy cattle dips for prevention of mastitis caused by Streptococcal infections.

Using sorghum wax to produce Distillers Milo Oil. Ethanol plants occasionally use sorghum (milo) as a feedstock, and in the process produce Distillers Milo Oil. ARS researchers at Wyndmoor, Pennsylvania have evaluated the chemical composition of Distillers Milo Oil and have determined that these oils are like Distillers Corn Oil and that both could potentially be used for biodiesel and animal feed applications. Also, Distillers Milo Oil contains significantly higher levels of wax (1-2%), which can be recovered as wax. This sorghum wax has similar physical properties to commercial imported carnauba wax. Ethanol plants that are fermenting sorghum to produce Distillers Milo Oil can also produce sorghum wax as an additional new valuable coproduct.

Resolving bacterial contamination of industrial fermentations with beneficial bacteria. Bacterial contamination of fuel ethanol fermentation reduces ethanol yields and can lead to stuck fermentations. Novel alternatives to antibiotics are needed to control bacterial contamination in industrial fermentations. ARS scientists in Peoria, Illinois, examined several hundred microorganisms as potential treatments to mitigate the deleterious effects of harmful bacterial contaminants. Dozens of beneficial bacteria were identified that fully mitigated a harmful contamination. This research could eliminate the use of antibiotics used in industrial fermentations and improve the quality of the animal feed co-product from biofuel production; thereby, could mitigate the emergence and spread of antibiotic-resistance.

Novel biomass and food processing enzyme. The growth and sustainability of the biorefining industry requires the development of more value-added products from abundant agricultural feedstocks, including lignocellulosics. ARS scientists in Peoria, Illinois, discovered a highly active bacterial ferulate esterase that is produced by a generally recognized as safe microorganism; thereby, it has added potential applications in food processing. This enzyme is currently used to convert low valued lignicellulosic biomass, including the lignin in corn fiber and corn stover, into ferulic acid, a high-value specialty chemical. Such enzymes have application in the biorefining industry in the breakdown of biomass, as well as the synthesis of valuable coproducts.

Development of an industrial method to measure insoluble and soluble starch in sugar products at a sugarcane factory or refinery. In recent years, starch impurity concentrations in sugarcane raw sugar have been increasing in the United States. ARS researchers in New Orleans, Louisiana, showed that existing starch methods used in the international sugar industry do not accurately measure total starch because they cannot efficiently solubilize the insoluble starch well and are also limited by the color of the factory product. At the request of industry, a rapid, precise, and accurate industrial method was developed, based on chemical assisted microwaving, to measure total, soluble, and insoluble starch at the factory and refinery. The industrial method compared favorably (less than 6.5% difference in accuracy and precision) with the USDA Starch Research Method but is much less expensive because the relatively expensive probe sonicator in the USDA Starch Research Method was replaced with less expensive and readily available chemicals. The new method costs only 4 cents per analysis. The method has already been submitted as a new method to the International Commission for Uniform Methods in Sugar Analysis.

Biodiesel

Biological antifungal alternative to thiabendazole for potato disease control. Fusarium dry rot, from Fusarium sambucinum infection, causes greater losses in storage and transit of both seed and commercial potatoes than any other postharvest disease. Chemical control by thiabendazole, currently the only chemical registered for use on food-grade potatoes, is no longer effective since 80% of pathogenic strains are resistant to thiabendazole. As a biological alternative, new microbial strains antagonistic to F. sambucinum were discovered as a co-product from the renewable lignocellulose bioconversion research program. Optimal strains capable of superior protection of potatoes to dry rot disease were developed by ARS scientists, Peoria, Illinois. Production on low cost renewable, sustainable biomass feedstocks is expected to lower costs and expedite application by potato growers. This new technology will benefit U.S. agriculture by providing a non-chemical, antifungal microbial alternative to thiabendazole and by serving as a commercial co-product of a renewable lignocellulose bioconversion industry with the effect of boosting economic viability.

Production of renewable crystalline biocoke as a coproduct from bio-oil. Previously, ARS Researchers at Wyndmoor, Pennsylvania produced a biomass-sourced (biorenewable) calcined coke (termed ‘biocoke’), using residues from the distillation of biomass-derived pyrolysis oil. This biocoke has properties that are similar to or better than analogous materials made from petroleum and therefore are highly desirable to producers of aluminum metal who use them and want to reduce their environmental footprint. However, the crystalline structure of the biocoke (crystallinity) was deficient and needed to be optimized for it to compete in the aluminum production anode market. We have devised a modified process for producing biocoke that has improved biocoke crystallinity, marking the first known time this has been accomplished using simple process steps. This led to the filing of a new patent application (DN: 0009.17), allowing for the initiation of a new MTRA with a major international research organization.

Pyrolysis

High-value phenols and furan coproducts from biomass catalytic pyrolysis. Small profit margins are associated with fuel refining, for both fossil-fuel and biomass refiners. However, in the fossil-fuel industry 80 percent of the profits comes from non-fuel petrochemicals. For biomass refiners to be successful, they too need to make high valued coproducts. Unfortunately, the current biomass conversion focus is on removing oxygen from the biomass, which produces non-oxygenated hydrocarbons, but it’s the oxygenated analogs that are the more valuable coproducts. ARS scientists at Wyndmoor, Pennsylvania have modified a zeolite catalyst traditionally used in biomass pyrolysis to increase the production of the valuable oxygenated chemicals (phenols and furans), and optimized conditions for their production. Findings from this research demonstrated that phenols can be produced not only from the lignin portion of the biomass of the lignocellulosic polymer, as traditionally thought, but also from the cellulose portion thanks to such catalyst modifications. Impact from this work expands the potential to produce valuable coproducts from biomass, with the potential to improve the profitability of fast pyrolysis biorefineries.