National Program 213: Biorefining
National Program Annual Report FY2014

Introduction

The USDA-ARS National Program for Biorefining (NP213) initiated new research objectives in 2014 for the various research Projects. 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

In FY2014, NP213 initiated project plans that were developed from comprehensive stakeholder input gleaned from the NP213 national stakeholder workshop. The workshop brought stakeholders and the NP213 research community together to prioritize the scope and direction of research in NP213 and to discuss current and future areas of impact for stakeholders.

These efforts are documented online at http://www.ars.usda.gov/research/programs/programs.htm?NP_CODE=213and include: the ***Retrospective Review Panel Executive Summary*** and the ***FY2014-FY2019Action 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 2014, 36 full-time scientists working at 4 locations across the U.S. actively engaged in 9 ARS-led and 49 cooperative research projects in NP213. The gross fiscal year 2014 funding for NP213 was $15 million.

The following scientists retired from the ranks in NP213:

- **Dr. Kevin Hicks**, **Dr. Michael Haas**, **Dr. Andrew McAloon**, all of Wyndmoor, PA.

The distinguished record of service of these scientists is recognized world-wide, and they will be missed in NP213.

The following scientists in NP 213 received prominent awards in 2014:

- **Rodney Bothast** (retired) Peoria, IL was awarded the Charles D. Scott Award by the Society of Industrial Microbiology at the 36th Symposium on Biotechnology for fuels and chemicals in April, 2014. This is the premiere award in the cellulosic ethanol area. Dr. Rodney Bothast is a former Research Leader of biofuels research.
- **Gillian Eggleston** and Post-doc **Marsha Cole,** New Orleans, LA, won two highly coveted International sugar research awards; first, the George and Eleanor Meade Best Paper Award of the Sugar Industry Technologists, Inc., 2014 Annual International Meeting and second, the Frank

Chapman Memorial Award for Best Poster Presentation at the 2013 Sugar Industry Technologists, Inc., Annual International Meeting in Guangzhou, China.

- **Gregory Glenn, Artur Klamczynski, and William Orts,** Albany, CA received the Federal Laboratory Consortium Award for “Outstanding Technology Development – Solution Blow Spinning, an alternative method to electrospinning for making nanofibers.”
- **Colleen McMahan**, Albany, CA, received the Best Paper Award; Natural Rubber and Resins Division from the Association for the Advancement of Industrial Crops for the paper, “2013-2014 Production of guayule natural rubber in Arizona, USA.”
- **Helen Ngo**, Wyndmoor, PA, received the Young Scientist Research Award from the American Oil Chemists Society
- **William Orts,** Albany, CA, received the Jim Hammar Memorial Award from the BioEnvironmental Polymer Society (BEPS) for lifetime achievement, scientific excellence, and service to the field of biopolymer development.

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

- 64 refereed journal articles published
- Two new patent applications and five new invention disclosures submitted
- Seven current cooperative research and development agreements with stakeholders
- Seventeen new material transfer agreements with stakeholders, and
- Five new scientific technologies developed.

In 2014 NP 213 scientists participated in research collaborations with scientists in: Argentina, Brazil, Canada, China, Colombia, Denmark, Finland, Japan, Netherlands, Pakistan, Spain, Thailand, and United Kingdom.

NP 213 Accomplishments for FY2014

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 2014 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

Novel yeast strains reduce the price of biomass conversion to ethanol. Traditional yeasts convert sugars in cereal grains to ethanol, but these yeasts cannot use the sugar xylose, which is the second most abundant sugar in corn stover, switchgrass, and lignocellulose feedstocks. In addition, the process of converting sugars to ethanol also results in toxic conditions that inhibit all yeast fermenting activities. *Saccharomyces stipitis* is a native pentose-sugar fermenting yeast that ARS scientists in Peoria, Illinois, cultured in an ethanol-challenged continuous culture system to force the development of robust yeast isolates. These isolates were able to overcome toxic conditions and produced ethanol using either highly acid- or base-pretreated corn stover or switchgrass. The novel yeast isolates had reduced growth lag time, significantly enhanced fermentation rates, improved ethanol tolerance and yield, and rapidly and economically generated recoverable ethanol at acidic pHs (which potentially inhibit ethanol fermentation). Compared to the parent yeasts, this new yeast isolates reduce ethanol selling costs by $0.31/gallon, an accomplishment that advances national efforts in developing renewable fuel systems to stimulate the rural economy, preserve the environment and reduce dependence on foreign oil.

Novel microbial oil has antibacterial activity. Antimicrobial resistance, a major health concern, has decreased the effectiveness of therapeutic drugs to treat and prevent infectious disease. As a result, antibiotic alternatives are needed to maintain the health and welfare of animals. ARS scientists in Peoria, Illinois collaborated with a scientist from Rangsit University in Thailand to test a novel oil produced by the fungus *Aureobasidium pullulans* for antibacterial activity. The oil, called liamocins, was produced through bioconversion of a variety of sugars and lignocellulosic feedstocks and was found to preferentially inhibit the growth of strains of the pathogenic bacteria *Streptococcus*. The antibacterial oil can improve animal health in the dairy, swine, and aquaculture industries, and can have impact in the biorefining industry by providing a new a high-value bioproduct.

Changing landfills into biorefineries. To provide sufficient quantities of biomass sources between growing seasons, ARS researchers in Albany, California, developed a large pilot scale biorefinery located at the Salinas, California, Crazy Horse Landfill that converts rural and urban solid waste into ethanol, biogas, compost, and/or value-added recyclables. Each ton of food processing waste at the landfill can be currently converted into 65 gallons of ethanol. Conversely, if the same biomass source is converted to liquefied natural (bio)gas (which has the same burn rate as 100 percent ethanol) it yields 108 gallons of transportation fuel, which can be used to power diesel turbines. Together, ARS and the City of Salinas are creating an “energy park” that converts both agricultural biomass and curb-collected garbage into bioenergy in the same biorefinery, which demonstrates the facility’s remarkable flexibility in handling and processing different feedstock supplies.

Cost-effective process technology for butanol production from corn stover. Butanol is an advanced biofuel that packs 30% more energy than ethanol on a per gallon basis. It is produced via fermentation of sugars; however, butanol must be removed as it is being produced because above a certain concentration, butanol inhibits its own production). Thus, the key to produce butanol economically from corn stover is a three-step process: 1.convert pretreated corn stover to sugars using enzymes, 2. ferment the sugars to butanol, and 3. recover butanol as it is generated. ARS scientists in Peoria, Illinois, have developed such a novel three-step process for butanol production from dilute acid-pretreated corn stover coupled with vacuum distillation to allow for continuous butanol recovery. The production cost for butanol from corn stover by this process was estimated to be at $3.42/gal whereas the production cost of butanol from corn was $4.39/gal. This newly developed fermentation/recovery process, for the first time, provides for a more cost-effective production of butanol.

Identifying ethanol-tolerant proteins in bacteria that convert feedstocks to ethanol. Lactic acid bacteria are used in the industrial fermentation of agricultural biomass to biofuels such as ethanol, but these bacteria are sensitive to the elevated concentrations of ethanol generated during the conversion process. Scientists in Peoria, Illinois examined a strain of lactic acid bacteria to identify proteins that confer ethanol tolerance to the bacterium. Twenty proteins were identified which varied in response to elevated ethanol concentrations. These results provide information on the production and regulation of the proteins involved in ethanol tolerance, and are useful in genetically improving microbial strains for a more efficient and a more complete conversion of agricultural biomass to biofuels and bioproducts.

Finding what limits enzymatic conversion of xylan to xylose. Xylan is the most abundant plant carbohydrate after cellulose, and maximizing its yield is necessary for commercial development of biorefineries. ARS scientists in Peoria, Illinois, discovered in three varieties of switchgrass a previously unreported xylan linkage that resists enzymatic cleavage by commercial enzymes. The presence of this bond prevents enzymatic hydrolysis of xylan and blocks enzymatic hydrolysis of adjacent sugar units. Enzyme researchers can now focus on discovery of an efficient enzyme that targets this bond, while plant breeders can focus on suppressing formation of this bond in the plant cell wall. The end result will be higher yields of xylose—possibly with less expensive enzymes—which will benefit current and future cellulose refiners.

Napier grass for bioenergy production. Napier grass (*Pennisetum purpureum* (L) Schum) is being developed as a bioenergy crop for production in the southeastern U.S. However, the agronomics for its bioconversion are not yet optimized. ARS scientists in Peoria, Illinois, and Tifton, Georgia, compared three different agronomic systems for production of Napier grass. ARS found that a two-cut regime significantly outperformed a one-cut system for production of sugars. These sugars are directly available for conversion to ethanol or other fermentation related products. Furthermore, differences in yields were correlated with other plant properties, which may suggest future strategies for further improving conversion efficiency. Napier grass has the highest reported production yield for any perennial grass suitable for cultivation in the Southeast. These findings will help companies considering construction of biorefineries in the Southeast to decide on the best feedstock options.

Recombinant ferulate esterase for application in breaking down lignocellulosic biomass. A variety of enzyme activities are required to degrade the complex structure of lignocellulosic biomass (woody material) into fermentable sugars. ARS scientists in Peoria, Illinois cloned the gene for an enzyme called ferulate esterase from a strain of *Lactobacillus*, and produced the recombinant enzyme in *Escherichia coli*. The enzyme shows good activity in breaking down recalcitrant woody material, and may prove suitable for industrial application in the conversion of biomass to sugars for production of fuel ethanol or other valuable fermentation products, as well as in the feed, textile, and pulp and paper industries.

Fermentation based process for making xylitol. Xylitol is a commercially valuable product that can be produced by fermentation of agricultural biomass-derived sugars; especially hemicellulose hydrolyzates that are rich in xylose. However, these feedstocks typically contain a mixture of xylose and arabinose, and xylitol-producing yeasts generally convert arabinose to arabitol. It is very difficult to separate xylitol and arabitol, which adds significantly to the cost of product purification and loss of yield. ARS in Peoria, Illinois, have identified a yeast strain that has the ability to produce xylitol from xylose, but no or little arabitol from arabinose in a mixture of xylose and arabinose. The process of making xylitol from corn stover hydrolyzate by the yeast strain was optimized in batch and fed-batch fermentations. This yeast strain has potential to be used in commercial production of xylitol from various hemicellulosic hydrolyzates. Xylitol has has been identified as one of the 12 value-added materials to be produced from biomass, thereby serving as key economic driver for biorefineries.

Component 2: Biodiesel

Sustainable biodiesel additives improves cold weather flow. The cold flow properties of fatty acid methyl esters (biodiesel) are relatively poor and detract from commercial viability of biodiesel as a fuel during cold weather. Synthetic cold flow improver (CFI) additives made from soybean, canola, and palm oils have been shown to increase the low temperature flowability of biodiesel. ARS scientists in Peoria, Illinois, and Wyndmoor, Pennsylvania, collaborated on the synthesis and testing of CFI additives obtained from non-food resources such as waste cooking oil. Results from this research benefit farmers who supply seed oils for biodiesel conversion by making the fuel more marketable during cooler seasons. Biodiesel fuel producers, distributors, and consumers will also benefit by improved flowability and performance in cold weather.

Improved solid regeneration process. ARS researchers at Wyndmoor, Pennsylvania successfully developed an efficient approach to recover, regenerate, and re-use the solid catalyst that starts the conversion of linear-chain fatty acids from unbranched to branched in the production of biodiesel. Because the catalyst is the most expensive component in the fatty acid conversion reaction, the ability to reuse the catalyst reduces process costs in the manufacture of cosmetics, paints, and lubricants while also reducing the amount of solid waste generated during the fatty acid conversion reaction. The catalyst is also considered a contaminant of biodiesel; thus its removal from the finished product is a desirable technology.

Novel microbial enzyme for biotechnological applications. Lipase is an enzyme that has numerous biotechnological applications in the detergent, food, and pharmaceutical industries. The fungus *Aureobasidium* is well known as a source of valuable bioproducts; however, little information has been available concerning lipase production by this organism. ARS scientists in Peoria, Illinois in collaboration with a visiting scientist from Rangsit Univeristy, Thailand, discovered that a certain genetic group of *Aureobasidium* strains produced relatively high levels of lipase. This work facilitates the identification of new sources of microbial lipases, which can be used in the production of biodiesel fuels.

Component 3: Pyrolysis

Untreated biochars remove mercury from simulated flue gas. Mercury emissions from coal-fired power plants must be reduced due to new regulatory standards. ARS scientists from the Southern Regional Research Center (SRRC) in New Orleans, LA, showed that un-activated biochars made from chicken manure were very efficient at removing mercury from a simulated flue gas in the laboratory. This was part of a larger test that involved biochars made from different raw materials under different pyrolysis conditions and with different post-treatment strategies. While previous studies have shown that acid-washed, steam-activated biochars made from chicken manure performed better than their unwashed, un-activated counter parts, significant cost savings can be realized using untreated biochars with only a minor drop in performance. The use of abundant chicken waste to make biochars for mercury removal represents a sustainable approach for managing mercury emissions in the future.

Deoxygenated bio-oils without catalysts. ARS scientists in Wyndmoor, PA have developed a process for producing deoxygenated bio-oil without the use of catalysts through a technology that relies on the recycling of tail gases to create a reactive atmosphere, called Tail Gas Reactive Pyrolysis (TGRP). TGRP has been demonstrated to be effective on several feedstocks including oak, forest thinning, switchgrass, horse manure, eucalyptus, sugar cane trash, and elephant grass. Bio-oils from the TGRP process are amenable to refinery operations including distillation, which is not possible for traditional fast pyrolysis oils. Results from TGRP bio-oil distillation can be predicted by computational modeling. This will ease refinery integration of TGRP bio-oils via accurate design of large-scale processes. A patent has been filed for this technology.