Conversion Technologies for Biofuels

Symposium as part of the S-1041 Annual Meeting, August 2-3, 2010
Eastern Regional Research Center, ARS, USDA, Wyndmoor, PA

Edited by
Kent Rausch, Vijay Singh and Mike Tumbleson
University of Illinois
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Charles A. Mullen* and Akwasi A. Boateng, Crop Conversion Science and
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BIOMASS GASIFICATION TAR REMOVAL AND SYNGAS CONDITIONING USING NOVEL CATALYSTS

Duo Wang and Wenqiao Yuan* Biological and Agricultural Engineering, Kansas State University, Manhattan, KS 66506 (785-532-2745) wyuan@ksu.edu

CATALYTIC BIOMASS CONVERSION AND BIO-OIL REFINING

Xiaoquan Wang¹, Zhengyi Du¹, Xianghong Lu², Chengguang Wang¹, Yiqin Wan¹, Yanling Cheng³, Xiangyang Lin³, Yuhuan Liu⁴, Paul Chen¹ and Roger Ruan¹,4*, ¹Center for Biorefining, Bioproducts and Biosystems Engineering, 1390 Eckles Avenue, University of Minnesota, St. Paul, MN 55108 ²College of Chemical Engineering and Material Science, Zhejiang University of Technology, Hangzhou 310014, China, ³College of Biological Science and Engineering, Fuzhou University, Fujian, China and ⁴MOE Bioenergy Center, Nanchang University, Jiangxi, China (612-625-1710) ruan001@umn.edu

PHENOLIC ACIDS, LIPIDS AND PROTEINS IN CORN FIBER GUM

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*Denotes the speaker or poster presenter.
S-1041 Objectives

A. Reduce costs of harvesting, handling and transporting biomass to increase the competitiveness of biomass as a feedstock for biofuels, biomaterials and biochemicals.

B. Improve biofuel production processes.

C. Identify, develop and evaluate sustainable processes to convert biomass resources into biochemicals, biocatalysts and biomaterials.

D. Identify and develop needed educational resources, develop distance based delivery methods, and develop a trained work force for the biobased economy.

Symposium Background and Purpose

During the 2009 S-1041 meeting in Richland, WA, it was decided a short symposium (with printed proceedings) related to the objectives of the S-1041 project would enhance the annual meetings and further inform participants on topics related to the project’s objectives. Eventually, these proceedings were to be posted on the S-1041 website. To this end, the first symposium was planned for the 2010 meeting at the Eastern Regional Research Center (ARS, USDA) and was to include speakers from the facility as well as the region surrounding the meeting.

The S-1041 Website

A complete description of the S-1041 multistate project, its objectives and an electronic version of this symposium proceedings can be found at:

http://nimss.umd.edu/homepages/home.cfm?trackID=9057
# S-1041 Meeting Agenda – Monday, August 2, 2010

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<td>Continental breakfast at ERRC</td>
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<td>8:00</td>
<td>Welcome remarks from S-1041 chair and ERRC director</td>
<td>Sue Nokes, 2010 Chair, S-1041</td>
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<td>Sevim Erhan, Center Director, ERRC</td>
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<td>8:05</td>
<td>Remarks by the administrative advisor</td>
<td>William Brown, Administrative Advisor</td>
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<td>8:10</td>
<td>Introductions of attendees: names and stations</td>
<td>All meeting participants</td>
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<tr>
<td>9:00</td>
<td>NIFA remarks</td>
<td>Carmela Bailey</td>
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<tr>
<td>9:45</td>
<td>Break</td>
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<td>10:00</td>
<td>Overview of the Eastern Regional Research Center</td>
<td>Sevim Erhan, Center Director, ERRC</td>
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<td>Tours and laboratory demonstrations</td>
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<tr>
<td>12:00</td>
<td>Lunch at ERRC</td>
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<tr>
<td>12:30</td>
<td>Breakout sessions</td>
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<td></td>
<td>Session I: first objective of choice</td>
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<td>1:30</td>
<td>Session II: second objective of choice</td>
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<tr>
<td>2:30</td>
<td>Summary reports from Sessions I &amp; II</td>
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<tr>
<td>3:00</td>
<td>Break</td>
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<tr>
<td>3:30</td>
<td>Station reports: 3 minutes per station (no slides)</td>
<td>Station representatives</td>
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<td>4:30</td>
<td>S-1041 business meeting</td>
<td>2010 chair: Sue Nokes (KY)</td>
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<td>Sites and dates for 2011, 2012 meetings</td>
<td>2011 chair: Mark Wilkins (OK)</td>
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<td>Secretary election</td>
<td>2012 chair: Samir Khanal (HI)</td>
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<td>Committee for 2011 symposium (Biobased Products)</td>
<td>2013 chair: elected as secretary in 2010</td>
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<td>5:00</td>
<td>Adjourn</td>
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<td>Dinner on your own</td>
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### Symposium Agenda – Tuesday, August 3, 2010

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker*/Coauthor(s)</th>
<th>Topic</th>
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<tbody>
<tr>
<td>7:30</td>
<td>Continental breakfast</td>
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<tr>
<td>8:30</td>
<td>Robert L. Fireovid* National Program Leader, Bioenergy Agricultural Research Service, USDA</td>
<td>Role of the Agricultural Research Service, USDA in Bioenergy</td>
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<tr>
<td>9:00</td>
<td>Carmela A. Bailey* National Program Leader for Agricultural Materials National Institute of Food and Agriculture, USDA</td>
<td>New Directions in Bioenergy Research and Development for the National Institute of Food and Agriculture</td>
</tr>
<tr>
<td>9:30</td>
<td>Ken J. Goddard* University of Tennessee Biofuels Farmer Education Team</td>
<td>Growing and Harvesting Switchgrass for Ethanol Production in Tennessee</td>
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<tr>
<td>10:00</td>
<td>Break</td>
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<tr>
<td>10:15</td>
<td>Charles A. Mullen* and Akwasi A. Boateng Crop Conversion Science and Engineering, ERRC</td>
<td>Distributed Fast Pyrolysis for Conversion of Biomass to Stable Refinable Crude Bio-Oils</td>
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<tr>
<td>10:45</td>
<td>Bob Keefe*, GEA/Niro</td>
<td>Integration of Membrane Filtration Technology in the Cellulose to Ethanol Process</td>
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<tr>
<td>11:45</td>
<td>Robert. A. Moreau*, David P. Johnston, Leland Dickey and Kevin B. Hicks Sustainable Biofuels and Coproducts Research Unit, ERRC</td>
<td>Development of a “Green” Aqueous Enzymatic Process to Extract Corn Oil from Corn Germ</td>
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<tr>
<td>12:15</td>
<td>Lunch</td>
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<tr>
<td>12:45</td>
<td>Jonathan Male*, PNNL/OBP/DOE Thermochemical Conversion</td>
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<tr>
<td>1:15</td>
<td>Michael Haas* ERRC/ARS/USDA In Situ Transesterification: Biodiesel Prodution by the Direct Transesterification of the Lipids Resident in Biological Materials</td>
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<tr>
<td>1:45</td>
<td>Kevin Hicks* ERRC/ARS/USDA Winter Barley Ethanol – A New Advanced Biofuel</td>
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<tr>
<td>2:15</td>
<td>Kent Rausch University of Illinois</td>
<td>Symposium wrap up</td>
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SYMPOSIUM PRESENTATIONS
ROLE OF THE AGRICULTURAL RESEARCH SERVICE, USDA IN BIOENERGY

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(301-504-4774) rlf@ars.usda.gov

The ARS Bioenergy Program is a flexible, long term research effort involving coordinated thrusts in feedstock development (FD), sustainable feedstock production systems (SFPS) and biorefining (B). The holistic nature of ARS bioenergy research ensures that bioenergy production is integrated into existing agriculture in ways that:

• provide consistent, attractive returns to producers,
• minimize adverse impacts on existing markets for food, feed and fiber and
• demonstrate good stewardship of soil, water air and other natural resources.

ARS has research capabilities in all three major bioenergy research areas (FD, SFPS, B), most notably spanning all aspects of FD and SFPS, for solving complex technical problems involving multiple agricultural industries (food, feed, fiber and fuels). These can be associated with natural resources, including carbon cycling and water utilization, that can be targeted at any agricultural region in the Nation. ARS has an unique ability to implement this integrated approach and enable the Nation to optimize bioenergy production as soon as possible.

Research program components include:

Feedstock development: enable new varieties and hybrids of bioenergy feedstocks with optimal traits
• sustainable feedstock production systems: enable new optimal practices and systems that maximize the sustainable yield of high quality bioenergy feedstocks and
• biorefining: enable new commercially preferred biorefining technologies.

We will integrate components within five regional feedstock centers: southeast, south central, east central, west, northwest, and focus in four promising feedstocks: perennial grasses, sorghum, energy cane and oilseed crops.
ARS regional feedstock centers will:
• Accelerate biofuels production in the region
• Utilize the network of ARS laboratories in the region
• Leverage capabilities of:
  o university partners
  o biorefiners and other corporate collaborators
  o agricultural producers
  o other federal laboratories
  o relevant NGOs
  o other ARS laboratories with special scientific and technical expertise
    ▪ feedstock teams (perennial grasses, energy cane, sorghum, oilseed)
    ▪ biophysical and microeconomic modeling team
    ▪ biorefining and coproducts team.

ARS bioenergy research will have many national programs involved:
• Bioenergy (213)
• Agricultural quality and utilization (306)
• Forages (215)
• Crop improvement and protection (301, 302, 304)
• Agricultural system competitiveness and sustainability (216)
• Soil management (202)
• Manure and byproduct utilization (206)
• Global change (204)
• Water availability and watershed management (211)
• Feedstock development: 26 projects (2 in NP213)
• Feedstock production: 26 projects (1 in NP2213)
• Biorefining: 18 projects (12 in NP213).

ARS bioenergy research with interagency coordination:
Interdepartmental:
• Biomass research and development board
  o Interagency working groups
• Scientist exchange program with DOE-OS
  o Bioenergy research centers
• Interagency working group on plant genomes
Intra USDA:
• Energy council coordinating committee
• Biobased products and bioenergy coordination council (BBCC)

Feedstock development
• Biological and molecular basis for plant traits
  o Understand molecular basis for key traits (cell wall structure, growth, biomass yield, conversion potential)
• Breeding and evaluation of new germplasm
  o Improved germplasm and varieties for energy crops

Sustainable feedstock production systems
• Region specific, sustainable practices to maximize feedstock harvest
  o Whole farm optimization tools to incorporate bioenergy feedstock production into farm operations
• Analytical tools to estimate potential feedstock amounts and the implications of harvest on natural resource base
  o Decision tools for farmers and biorefinery operators
• On farm utilization of biorefinery coproducts
  o Physical, chemical and biological value of byproducts as soil amendments and nutrients

Biorefining
• Biocatalytic (ethanol and butanol)
  o starches and sugars (1st generation)
  o cellulosic (2nd generation)
• Biodiesel
  o fuel quality (cold flow, oxidative stability)
• Thermochemical
  o Pyrolysis (CHP, advanced biofuels)

ARS Strengths
• Biorefinery coproducts/byproducts
  o for each biorefining platform
• Biocatalysis
• Technoeconomic analyses
  o identify research and development goals and priorities
• Early stage technology transfer plans
  o pilot facilities
Table 1. ARS spending for bioenergy research (thousands of dollars).

<table>
<thead>
<tr>
<th></th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011 (proposed)</th>
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<td>$21,339</td>
<td>$32,359</td>
<td>$33,746</td>
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<tr>
<td>Feedstock Development</td>
<td>--</td>
<td>21%</td>
<td>18%</td>
<td>19%</td>
</tr>
<tr>
<td>Sustainable Feedstock</td>
<td>--</td>
<td>26%</td>
<td>29%</td>
<td>42%</td>
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<tr>
<td>Production</td>
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<tr>
<td>Biorefining</td>
<td>--</td>
<td>53%</td>
<td>53%</td>
<td>39%</td>
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For more information on bioenergy:

For more information on biobased products:
NEW DIRECTIONS IN BIOENERGY RESEARCH AND DEVELOPMENT FOR THE NATIONAL INSTITUTE OF FOOD AND AGRICULTURE


With the passage of the 2008 Food Conservation and Energy Act, the National Institute of Food and Agriculture (NIFA) was established. The mission of NIFA is to advance knowledge for agriculture, the environment, human health and well being, and communities by supporting research, education and extension programs in the land grant university system and other partner organizations. Within this mission, bioenergy has been designated as a priority topic area and is part of the Institute of Bioenergy, Climate and Environment. The goal is to ensure energy independence through clean, biobased energy systems and to ensure sustainable, adaptive agroecosystems in response to climate change. To accomplish this goal, biomass conversion technologies will be supported in the context of a sustainable supply chain.

Sustainability is an overarching theme for all bioenergy programs in NIFA. From an agricultural perspective, sustainability is defined as satisfying America’s needs for food, fiber, feed and fuel, while at the same time maintaining or enhancing environmental quality, rural economic viability and quality of life. To address sustainability in its fullest sense, we have taken holistic approaches to bioenergy: 1) the Agriculture and Food Research Initiative (AFRI) for Sustainable Bioenergy and 2) the Biomass Research and Development Initiative (BRDI). These two programs are offered as competitive grant programs to attract the best scientists and engineers, and requires them to form a consortia of experts that can move technologies to implementation and successful commercialization.

For AFRI support, projects must be coordinated, regional systems approaches to produce biofuels that are economically, environmentally and socially feasible. The focus is on sustainably producing regionally appropriate advanced biofuels, biopower and biobased products
from dedicated energy crops while minimizing impacts on existing agriculture sectors and the environment. This systems based approach leverages existing efforts in government agencies, industry and academia. Five crops of interest for this program include perennial grasses, energy cane, woody biomass, sorghum and oil crops (including algae). BRDI targets the development of biofuels, biopower and biobased products by requiring integration of feedstock development, feedstock conversion and analysis of the economic, environmental and social implications of the proposed technology. NIFA anticipates these programs will identify needs and technology gaps and will provide valuable information for informed policy making.

Traditionally, NIFA has supported biomass conversion as a single focus research activity and technologies have included innovative biochemical and thermochemical routes. Projects have not yet been funded under the new paradigm of addressing sustainability and linking conversion with feedstock development and production but a good example of a project that has developed an innovative holistic approach is the Biomass Based Energy Research project. This project is a consortium of Oklahoma State University, Mississippi State University, University of Oklahoma and Brigham Young University. Researchers will address the value chain from biomass production, harvest, storage, transportation, processing, conversion and waste disposal. The multidisciplinary project areas include feedstock development, gasification and syngas conditioning, syngas fermentation, microbial catalyst development, and process modeling and economics. End products are ethanol, potentially valuable coproducts and identification of technology gaps that require further research. Preliminary estimates suggest at least three energy units of output for one energy unit of input from a technology that links thermochemical conversion with a fermentation process.
GROWING AND HARVESTING SWITCHGRASS FOR ETHANOL PRODUCTION IN TENNESSEE

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Switchgrass is a warm season perennial grass native to North America. The plant can reach heights up to 10 ft with an extensive root system. Once established, switchgrass well managed for biomass should have a productive life of 10 to 20 yr. Within the stand, switchgrass is an extremely strong competitor. However, it is not considered an invasive plant.

Switchgrass adapts well to a variety of soil and climatic conditions. It is most productive on well drained soils of medium fertility and a soil pH at 5.0 or above. The high cellulosic content of switchgrass makes it a favorable feedstock for ethanol production. It can yield sufficient biomass to produce approximately 500 gal ethanol/acre.

While the Tennessee Biofuels Initiative includes a demonstration plant to make ethanol from switchgrass, the market for switchgrass as an energy crop remains limited. Producers will need to be located within 30 to 50 miles of a cellulosic ethanol plant. Producing switchgrass for energy generally occurs under some form of contractual arrangement with the end user. To reap potential benefits from using switchgrass for cellulosic ethanol production, the system of production must be profitable for farmers and energy producers, as well as cost effective for consumers.

VARIETIES

There are two main types of switchgrass. The upland varieties usually grow 5 to 6 ft tall and are adapted to the Midwest. Lowland varieties are adapted for the South and grow from 7 to 10 ft tall. The most common lowland varieties adapted to Tennessee are Alamo and Kanlow; both are recommended by the University of Tennessee. Alamo has been used in the majority of recent University of Tennessee research on using switchgrass for biomass.
SEEDING RATE

Switchgrass seed is very small and much of it will not germinate right after it is harvested. However, aging, treating with water and chilling temperatures (stratification) or storing it under the correct conditions will break dormancy. When establishing switchgrass, buying quality seed is an important consideration. Switchgrass seed normally is sold on the basis of pure live seed (PLS). The germination rate will vary; therefore, it is critical to plant according to the PLS. In calibrating seeding equipment, take into account the percentage of pure seed and the germination rate. The University of Tennessee recommends 6 lb PLS/acre.

FERTILIZER AND pH

Although switchgrass is adapted to nutrient deficient soils, soil fertility is important and soil testing is recommended to determine pH and nutrient availability. If soil test values are medium or high in phosphorous (P) and potassium (K), no additional P2O5 or K2O is recommended. If the soil test is low in P, an annual application of 40 lb P2O5/acre is recommended. If soil is low in K, an annual application of 80 lb K2O/acre should be applied. If the soil pH is 5.0 or above, no lime is recommended.

These recommendations are for long term reasonable yields of switchgrass for biomass, with switchgrass harvested after a killing frost over a period of 10 yr. Being a perennial, switchgrass is thought to translocate much of the aboveground nutrients back into the crown root system, resulting in low input needs after establishment. Nitrogen (N) should not be applied until the stand is established and weeds controlled. In the first yr, do not apply nitrogen. It increases competition from annual grasses and broadleaf weeds. Beginning in the spring of the second yr, 60 lb N/acre are recommended.

PLANTING DATE

Planting dates can range from late April to mid June. Switchgrass is a warm season grass and establishes and grows best under warm conditions.

PLANTING METHODS

Switchgrass can be planted into a tilled seedbed or no tilled. It appears no till planting with a no till drill in fields not bedded from past row crops is the ideal way to plant. Switchgrass should be planted when sufficient soil moisture is available for emergence of the seeds. A planting depth of ¼ in or less, with good seed coverage at that depth, is critical. This usually is easier to achieve in no tilled soil conditions. The drill should have small seed boxes suitable for accurately metering the seed.
WEED CONTROL

In the establishment year, switchgrass does not compete well with grasses such as fescue, crabgrass, johnsongrass and broadleaf weeds. Appropriate weed control measures vary according to previous cropping history and specific weed varieties. Evaluate fields before and after planting and check with an Extension agent for control options.

Few herbicides are labeled for weed control in switchgrass on nonConservation Reserve Program (CRP) land in Tennessee. Therefore, it is critical to control weeds prior to planting. Most often, a glyphosate herbicide (eg, Roundup®, Gly-4 Plus®) is used to kill existing cover. The guideline switchgrass budgets include two burndowns with a glyphosate herbicide. The preferred method to establish switchgrass in a pasture or hay field is to spray with a glyphosate herbicide in the fall prior to planting. Before the sod is sprayed, the field should be grazed, mowed or hayed and regrowth allowed to reach 6 to 10 in. This ensures the herbicide comes into contact with an actively growing plant. Cimarron®, formerly named Ally® from DuPont™, is labeled for postemergence application on switchgrass for control of broadleaf weeds.

Efforts are being made to gain regulatory approval for the use of an additional herbicide (Accent) for grass suppression in Tennessee. Observations in Tennessee on weed control in switchgrass indicate grass competition is more severe than broadleaf competition. Once switchgrass is well established and properly managed, it is competitive against weeds.

RESEEDING SWITCHGRASS

Switchgrass establishment will not always be successful in the first year. Weed pressure, seed quality, incorrect planting procedures, level of soil moisture within the first four to five wk of planting and other factors can contribute to not achieving an acceptable stand of switchgrass. The expected probability of needing to reseed is assumed to be 20%. Guideline expenses for establishment include reseeding costs.

HARVESTING

Switchgrass can be harvested as a one or two cut system. When cut twice, the first cutting would occur when switchgrass is in late boot to early seed head emergence, which is usually in late June or early July. The second cutting would be at the end of the season and can occur when the plant goes into dormancy (usually after the first killing frost).

In the one cut system, switchgrass is harvested once after the first killing frost. Total biomass yield for the year is similar in the one and two cut system. The choice should be influenced by management timing, costs and needs of the buyer relative to cutting time. Single harvest of switchgrass after the aboveground growth is killed by frost will reduce nutrient removal and fertility needs. Switchgrass should be cut at least 6 in high. The taller stubble will
reduce punctured tires by allowing equipment tires to push over the stubble. Furthermore, switchgrass stand survival, vigor and yield consistency in later years is impacted by cutting time and height. Switchgrass can be harvested with conventional hay equipment. Many farmers have large round balers and prefer to use their existing equipment. Round bales are acceptable. However, large rectangular bales (3x4x8 ft or 4x4x8 ft) are easier to handle, store and transport.

**YIELDS**

In test plots, switchgrass has produced more than 10 tons of dry matter/acre/yr. However, on a commercial scale, it is more reasonable to expect 6 to 8 tons/acre. In the first yr of production, yields are estimated to be 30% (2 tons) of the full yield potential. Second yr yield is normally 70% (5 tons) of full production. In the third yr, yields should be at the 100% yield level of 7 tons/acre. Over the first 5 yr of an anticipated contract period, yields are estimated to average 5.5 tons/yr. Land quality, weather conditions, stand vigor, weeds and overall management will impact yield levels for a given switchgrass field.

**PEST AND DISEASE MANAGEMENT**

Switchgrass has resistance to a wide variety of insects and diseases. Researchers and farmers have not experienced significant insect and disease problems in switchgrass trials conducted in Tennessee. As switchgrass acreage expands, it is not unreasonable to expect problems to develop over time.

Visit the UT Extension Web site at www.utextension.tennessee.edu/Biofuels Initiative.
DISTRIBUTED FAST PYROLYSIS FOR CONVERSION OF BIOMASS TO STABLE REFINABLE CRUDE BIO-OILS

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The focus of ARS’ biomass fast pyrolysis research at ERRC is related to on or near the farm pyrolysis of agricultural feedstocks. This includes: 1) quantifying the effect of various feedstock traits (composition, maturation, postharvest handling, pretreatment) on pyrolysis products yield and composition, 2) development of catalytic and noncatalytic process for farm scale production of stable bio-oil that meets boiler fuel or petroleum refinery feedstock specifications and 3) development of processes for coupling of production and activation of biochar. An overview of the current research on pyrolytic conversion of lignocellulosic biomass to biofuels will be presented.

Pyrolysis, gasification and combustion are the major thermochemical processes available for the conversion of biomass to renewable energy. The familiar combustion process utilizes biomass and oxygen and can be used to produce heat and power. Gasification utilizes biomass and substiochiometric amounts of oxygen to produce syngas, a mixture of carbon monoxide and hydrogen, that can either be burned or used to synthesize hydrocarbons or alcohols through Fischer-Tropsch or similar processes. In the pyrolysis process, heat is used alone in the complete absence of oxygen to break down the polymeric structure of the biomass into condensable vapors, which are collected as pyrolysis liquid also known as bio-oil (pyrolysis oil, biocrude) (Huber et al 2006, Mohan et al 2006). Bio-oil can be upgraded to either diesel or gasoline range

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hydrocarbons or to a petroleum refinery feedstock to produce those fuels in existing oil refineries. In addition to bio-oil, a solid coproduct, biochar, consisting mostly of carbon and inorganic materials (ash) contained in the biomass plus a gaseous fraction, syngas, are produced during pyrolysis. The ratio of the three products depends on several factors, but the yield of bio-oil usually is maximized in “fast pyrolysis” processes that are characterized by high heating rates and quenching rates, with the reaction temperatures of 450 to 550°C. Processes with moderate temperatures and slower heating rates maximize the yield of biochar; they are called “slow pyrolysis” (torrefaction) processes.

Pyrolysis oils are collectively a highly oxygenated, complex mixture of several hundred organic compounds including acids, esters, alcohols, ketones, aldehydes, anhydrosugars, furans and phenols. Experience at ERRC includes production of bio-oil by fast pyrolysis of numerous agricultural feedstocks, including woody feedstocks, grasses, legumes and agricultural residues. Specific feedstocks include switchgrass, alfalfa stems, corn cobs, corn stover, guayule bagasse, barley straw, barley hulls, soybean straw and several other feedstocks (Boateng et al 2007, Boateng et al 2008, Mullen et al 2010a, Mullen et 2010b, Boateng et al 2009, Boateng et al 2010a).

Analyses of some bio-oils produced at ERRC are listed in Table 1. Most of the oils produced from agricultural residues are highly viscous and although their appearance resembles that of petroleum crude, they consist of partially oxygenated hydrocarbons and therefore their energy content (heat of combustion) is only 50% that of petroleum derived fuels. Bio-oil is acidic, corrosive and unstable because of the high concentrations of reactive oxygenated molecules. During storage, oligomerization of these molecules causes the molecular weight and viscosity of bio-oil to increase. Because of these factors, bio-oil must be upgraded to reduce its oxygen content to be fungible as transportation fuel or petroleum refinery feedstock. Upgrading processes can occur during (in situ) or after (ex situ) production of bio-oil.

In-situ upgrading strategies employed at ERRC include catalytic pyrolysis to produce partially deoxygenated bio-oils which may be thermally stable and potentially reduce the hydrogen requirement of further upgrading. On a small scale, Boateng et al (2010b) reported that increased production of aromatic hydrocarbons in the pyrolysis of biomass was increased by the addition of an acidic zeolite (HZSM-5) (Mullen and Boateng 2010). Ex-situ bio-oil can be deoxygenated by cracking over catalysts (eg, zeolites) or by catalytic high pressure hydrogenation (Elliott 2007).

Success in upgrading pyrolysis oil to transportation fuel has been demonstrated by industry groups such as UOP and Boeing when in 2009 a hydroplane was operated using fuel comprised of 98% upgraded natural oils and 2% upgraded pyrolysis liquids to provide the required aromatics. Bio-oil can be used as a boiler fuel “as produced” or as fuel for other
external combustion applications; however, even in these cases combustion of bio-oil can prove difficult and additional research is required to perfect the use of bio-oil in such applications.

Table 1. Analyses of biomass fast pyrolysis bio-oils produced at ERRC.

<table>
<thead>
<tr>
<th></th>
<th>oak</th>
<th>switchgrass stems</th>
<th>alfalfa stems</th>
<th>corn stover</th>
<th>barley straw</th>
<th>barley hulls</th>
<th>chicken litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total water wt%</td>
<td>22.3</td>
<td>15.8</td>
<td>28.6</td>
<td>9.2</td>
<td>26.7</td>
<td>13.8</td>
<td>20.1</td>
</tr>
<tr>
<td>pH</td>
<td>2.6</td>
<td>3.1</td>
<td>-</td>
<td>2.9</td>
<td>2.4</td>
<td>2.5</td>
<td>6.9</td>
</tr>
<tr>
<td>Elemental Analysis (db)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (wt%)</td>
<td>58.1</td>
<td>47.5</td>
<td>56.8</td>
<td>53.9</td>
<td>50.8</td>
<td>54.4</td>
<td>55.6</td>
</tr>
<tr>
<td>H (wt%)</td>
<td>6.1</td>
<td>6.9</td>
<td>7.8</td>
<td>6.9</td>
<td>3.2</td>
<td>5.3</td>
<td>7.2</td>
</tr>
<tr>
<td>N (wt%)</td>
<td>1.5</td>
<td>0.4</td>
<td>3.7</td>
<td>1.2</td>
<td>1.4</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>S (wt%)</td>
<td>0</td>
<td>-</td>
<td>0.07</td>
<td>&lt;0.05</td>
<td>0.0</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>O (wt%)</td>
<td>34.3</td>
<td>45.2</td>
<td>31.3</td>
<td>37.9</td>
<td>44.4</td>
<td>38.5</td>
<td>29.2</td>
</tr>
<tr>
<td>HHV (MJ/kg)</td>
<td>18.1</td>
<td>18.4</td>
<td>20.6</td>
<td>24.3</td>
<td>17.7</td>
<td>20.8</td>
<td>23.3</td>
</tr>
<tr>
<td>HHV (MJ/kg, db)</td>
<td>23.3</td>
<td>21.9</td>
<td>28.9</td>
<td>26.7</td>
<td>24.2</td>
<td>24.1</td>
<td>29.2</td>
</tr>
</tbody>
</table>

Despite the need for ongoing research to improve the fuel properties and stability of bio-oil, pyrolysis offers several advantages over other conversion methodologies. One is that in comparison with biochemical conversion methods, thermochemical processes offer much higher feedstock generality. That is because biomass sources can be converted to bio-oil and biochar with little change in the process. Pyrolysis also can be a self sustaining process when some of the coproducts (syngas and/or biochar) are used to provide the energy for the pyrolysis reaction.

Another major advantage of pyrolysis is the production and use of biochar as a soil amendment. Soil application of biochar may enhance soil quality and be an effective means of sequestering large amounts of carbon, thereby helping to mitigate global climate change through carbon sequestration (Hansen et al 2008, Laird 2008). Use of biochar as a soil amendment could offset many of the problems associated with removing some crop residues from the land. Biochar is highly absorbent and therefore increases the soil’s ability to retain water, nutrients and agricultural chemicals, preventing water contamination and soil erosion. These attributes of biochar may be related to their surface areas and porosities, making slow pyrolysis which tend to produce high surface area biochars better for such applications.

Fast pyrolysis biochars tend to have low surface areas due to short residence times of the biomass in the reactors. For example, biochars from corn cobs and corn stover have been shown to have surface areas of 0 and 3.1 m²/g respectively (Mullen et al 2010b). Nonetheless, despite
the low surface areas attained during fast pyrolysis, these chars and their activated counterparts have high ion exchange capacities for metal adsorption from aqueous solutions. For example, biochar produced by fast pyrolysis of corn stover was able to remove 80% of the copper from a 1M aqueous solution of Cu(II) ions, showing that biochars may find use in removing metal pollutants from water (Mullen et al 2010b).

To develop the high surface areas that may be required for soil applications, research approaches at ERRC include ex situ steam activation of fast pyrolysis biochars. Steam activation of biochar produced by the fast pyrolysis of switchgrass and alfalfa stems increased the surface area from 0.6 to 220 m$^2$/g and 2.97 to 250 m$^2$/g respectively (Lima et al 2008). When biochars are sequestered or applied to the soil, carbon balance requires a replacement plant will be deficient of the amount of carbon in the char for metabolic growth and has the potential to balance this carbon by removing carbon dioxide from the atmosphere. Since biochar may remain sequestered in the soil for thousands of years, the fast pyrolysis process is potentially a carbon negative process. An idealized scenario how bio-oil production could be carbon negative based on data produced in ARS’ experiments on fast pyrolysis of soybean straw is depicted in Fig. 1 (Boateng et al 2010a).

Perhaps the most important advantage for the pyrolysis process is in transportation and logistics costs. Because pyrolysis is amenable to small scale systems, a distributed network of pyrolyzers can provide on site conversion (eg, on a farm) of biomass to bio-oil and supply a centralized facility for upgrading to transportation fuels. Furthermore, because bio-oil is dense (specific gravity greater than water), it can be more cost effectively transported for further processing than its low bulk density biomass precursor, offering a major cost advantage in a commercial biorefinery (Wright et al 2008). Most other conversion methods require large plants to be economical and therefore require delivery large amounts of low density biomass from a large area frequently, adding cost to the operation. The distributed processing model for bio-oil production by fast pyrolysis is depicted in Fig. 2. Developing and advancing technology at a scale that fits this integrated distributed pyrolysis concept is the focus of the pyrolysis research program at ERRC.
Figure 1. Potential bioenergy production carbon footprint for soybean straw pyrolysis (from Boateng et al 2010a).
Figure 2. Concept of integrated distributed pyrolysis system (adapted from Hansen et al 2008).
LITERATURE CITED


INTEGRATION OF MEMBRANE FILTRATION TECHNOLOGY IN THE CELLULOSE TO ETHANOL PROCESS

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ABSTRACT

High oil prices and the slowdown in grain based ethanol production due to the elevated cost of corn along with public pressure to minimize the impact of using a food supply to produce ethanol, have contributed to creating the perfect storm of interest in producing ethanol from renewable sources such as biomass based cellulose. These factors, along with the government’s passage of the Renewable Fuels Standard (RFS) included as part of H.R.6, the Energy Independence and Security Act (EISA) signed in December, 2007 (which has been modified multiple times) calling for a significant increase in ethanol usage nationwide from both grain and cellulosic based sources, have generated interest in developing efficient, cost effective manufacturing processes to produce ethanol from cellulosic feed stocks.

With yields of sugars from cellulosic sources less than those from corn, perhaps the biggest challenges facing this industry is to develop an overall process that will isolate 5 and 6 carbon sugars which ultimately will be the source for fermentation in producing ethanol along with doing it in a cost effective manner that will be sustainable. Membrane filtration has proven to be a versatile separation technology that can play an important role in many steps within the cellulose to ethanol (CTE) process.

INTRODUCTION

The production of fuel grade ethanol has exploded in recent years due to the push to get away from the reliance on fossil fuels such as gasoline. Until recently, the primary source for ethanol has been corn. However, ethanol derived from cheaper and more replenishable sources such as cellulosic biomass like corn stover, switchgrass, cereal straws and wood chips has emerged as a major factor in this industry.
The main potential advantages of ethanol produced from cellulosic biomass sources are: 1) cellulosic sources typically are waste products that are available and renewable, 2) no depletion of food supply, 3) cogeneration and burning of the residual lignin produces steam and electricity and 4) up to 90% reduction in green house gases compared to gasoline. The process to access the cellulose from biomass and hydrolyze the sugars required for the fermentation and ethanol production involves many steps. Membrane filtration offers the opportunity to improve the efficiency, and therefore reduce the overall processing costs, in many of these steps.

Key advantages membrane filtration technology offer to the CTE process are: 1) specific separations down to the molecular level, 2) the ability to purify and concentrate fermentation precursors such as 5 and 6 carbon sugars to improve the efficiency and yield of ethanol production and 3) generation of new, high quality coproducts. Overall, membrane filtration is a widely accepted technology in the food, beverage and dairy industries for process applications generating commercial products. Membrane technology also is used and under consideration for coproduct or waste stream applications in these same industries.

Membrane technology has four general classifications, microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). Membrane pore sizes (alternately described by molecular weight cut offs (MWCO)) range up to a couple microns for the most open MF membrane to less than one thousandth of a micron (60 MWCO) for RO membranes. Membrane applications can be classified as: 1) clarifications, ie, separating suspended and colloidal components from dissolved components, 2) component separations based on molecular size and 3) dewatering or concentration, ie, removal of water or other solvents from all other components.

An overview of how membrane filtration compares to more typical separation technologies such as centrifugation, electrodialysis and ion exchange is depicted in Fig. 1. A membrane’s composition usually is described as being either polymeric or inorganic. While more than a hundred polymers have been used for membranes, there are 5 to 10 polymer types that make up most of the polymeric membranes widely used and available today. Different polymeric membranes have different membrane capabilities (pore sizes, flux rates) and also process stream capabilities and compatibilities (pH and temperature ranges, solvent stability, oxidizer stability). The composition and type of membrane required for different applications are important factors when evaluating their capability of being used within the CTE process as many potential process areas are demanding, either in terms of the level of suspended solids present or process conditions such as pH and temperature.
Figure 1. Common separation mechanisms.
Polymeric membranes cover the complete range of pore sizes from MF to RO. Polymeric membranes can be configured as spiral, tubular, hollow fiber or flat sheets, with spiral the most widely used configuration for process applications dealing with soluble compounds. Inorganic membranes predominantly have supporting ceramic or stainless steel layers. The actual membrane layer is usually a ceramic material. Inorganic membranes are limited to MF and UF pore sizes. Inorganic membranes can be described as robust, capable of tolerating pH extremes, temperature extremes, organic solvents and oxidizers. Inorganic membranes almost always are configured as tubular membranes.

Figure 2. Filtration spectrum.
Without question, ethanol represents a cleaner and more renewable energy source alternative to gasoline. The increasing use of, and reliance on, ethanol as a fuel requires that many types of biomass feedstocks be used for ethanol production. The biochemical process involves the breakdown and conversion of biomass sources to sugar polymers to ethanol. Advancements in the biochemical process and related equipment technologies continue to improve economical viability, resource efficiency and environmental impact. Membrane filtration technologies such as MF, UF, NF and RO can provide important process solutions in various steps of the biomass processes.

Generally there are four types of organic materials that can be used in a typical biomass to ethanol biochemical conversion process, these being monomeric sugars, starch, cellulose and hemicellulose. Monomeric sugars (as found in sugarcane, sugar beets, fruits) can be fermented easily into ethanol. However, the cost of production and/or competition from other uses makes...
these sources of biomass either too expensive or too low in profit in the US. An advantage of the processing of starch, cellulose and hemicelluloses is their efficient breakdown into sugars for fermentation. Starch is a biopolymer of glucose and can be broken down chemically and/or enzymatically to glucose and then fermented into ethanol.

Steps in the biochemical production process of ethanol from cellulosic (cellulose or hemicellulose) biomass are:

**Pretreatment:** The biomass is pretreated to degrade lignin components and to solubilize and make more accessible the cellulosic components for hydrolysis. Pretreatment may include one or more of the following: physical (grinding/milling, steam explosion), chemical (dilute acid, alkaline, hot water) and biological.

**Hydrolysis:** Next, the cellulosic components in the biomass are hydrolyzed to simple sugars. Enzymatic hydrolysis is the most preferred method but other methods like concentrated acid and dilute acid hydrolysis also can be utilized. Hydrolysis results in both 5 and 6 carbon sugars being produced.

**Separation/Purification:** The hydrolysate stream may be purified to provide a better product for fermentation. Purification includes the removal of unwanted fibers and other suspended matter, and/or the removal of unwanted dissolved components. Residual fiber material can be utilized for power generation.

**Fermentation:** The 5 and 6 carbon sugars are fermented into ethanol by yeast or bacteria. Typically the yeast and bacteria have been modified genetically to maximize fermentation efficiency.

**Ethanol Recovery:** Ethanol almost always is recovered from the fermentation product by the use of distillation in combination with other technologies (eg, molecular sieves) to increase purity above the azeotrope limit. Stillage off the distillation process will be processed to concentrate and recover suspended materials (wet grains) or dissolved materials as coproducts.

Steps of the biochemical process are shown in Fig. 4.
Figure 4. The biochemical process.

Membrane filtration technology can be used to facilitate or improve various aspects of the above steps. Examples include: 1) clarification of the pretreated liquor prior to hydrolysis with the concentrated fiber and suspended solids sent on to the byproduct recovery processes, 2) clarification of the hydrolyzate stream prior to fermentation, 3) concentration of 5 and 6 carbon sugars prior to fermentation and 4) polishing of evaporator condensate to produce high quality water for reuse within the plant.
In Fig. 5 are highlighted the basic steps in the CTE process along with examples of where membrane filtration can be applied. Along with the ability to effectively accomplish key separation and/or concentration steps within the CTE process, membrane filtration can have environmental benefits as well when applied to water recovery and waste water treatment applications, thereby minimizing fresh water usage and waste discharges.

Ethanol production plants use roughly 4 gallons of water to make each gallon of fuel, putting tremendous pressure on water supplies in many regions throughout the country. Membrane filtration, particularly RO, can be utilized to purify and recover up to 90% of the water used in some of the process steps, significantly lowering the overall volume of water needed (Fig. 6).
Figure 6. RO system treating evaporator condensate.

SUMMARY

Membrane filtration will not be the panacea to all CTE processors’ challenges but without question, the technology represents the opportunity to improve yields and operating efficiencies, as well as reducing operating costs, all of which are major hurdles currently facing the biomass industry.
THERMOCHEMICAL CONVERSION OF BIOMASS TO ADVANCED BIOFUELS, U.S. DEPARTMENT OF ENERGY, BIOMASS PROGRAM

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INTRODUCTION

In State of the Union addresses in 2006 and 2007, then President Bush outlined Advanced Biofuels Initiative, which seeks to break our national dependence on imported oil by accelerating the development of domestic, renewable biomass fuels to supplant petroleum based transportation fuels. In President Obama’s addresses to the joint session of congress, in both 2009 and 2010, he mentioned the need for continued investment in advanced biofuels. Advanced biofuels are defined as renewable fuels, not including corn based ethanol, that offer life cycle greenhouse gas emissions at least 50 percent below baseline lifecycle greenhouse gas (GHG) emissions (Energy Independence and Security Act 2007).

The Energy Independence and Security Act (EISA) of 2007 specifies a target quantity of 36 billion gallons of renewable fuels to be available in the US for domestic consumption by 2022. Of this amount 21 billion gallons must be from advanced biofuels and within these 16 billion gallons are expected to be cellulosic biofuels, 1 billion from biomass based diesel and the rest from other sources. The Department of Energy (DOE) Biomass Program currently is focused on cellulosic ethanol which has the potential to lower lifecycle GHG relative to baseline lifecycle GHG emissions by ≥ 80%. The investment in cellulosic ethanol made by DOE and the federal government is helping to make cellulosic biofuel technology a reality; together with significant research, development, demonstration and industrial partners this enable the technology to enter the marketplace (POET 2009).
THERMOCHEMICAL CONVERSION RESEARCH

The DOE Biomass Program takes a holistic approach to research, development, demonstration and deployment (RDD&D) on the entire biomass to biofuels supply chain. Within the program, conversion of biomass research area is a pivotal component and is divided into biochemical and thermochemical conversion of biomass research areas. Thermochemical conversion of biomass research and development enables technology to convert biomass to fuels, chemicals and power via thermal and chemical processes such as gasification, pyrolysis and other catalytic conversion processes. Intermediate products include clean synthesis gas (a mixture of hydrogen and carbon monoxide, resulting from gasification), bio-oil (a liquid product from pyrolysis), biochar (a solid coproduct from pyrolysis) and gases rich in methane, ethane or hydrogen. These intermediate products can be upgraded to products such as ethanol, other alcohols, renewable gasoline, renewable diesel, renewable jet fuel, ethers, chemical products or high purity hydrogen. Or they may be used directly for heat and power generation. Some of these products are direct substitutes for fossil fuel based intermediates and products which are compatible with existing fossil fuel processing and distribution infrastructure.

Each research platform funds RD&D across the associated conversion process unit operations, advancing technologies and ensuring specific technical targets are met. Specifically, thermochemical conversion of biomass RD&D focuses on the following areas for gasification of biomass:

- feedstock processing interface,
- gasification,
- gas cleanup,
- gas conditioning,
- fuel synthesis and
- throughout the entire system engineering and process optimization.

Regardless of the end biofuel, the feedstock interface addresses the main biomass properties that affect the long term technical and economic success of a thermochemical conversion process: moisture content, fixed carbon and volatiles content, impurity concentrations and ash content. High moisture and ash content reduce the usable fraction of delivered biomass.

Biomass gasification is a complex thermochemical process that begins with the thermal decomposition of a lignocellulosic feedstock. This is followed by partial oxidation or reforming of the fuel with a gasifying agent, usually air, oxygen or steam, to yield raw syngas (producer gas). Raw syngas composition and quality are dependent on a range of factors, including feedstock composition, type of gasification reactor, gasification agents, stoichiometry, temperature, pressure and the presence or lack of catalysts.
Gas cleanup and conditioning is the removal of contaminants from biomass derived raw synthesis gas. Research is focused on gas cleaning and conditioning catalysts and technology for cost effective removal of contaminants such as tars, particulates, alkali and sulfur. Interactions among catalysts used for gas cleanup and conditioning, and gasification conditions and feedstock are not well understood. These interactions require careful attention to trace contaminants and are important for efficient cleanup and conditioning of raw syngas in conjunction with optimal lifetimes of the catalyst(s).

The “cleaned and conditioned” synthesis gas composed of carbon monoxide (CO) and hydrogen (H) in a given ratio can be converted to mixed alcohols or Fischer-Tropsch hydrocarbons. The commercial success of mixed alcohol synthesis or hydrocarbon liquids has been limited by poor selectivity and low product yields. Research focused on more robust catalysts with increased productivity and selectivity with a biomass feedstock together with extended lifetimes are required to enable viable capital and operating costs.

Thermochemical conversion technologies and process integration currently present large scale up risks because of lack of high quality controlled process data on integrated systems carried out for extend periods of time required for industrial operations. Process integration work is essential for characterizing the complex interactions that exist among many of the processing steps, identifying impacts of trace components on catalytic and thermal systems and enabling the generation of predictive engineering models that can guide process optimization and scale up.

Technoeconomic modeling is utilized to determine the impact of thermochemical conversion technical targets on the overall conversion cost of the final fuel. Current cost targets for gasification of biomass for a modeled feedstock (woody feedstocks) are listed in Fig. 1. The state of technology status and projection is a modeled production cost at 2,000 dry tons feedstock/day of an nth plant using programmatic data from the thermochemical gasification conversion R&D. These cost targets currently are being updated with hopes to publish the updated targets in 2010 dollars in fiscal year 2011.
A growing body of work is suggestive the next thrust of biomass derived fuels should be to hydrocarbons that are compatible with current fuel infrastructure (Bioenergy, Biofuels, Huber). Should this vision be realized, the result would be higher levels of energy efficiency, energy density, cost efficiency and a potential to reach to a broader cross section of vehicles. Biofuels that are infrastructure compatible range from: ≥ C4 carbon chain biomass derived alcohols, renewable gasoline, renewable jet fuel, renewable diesel, algal derived fuels and others. One possible route that yields both renewable gasoline and renewable diesel is that of fast
pyrolysis. Specifically, thermochemical conversion of biomass RD&D focuses on the following areas for the fast pyrolysis of biomass:

- feedstock processing interface,
- pyrolysis,
- bio-oil clean up and stabilization,
- fuel processing and
- throughout the entire system engineering and process optimization.

There are some commonalities with the feedstock interface outlined for gasification and these will not be elaborated on further here, only to mention particle size required for fast pyrolysis typically is smaller than for gasification. Pyrolysis is the thermal decomposition of biomass in the absence of oxygen to produce a bio-oil intermediate that superficially resembles No. 4 fuel oil. The pyrolysis of biomass has been studied for some time; however, the resulting bio-oil is unstable and highly reactive. Improvements in pyrolytic processing with or without catalysts are needed to yield higher quality bio-oil, lower subsequent upgrading costs and allow for greater commercial viability. New methods and catalysts to clean and stabilize the bio-oil are needed to ensure the product is less reactive and stable for at least of six months, these advances include improved catalysts for deoxygenation and techniques for removal of solids from bio-oil.

Additional processing of the bio-oil is required to enable bio-oil to become a feedstock suitable for use in a petroleum refinery at several entry points. There is a need for hydrotreating and hydrocracking catalysts that are selective to the desired end product, robust with respect to the bio-oil impurities and have high conversion rates and long lifetimes. The development of robust catalysts for upgrading and hydrotreating bio-oils to produce liquid transportation fuels is vital for the success of these processes.

A technical based cost target for fast pyrolysis has been developed based on a fast pyrolysis design case (PNNL), by 2017, a biomass based thermochemical route that produces gasoline and diesel blendstocks will achieve a conversion cost of $1.56 per gallon of total blendstock ($1.47/GGE, 2007 $).

Conversion research has been focused towards production of cellulosic ethanol; however, as the expected commercialization of cellulosic ethanol draws nearer (POET), the Biomass Program is diversifying its research portfolio to include other biofuels as illustrated here with one such example and it is anticipated other routes will be added in future years to enable a diverse portfolio that can be optimized for the different regional needs of the nation. There are critical roles for all aspects of biology, chemistry and engineering research to facilitate cellulosic biomass derived advanced biofuels entering the market. With continued research, development and demonstration to remove barriers to market entry in the US, such as cost and ease of use, a vibrant advanced biofuels market will be enabled.
LITERATURE CITED


POET plant produces cellulosic ethanol 1/12/2009.
DEVELOPMENT OF A “GREEN” AQUEOUS ENZYMATIC PROCESS TO EXTRACT CORN OIL FROM CORN GERM

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In 1994, an Yugoslavian group reported an enzymatic method that resulted in an 80% yield of corn oil from wet milled corn germ (Table 1) using commercial enzyme preparations. In their first paper (Karlovic et al 1994) they used Pectinex Ultra SP-L, a pectinolytic enzyme preparation from \textit{Aspergillus niger} and in their second paper (Bocevska et al 1993) they obtained higher yields using Cellulclast\textsuperscript{TM}, a cellulase enzyme preparation from \textit{Trichoderma reseei}. Both processes started with corn germ from wet milling; the corn germ was used while still wet, not oven dried. Part of the process involved “hydrothermal” treatment (the equivalent of an autoclave treatment of the corn germ) prior to the addition of enzyme.

We developed an aqueous enzymatic oil extraction (AEOE) method (Table 1) to extract corn oil from wet milled corn germ (routinely dried to 3% moisture at the corn wet mill) (Moreau et al 2004). The basic steps in the method involved “churning” the corn germ with various enzymes and buffer for 4 hr at 50°C, and an additional 16 hr at 65°C, followed by centrifugation and removal of the surface oil layer. No hexane or other organic solvents were used in this process. Using oven dried corn germ samples (6 g) from a commercial corn wet mill, corn oil yields of 80% (relative to hexane extraction) were achieved using three different commercial cellulases. Using this method in the absence of enzyme resulted in corn oil yields of 37% (Moreau et al 2004). A 4 fold scale up of the method (to 24 g germ) resulted in oil yields of 90%. Most of the remaining 10 to 20% of oil is probably in a white emulsion layer located directly below the floating oil layer, after centrifugation. In another study, we scaled the process to 100 g wet milled corn germ and achieved oil yields of 36 and 72%, without and with enzyme (cellulase, Multifect GC), respectively (Dickey et al 2008). An alternative approach to scaling up the AEOE corn germ process has been to use a bubble column to enrich the corn oil as it is released from the corn germ (Dickey et al 2009). Using this approach with wet milled corn germ, we enriched the foam oil 4 fold, so only 25% of the original volume of AEOE solution
needed to be centrifuged to float the free oil. Adding this bubble column approach reduced the cost of AEOE from corn germ by reducing centrifugation costs.

Three commercial cellulases (Multifect GC and GC 220 from Genencor and Celluclast from Novozymes) were evaluated using our protocol; all three resulted in oil yields of 80% (Moreau et al 2004). An additional cellulase (Sigma C1794) and two xylanases (Multifect Xylanase from Genencor and Sigma X 2753) resulted in yields of 54 to 66%. Six other enzymes (including three cellulases, two proteases and one pectinase) resulted in yields of 30 to 44%; whereas, an oil yield of 27% was achieved using this protocol with no enzyme. Six of the seven most effective enzymes were from the fungus Trichoderma. Although these enzymes are marketed as cellulases and xylanases, all of them are mixtures of enzymes and contain other enzyme activities that may contribute to their ability to extract corn oil.

Karlovic et al (1994) noted that unlike most oilseeds, arabinoxylans are the most abundant carbohydrate polymer in corn germ. Because of this, it is reasonable that enzyme preparations that combine xylanase and cellulase activities may be the most effective (Table 1). Lipid bodies, the triacylglycerol containing organelles in seeds, are surrounded by a “half unit” membrane that is comprised of a phospholipid monolayer and a structural protein called “oleosin”. Therefore, we can hypothesize that proteases and/or phospholipases may be useful enzymes for aqueous enzymatic oil extraction. Others reported that protease alone is useful for AEOE for some oilseed species; however, the two proteases tested in our study (Moreau et al 2004) had no effect on oil yields. To our knowledge, no one has evaluated phospholipases for their efficacy at enzymatic oil extraction. However, because phospholipases could degrade all cellular biomembranes, and some phospholipases could release lysophospholipids (which are known to act as surfactants), their use may be problematic.

When the oil quality of hexane extracted vs our aqueous enzymatic extracted corn oils was compared, the two compositions were similar (Moreau et al 2004). Bocevska et al (1993) reported their AEOE corn oil was of high quality. The low levels of free fatty acids generated during AEOE were indicative that lipolytic activity was minimal in our oven dried wet milled corn germ (Moreau et al 2004) and in the hydrothermally treated wet milled corn germ (Bocevska et al 1993, Karlovic et al 1994). If this aqueous enzymatic method was used with wet corn germ instead of oven dried corn germ, hydrothermal treatment may be necessary. Phytosterol levels of (free and esterified) were lower in the aqueous enzyme extracted oil than in hexane extracted oil.

In a follow up study (Moreau et al 2009), we evaluated the AEOE method developed for wet milled corn germ (Moreau et al 2004) and instead of wet milled corn germ we used commercial dry milled corn germ and experimental enzymatically milled germ (E-germ). We found no oil was obtained from dry milled corn germ or E-germ using the AEOE method with only cellulase (Moreau et al 2004). To obtain oil with both dry milled corn germ and E-germ,
we found we needed to cook the corn germ in a conventional oven or microwave oven or add a second enzymatic step, an alkaline protease treatment, after the acidic cellulase treatment step (Dickey et al 2009). A possible explanation for the cooking effect was reported in a previous paper from our laboratory (Dickey et al 2007). We presented microscopic evidence suggesting that when corn germ was heated in a conventional oven or a microwave oven, oil body membranes were disrupted, oil from ruptured oil bodies coalesced and oil yields via mechanical pressing were increased (Dickey et al 2007). We postulated that conventional oven cooking or microwave oven cooking of the germ similarly ruptured the oil bodies and enabled the oil to be released by treatment with acidic cellulase. Similarly, treatment of the acidic cellulose digested dry milled germ or E-germ with a second alkaline protease step also released oil from corn germ (Dickey et al 2009).

In summary, our new aqueous enzymatic extraction processes results in oil yields of greater than 90% from wet milled corn germ and from E-germ. This yield is higher than the 80% yield of corn oil from corn germ previously reported (Karlovic et al 1994). Care needs to be taken in comparing our yields with those previously reported because in the earlier report wet (undried) corn germ was used as a feedstock for oil extraction but in our present method factory dried germ was used. Also, the aqueous enzymatic extraction process used by Karlovic et al (1994) included an essential “hydrothermal pretreatment” step; whereas, our method resulted in high yields without a hydrothermal pretreatment step. If we had used wet (fresh, not heat dried) corn germ, it is possible the hydrothermal pretreatment may have been necessary. No precautions were taken to limit the growth of microbes during our aqueous enzymatic process. We recognize the development of a successful aqueous enzymatic oil extraction process will require the implementation of strategies to limit microbial growth. Since several cellulase preparations appear to result in high oil yields, we anticipate some of the new cellulolytic enzymes being developed for biomass hydrolysis and fermentation may result in even higher oil yields and may be less costly to produce.
Table 1. A comparison of the published aqueous enzymatic oil extraction (AEOE) methods for the extraction of corn oil from corn germ.

<table>
<thead>
<tr>
<th>Dry Milled Germ (DMG) or Wet Milled Germ (WMG) or E-germ</th>
<th>Germ Dried in Oven? (% moisture)</th>
<th>Hydrothermal Processing Step (112°C)</th>
<th>Enzyme used</th>
<th>Oil Yield Free oil (wt %)</th>
<th>Oil Quality</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>WMG</td>
<td>N (53%)</td>
<td>Y</td>
<td>Pectinex Ultra SP-L</td>
<td>NR</td>
<td>Excellent</td>
<td>Karlovic et al 2004</td>
</tr>
<tr>
<td>WMG</td>
<td>N (53%)</td>
<td>Y</td>
<td>Celluclast</td>
<td>80%</td>
<td>NR</td>
<td>Bocevska et al 1993</td>
</tr>
<tr>
<td>WMG</td>
<td>Y (3%)</td>
<td>N</td>
<td>Celluclast or Multifect GC or GC 220</td>
<td>80-90%</td>
<td>Excellent</td>
<td>Moreau et al 2004</td>
</tr>
<tr>
<td>WMG</td>
<td>Y (3%)</td>
<td>Y</td>
<td>Multifect GC</td>
<td>72%</td>
<td>NR</td>
<td>Moreau et al 2004</td>
</tr>
<tr>
<td>WMG</td>
<td>Y (3%)</td>
<td>Y</td>
<td>Multifect GC</td>
<td>75%</td>
<td>NR</td>
<td>Dickey et al 2008</td>
</tr>
<tr>
<td>DMG</td>
<td>N (15%)</td>
<td>N</td>
<td>GC 220 + Alcalase</td>
<td>65%</td>
<td>NR</td>
<td>Moreau et al 2009</td>
</tr>
<tr>
<td>E-germ</td>
<td>N (~50%)</td>
<td>N</td>
<td>GC 220 + Alcalase</td>
<td>90%</td>
<td>NR</td>
<td>Moreau et al 2009</td>
</tr>
</tbody>
</table>

NR = not reported.
LITERATURE CITED


BIOCHEMICAL CONVERSION
U.S. DEPARTMENT OF ENERGY, BIOMASS PROGRAM

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PROGRAM OVERVIEW

Over the last 5 years, the national concern over energy security has been a common topic of discussion. As such, Congress has enacted several laws to spur the development of renewable alternatives to petroleum. Specifically, the Energy Independence and Security Act (EISA) of 2007, specifies a target quantity of 36 billion gallons of renewable fuels to be available in the US for domestic consumption by 2022. Of this amount, 21 billion gallons must be from advanced biofuels including cellulosic biofuels, biomass based gasoline and diesel, and other forms of biofuels.

The U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy’s Biomass Program is in an unique position to address and foster the development of biomass to biofuel conversion processes. The Biomass Program takes an holistic approach to funding research, development, demonstration and deployment (RDD&D) projects on biomass to biofuels technologies and from feedstocks to distribution and end use. The Program is managed by research area (Fig. 1) and is performed by a variety of partners, which includes the national laboratories, academia and industry stakeholders.
BIOCHEMICAL CONVERSION RESEARCH

The conversion research area is divided into the biochemical and thermochemical conversion platforms. Each research platform funds RD&D across the associated conversion process unit operations, advancing technologies and ensuring specific technical targets are met. Specifically, the biochemical platform funds RD&D in the following areas:

- feedstock/biochem processing interface,
- pretreatment,
- hydrolysis,
- fermentation
- and process integration (including engineering and process optimization).

To date, the research on these platforms has been geared towards production of cellulosic ethanol; however, as the expected commercialization of cellulosic ethanol draws nearer, the program is diversifying its research portfolio to include other biofuels.

Regardless of the end fuel, most biochemical conversion processes begin with a pretreatment process. To start with the pretreatment process would be premature, as the feedstock and biochemical processing interface addresses issues at a crucial logistics interface. By characterizing the feedstock both before and after it has been transported and preprocessed, the impact of the feedstock delivery can be analyzed. This characterization of the preprocessed feedstock(s) is essential determining the impacts of preprocessing on downstream conversion processes. Current activities under this area include but are not limited to: 1) determining chemical compositions and physical formats of agricultural residues and 2) qualifying differences in feedstock specifications into significant and nonsignificant attributes.
Within the pretreatment focus area, research is concentrated on investigating and improving the performance of multiple pretreatments resulting in an overall reduction in pretreatment cost. Task performers are attempting to increase solids loading potential without decreasing conversion efficiency and reducing sugar loses due to degradation. Work also is ongoing on reducing or eliminating the need for the costly conditioning step. In recent years, the National Renewable Energy Laboratory (NREL) demonstrated their improvements in overall xylose yields (from 70 to over 80%) were reproducible at pilot scale\(^1\).

Critical work in enzyme and fermentative organism development is underway. These fundamental activities are being performed in an applied manner. That is to say, the performers are using advanced and traditional methods to improve their selected organisms to perform at higher efficiency and lower cost at process relevant conditions. This work is ongoing and was competed for within industry. The first of these projects are approaching their respective the close dates and the program hopes to be able to demonstrate success at pilot scale. For these projects, the program implemented a benchmark and validation process to access their starting technology and monitor and evaluate their progress. This process has proven to be a success and will be duplicated for future projects in this area.

The work on the individual process steps described cannot be performed independently, as each step is dependent on the prior step. Process integration is a vital part of the biochemical portfolio that helps to identify potential barriers to processing and reduce the risk associated with scale up. Activities including assessing water recycle loops, evaluating process power requirements, identifying promising technology options and configurations, and performance demonstration. The majority of this work has been performed at the Process Development Unit (PDU) User Facility at the National Renewable Energy Laboratory. Additionally through Recovery Act funding, the program approved and is funding the building of a new smaller advanced biofuels PDU at Lawrence Berkeley National Laboratory. This second user facility is expected to open in Q2 of FY 2011.

Technoeconomic modeling is utilized to determine the impact of the biochemical conversion platform targets on the overall production cost of the final fuel. Current cost targets for the platform for a modeled feedstock (corn stover) are illustrated in Fig. 2. These cost targets currently are being updated and the program hopes to publish the updated targets in 2010 dollars by early fiscal year 2011.

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BEYOND THE BIOCHEMICAL CONVERSION PLATFORM

Beyond the efforts funded by the biochemical conversion platform, there are demonstration and deployment activities ongoing on integration and scale up of biochemical technologies and processes. These efforts are managed by the Integrated Biorefinery Team. An interactive map of the US with details on all of the Integrated Biorefinery Projects is available on the Biomass Program website:

http://www1.eere.energy.gov/biomass/integrated_biorefineries.html
Figure 2: Biochemical Conversion Cost Targets for Corn Stover to Ethanol (07$s)

*(Note: Unit operation cost contribution estimates are based on process concept targets; For “Processing Subtotal,” please see footnote on Table B5 in Appendix B for comments on rounding of numbers and subsequent summation).
IN SITU TRANSESTERIFICATION: BIODIESEL PRODUCTION BY THE DIRECT TRANSESTERIFICATION OF THE LIPIDS RESIDENT IN BIOLOGICAL MATERIALS

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In contemporary biodiesel production the acylglycerols in a biological material such as an oilseed are recovered by pressing or solvent extraction, purification and transesterification to produce simple fatty acyl esters, most typically the methyl esters. In an effort to reduce the complexity and attendant cost of this process we explored the possibility of directly transesterifying the acylglycerols resident in biological materials. This eliminates the need for oil extraction and purification prior to transesterification and could reduce process costs. Our initial experiments were conducted with soybeans which were converted to thin flakes to reduce diffusional distances; subsequently they were incubated in alkaline methanol, the most commonly used solution for biodiesel production. During incubation at room temperature and ambient pressure fatty acid methyl esters (FAME) appeared in the liquid phase. Unreacted acylglycerols did not transfer to the liquid phase.

By optimizing the reaction, conditions sufficient to convert greater than 90% of the acylglycerols to FAME were identified (Haas et al 2004). However, a 181 fold molar excess of methanol over substrate fatty acids in the substrate was required. Predrying the substrate reduced the methanol requirement by 60%; following removal of a slightly excessive free fatty acid content, the resulting FAME preparation met existing standards for biodiesel (Haas and Scott 2007). The reaction has been applied to produce FAME from the acylglycerols in soybeans, distillers dried grains with solubles (Haas et al 2007), meat and bone meal (Haas et al 2007), rice bran (Ozgul-Yucel and Turkay 2003), sunflower seed (Harrington and D’Arcy-Evans), algae (Haas et al in preparation), corn germ (Haas et al unpublished), peanuts (Haas et al unpublished) and canola (Haas et al unpublished).

A technoeconomic model was produced to estimate capital and process costs of biodiesel production from soybeans via in situ transesterification (Haas et al 2006). This was compared
with a similar model constructed for biodiesel production via the conventional route involving transesterification of refined oil. The \textit{in situ} method resulted in a predicted process cost 24% greater than the traditional method. The elevated cost was due to excessive energy use in the recovery of the large amount of unreacted alcohol required by the reaction. Subsequently, it was found that by passing the soybeans through an extruder prior to \textit{in situ} transesterification the methanol requirement, and hence the process cost, could be reduced, resulting in a predicted process cost lower than using conventional biodiesel production (Haas et al submitted for publication).

In few (if any) situations will the funds generated by sale of the oil from an oilseed, or of biodiesel produced from that oil, be sufficient to ensure profitability of operation. Value must be recovered from the meal component of the oilseed. Generally these meals are high in protein and used in animal diets. Animal feeding studies were conducted to determine whether the \textit{in situ} transesterification process affected nutritional suitability of soybean meal. In studies conducted with trout (Barrows et al 2008) and chickens (Haas et al in preparation), animals fed diets containing soybean meal that had been subjected to \textit{in situ} transesterification had feed efficiencies comparable to test groups receiving diets formulated with conventional soybean meal. Therefore, \textit{in situ} transesterification is a viable method to reduce the cost of biodiesel production while retaining the feed value of the meal coproduct.
LITERATURE CITED


WINTER BARLEY ETHANOL – A NEW ADVANCED BIOFUEL

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The Energy Independence and Security Act (EISA) of 2007 set an ambitious goal for the US to produce and use 36 billion gallons annually of renewable fuels by 2022. Of this quantity, only 15 billion gallons may come from conventional sources, such as corn; the remainder must be made from advanced and cellulosic biofuels. The Renewable Fuel Standard 2 (RFS2) of 2010 sets rules for defining the Life Cycle Green House Gas (LCGHG) emission requirements for all classes of biofuels; it contains a controversial rule that penalizes conventional fuel ethanol from corn by assessing additional GHG emission penalties for unmeasurable indirect land use changes as well as those resulting from direct land use.

Some advanced biofuels pathways have been defined in the RFS2 but new pathways can be developed for new advanced biofuel products as long as both direct and indirect LCGHG emissions are 50% (advanced) or 60% (cellulosic) less than emissions from conventional gasoline. The EISA requires increasing amounts of both conventional and advanced biofuels each year as we approach 2022. Reaching the cap of 15 billion gallons per year of corn ethanol probably will not be an issue. In fact, at this writing, the US already has enough capacity to produce nearly this amount.

However, reaching the goals for cellulosic and advanced biofuels for the current year already has become a challenge. In 2010, the EPA dramatically downsized the required cellulosic biofuel quota because the amount that could be produced was far short of the goals set by Congress 3 years ago. Looking forward, goals for 2011 and beyond look equally challenging.

Why is the US having a difficult time reaching the cellulosic biofuel goals set in 2007? The reason is that cellulosic biofuels are difficult to produce. Nature created both structural and storage type carbohydrates. Storage carbohydrates such as starch and storage lipids such as triacylglycerols were designed for easy assembly and disassembly. We know how to take these
molecules apart to make monomeric sugars or fatty acids which can be converted readily to fuel ethanol and biodiesel.

Structural carbohydrates (e.g., lignocellulosic cell walls) were designed by nature as durable composites that could resist everything, except for fire, that nature (and man) could use to try to decompose them. For this reason, many think thermochemical conversion processes have a better chance than biochemical processes for the eventual production of economically viable biofuels. Regardless of which will be more successful in the long run, most researchers agree that it will be 5 to 10 years before lignocellulosic biofuels will be as inexpensive to produce as conventional biofuels.

Currently, billions of dollars are being spent to design lignocellulosic energy crops that we do not know how to convert economically to biofuels. This is necessitating the additional expenditure of billions of dollars for research methods to deconstruct these cell walls. Rather than focusing exclusively on this daunting set of research tasks, researchers should be focusing on the production of energy crops that contain readily convertible storage carbohydrates and lipids. Use of these crops could help us reach the current and midterm RFS goals while the more complex cellulosic technologies are being developed.

We propose, across the US, there are possibilities for more easily convertible feedstocks than lignocellulose to produce renewable biofuels that meet EPA’s requirements for advanced biofuels. For the Mid Atlantic States, a model feedstock candidate is winter barley and the fuel is winter barley ethanol.

Winter barley can be grown in the Mid Atlantic States from Southern Pennsylvania through North Carolina. The mild winters allow the growth of an additional crop, winter barley, on winter fallow ground that would not be in use otherwise. The planting of winter barley occurs after harvest of corn in year 1 and harvest occurs before planting of soybeans in year 2 and does not interfere with production of full yields of both summer crops. This additional crop is important for regional farmers who need additional farm income.

This use of barley as a winter cover crop has been hailed by the Chesapeake Bay Commission (2007, 2008) as a preferred way to prevent nutrients and sediment from migrating from fallow winter fields into the Chesapeake Bay and therefore has a beneficial environmental impact. Finally, production of winter barley by regional farmers can produce, for the first time, a regional feedstock for the production of fuel ethanol and high protein animal foods.

In 2001, when the concept that winter barley could be a potential feedstock for regional fuel ethanol plants was presented to researchers at the Eastern Regional Research Center (ERRC) by barley breeders and extension staff from the Virginia Polytechnic Institute and State University (Virginia Tech), initial studies were conducted to determine the feasibility of the concept. These studies were promising and since 2001, ERRC researchers have worked with
Virginia Tech breeders to produce and evaluate hundreds of promising winter barley cultivars (Griffey et al 2010) of which outstanding high starch varieties such as Thoroughbred, Doyce, Eve and Dan have been released publicly.

ERRC researchers partnered with Genencor, a Danisco Division, to develop new concepts for converting these new, high starch cultivars into ethanol with better yields per gallon than traditional fermentation processes allowed. Of particular interest is the EDGE (Enhanced Dry Grind Enzymatic) barley ethanol process developed by ERRC and Genencor researchers (Nghiem et al 2010) which not only converted the starch in barley to ethanol but also converted mixed linkage $\beta$-1,3;1,4-glucans present in barley into fuel ethanol. These polysaccharides had created major obstacles to those who previously tried to make fuel ethanol from barley, due to the high viscosity $\beta$-glucans produced in traditional barley mashes. The EDGE process solved the viscosity problem and resulted in higher ethanol yields, both critical to using barley to produce fuel ethanol in an economical manner.

Fuel ethanol from any source is a commodity. To produce fuel ethanol economically, valuable coproducts also must be produced to improve the overall profitability of the enterprise. Barley grain contains superior amino acid profiles and slightly higher protein levels than corn grain. For that reason, barley distillers dried grains with solubles (DDGS) may command a premium price to corn DDGS and, in fact, have been referred to as barley protein meal by Land of Lakes, a major DDGS marketer. In addition to DDGS, barley contains valuable nutraceuticals such as tocopherols, tocotrienols and phytosterols (Moreau et al 2007a, 2007b) that, if isolated and purified economically, could become major coproducts. By abrasive milling and size fractionation processes, Moreau et al (2007a, 2007b) demonstrated how lipid rich fractions from barley could be isolated and extracted to yield barley oils enriched in many of these health promoting nutraceutical compounds.

Most varieties of barley are of the covered or hulled type. These hulls contain silica enriched phytoliths, monoliths of abrasive silica, which protect the kernel from insects and other pests. These organs are quite abrasive and create major damage to grain handling equipment. For this reason, many new varieties developed by Virginia Tech are naked or hulless varieties, which lose their hulls during harvest. These varieties are higher in starch and protein content than hulled varieties and are therefore improved for ethanol feedstock. Unfortunately, most hulless varieties yield fewer bushels per acre and therefore, are not favored by growers, who are paid by the bushel. For this reason, we have developed processes to remove the hull from hulled cultivars at the ethanol plant, using dry fractionation processes (Flores et al 2005). These and related processes can produce a hulless kernel with higher levels of fermentables for better ethanol yields.

Having the hull already brought into the ethanol plant as a captive biomass source may present some additional uses. We developed a process to produce cellulosic ethanol from barley...
hulls (Kim et al 2008), a process that could produce 11 to 16% more ethanol than an ethanol plant producing only ethanol from the barley starch. In addition, workers in our group have shown that barley hulls, barley straw and nonfeed grade barley DDGS can be converted to refinable, crude bio-oil and biochar using pyrolysis technology. Crude bio-oil made by this process can be refined into green gasoline and diesel using the existing petroleum refining industry’s infrastructure. The biochar produced in this process contains plant nutrients and a sequestered form of carbon. Application to soils may yield both direct and indirect benefits for growers and the environment.

ERRC researchers working with researchers at Genencor/Danisco and Virginia Tech led a vision for a new East Coast winter barley ethanol industry since 2001. It has been said that “A vision without an action is merely a dream; action without vision just passes the time; vision with action can change the world” (Barker, date unknown). In 2007, a new company called Osage Bio Energy (OBE) was started to put the action into the winter barley ethanol dream. OBE management took the winter barley ethanol story to Wall Street and received $300 million from First Reserve Corporation in 2008 to develop the first winter barley biorefineries.

In summer of 2010, the nation’s first winter barley ethanol plant will start up in Hopewell, VA, producing 65 mgd of ethanol. Life cycle studies performed by Dr. Sabrina Spatari in conjunction with OBE and ERRC researchers appear to confirm that in the unique OBE facility, the winter barley ethanol reduces life cycle green house gases more than 50% compared to conventional gasoline. Since winter barley is grown on land that otherwise would be fallow, and does not compete with food or feed production, it will not be penalized with an indirect land use change emissions penalty. When EPA officially approves this new advanced biofuels pathway, the OBE facility in Hopewell, VA will become the nation’s largest commercial scale advanced biofuel plant.

This demonstration of a new platform to produce advanced biofuels from winter crops can now be used as a model across the US. While winter barley may not be feasible in certain regions, other crops such as pennycress and camelina or others may be. Use of winter cover crops in the Mid West would not only create new advanced biofuel feedstocks urgently needed but also may help clean up the regional watersheds and even the dead zone of the Gulf of Mexico. Whereas “Vision with action can change the world”, vision without action may doom the world, or at least the RFS2. It is hoped these successes will result in other regional successes throughout the US and the world.
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POSTER PRESENTATIONS
A FLUX BALANCE APPROACH OF HEMICELLULOSE FERMENTATION TO ETHANOL BY INDUSTRIAL YEAST *Saccharomyces cerevisiae*

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A major challenge in ethanol production using lignocellulosic feedstocks is the inefficient utilization of hemicellulose, which accounts for 30 to 40% of the biomass. Xylose, which is not fermented by industrial yeasts, is the major product of hemicellulose hydrolysis from lignocellulosic feedstock. So, utilization of this carbon source would increase the ethanol yield from 60 to 90 gal/dry ton (Van Vleet and Jeffries 2009). Isomerisation of the available xylose to xylulose using commercially produced xylose isomerase *in vitro* is an approach to increase conversion of xylose to xylulose in yeasts (Chiang et al 1981, Chandrakant and Bisaria 2000).

The resulting xylulose can be utilized by yeast. Metabolic fluxes under experimental xylulose utilization conditions can be found using a flux balance based modeling approach. This approach relies on the stoichiometry of the yeast reaction network and the constraints determined by steady state growth of the organism in a defined media. A media replicating the hemicellulose hydrolysate in composition was designed and isomerized. Sugars (glucose, xylose and xylulose), sugar alcohols (ethanol, xylitol and glycerol) and yeast cell mass were monitored during the growth. The data from fermentation experiments were used to conduct a flux balance analysis of the yeast using the *Saccharomyces cerevisiae* iND750 model. The model outputs were comparable to the experimental production rates of products. Under the experimental conditions, the model gives an increase of 1.036 mmol/hr·g biomass of ethanol production by the consumption of xylulose.
LITERATURE CITED


EFFECT OF HARVEST DATE ON RELEASE OF SUGAR MONOMERS AND BYPRODUCTS FROM *Panicum virgatum* VAR. ALAMO

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The biofuels industry will rely on multiple feedstocks that will need to be supplied to biorefineries to provide a year round supply of biomass for producing valuable products and coproducts. Therefore, due to variables in biorefinery demand and weather, harvesters might find it advantageous to harvest their crops at various times in the season. The objective was to determine the effects of harvest date on the release of monomeric sugars and subsequent byproducts using dilute sulfuric acid hydrolysis followed by enzymatic hydrolysis of switchgrass (*Panicum virgatum* var. Alamo).

Thus far, only the dilute acid hydrolysis of switchgrass harvested in July has been conducted (Fig. 1). These results will be coupled with the enzymatic hydrolysis results to determine the optimum conditions for maximum monomeric sugar and minimal byproduct release. These conditions will be applied to switchgrass harvested in July through February. Dilute acid hydrolysis has been carried out using 1 g of switchgrass, ground to a size 20 mesh, and 20 mL of 0.98% aqueous sulfuric acid in a stainless steel reactor at 140 to 200°C for 10 to 120 min. Optimum hydrolysis conditions were 140°C for 70 min. Future work with enzymatic hydrolysis will show if these are the optimum pretreatment conditions and whether there are differences in monomer release and byproduct formation among harvest dates.
Figure 1. Concentration of monomer (A) and byproduct (B) after 40, 60, 70, 85, 100 and 120 min hydrolysis at 140°C.
ANNUAL CEREAL FORAGES FOR FUEL ETHANOL 
FEEDSTOCK AND LIVESTOCK FOOD

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Corn grain is the major feedstock for fuel ethanol production in the US. However, only 
small amounts of corn grain are produced in Montana due to the cool weather and short growing 
season. An alternative feedstock needs to be explored for fuel ethanol production. There is an 
abundant but underutilized supply of agricultural residues and herbaceous grasses available in 
Montana. We developed production systems to maximize biomass yield and evaluated annual 
cereal hay and straw as well as warm season grasses for potential fuel ethanol feedstock and 
livestock food.

A double cropping system was developed, where winter triticale was planted in the fall 
and harvested in mid June for hay or silage, followed by sweet sorghum or sweet stem pearl 
millet planted into the field for a second harvest in late September. Enzymatic hydrolysis and 
fermentation were conducted for chemical pretreated and ensiled wheat, barley and triticale 
straws and for barley, triticale, pearl millet and sweet sorghum hays. Hay qualities and silage 
feedstocks were measured for animal food.

The double cropping system produced 47 to 56% more total biomass than planting winter 
triticale alone as single cropping for grain and straw. Enzymatic hydrolysis of chemically 
pretreated solids (with 2.0% NaOH or H2SO4) was conducted with Celluclast 1.5L-cellubiase 
and Spezyme® CP-xylanase enzyme combinations. Glucan and xylan conversions during 
hydrolysis were 78 to 100% and 74 to 84%, respectively. Ethanol yield after fermentation of the 
hydrolysate from different feedstocks with Saccharomyces cerevisiae ranged from 0.27 to 0.34 
g/g glucose or 52.0 to 65.8% of theoretical maximum ethanol yield. Overall sugar conversion 
was 18.5 to 59.2% (db) for ensiled feedstocks. Ethanol yield from the ensiled feedstocks ranged
from 0.21 to 0.28 g/g (reduced sugar basis) or 40.1 to 54.9% of the theoretical maximum ethanol yield. Winter triticale, sweet sorghum and sweet stem pearl millet hays had a good relative feed value (>91%). Hays had 32 to 35% ADF, 59 to 65% NDF and 12 to 19% protein content.

Biofuel feedstock production may be integrated with existing livestock production systems in Montana and multiproduct crops may be used both for biofuel feedstock and livestock food.
Continuous use of petroleum derived fuels is recognized as unsustainable due to depleting supplies and the accumulation of greenhouse gases in the environment. Renewable, carbon neutral transport fuels are needed for environmental and economic sustainabilities. Algae have been demonstrated to be one of the most promising sources for biofuel production. However, large scale algae production and harvesting for energy manufacturing are too costly using existing methods. The approach of growing algae on solid carriers is innovative and can lead to cost effective manufacturing of algae biofuels. As cells approach the solid surface, many factors come in to influence microbial attachment such as the surface wettability, free energy, polarity, roughness and topography. Surface wettability plays an important role in the initial cell attachment. For further contact, surface free energy and polarity are related directly to cell substratum attachment strength. Surface roughness and texture are species specific parameters and have been applied in attachment studies.

We studied the effects of carrier material and design on microalgal cell attachment via theoretical modeling and experiments. Adhesion of a freshwater algal species *Scenedesmus dimorphus* to a variety of material surfaces (polymers, glass and metal) was investigated. The number of attached cells was related to surface characteristics, especially surface interaction energy in water (Cui et al 2010a). Nylon and stainless steel showed the most cell attachment, which was in agreement with the prediction from the thermodynamic theory. The effect of surface texturing on algae cell attachment to stainless steel surface also was studied. Laser machined stainless steel samples with microsized dimples showed more cell attachment than as received smooth samples, which indicated that surface modification could enhance cell attachment to stainless steel carriers (Cao et al 2009). The DLVO theory and thermodynamic models were employed to understand and explain this phenomenon (Cui et al 2010b).
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DILUTE ACID PRETREATMENT OF SWEETGUM PURE WOOD

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Sweetgum (*Liquidambar styraciflua* L.), has a widespread distribution in the southeastern US, grows as understory in managed pine tree forest and has the potential of being used as biomass in the production of cellulosic ethanol. Biomass is pretreated, which involves breaking down the cell wall to prepare for enzymatic hydrolysis so monomeric sugars can be released and fermented into biofuels. The overarching goal of this research project is to determine whether sweetgum can be mixed with other forest residue and remain a viable biomass source. The short term objective of this research project was to maximize the yield of hemicellulosic sugars extracted from pure sweetgum wood (not contaminated with other forest residue) using dilute sulfuric acid pretreatment.

Tested material consisted of sweetgum cultivated at the University of Arkansas Pine Tree Branch Station, AR. Wood chip samples were ground to a 10 mesh size. One g ground material and 20 mL 1% H₂SO₄ were added to a thick walled stainless steel reactor and placed in a fluidized sand bath at 140°C for 10, 30, 60, 90 and 120 min. Because experiments were conducted in nonstirred reactors, 3 biomass loading configurations were tested: biomass at the bottom (bottom), acid/biomass/acid (sandwich) and biomass at the bottom (vortex). After pretreatment, reactors were removed from the sand bath and cooled by running water at 4°C for 30 min. Calcium carbonate was added to the extract for neutralization before HPLC analysis. Samples were analyzed with a Waters 2695 HPLC equipped with a Shodex SP-G precolumn and SP0810 column (85°C) with H₂O as eluent at a flow rate of 0.2 mL/min. Detection was obtained with a Waters 2414 refractive index detector.

Xylose recovery was calculated from 2009 baseline analysis of Arkansas grown sweetgum material by the National Renewable Energy Laboratories (Fig. 1). The bottom loading procedure resulted in the highest xylose recovery and pretreating material for 90 min at...
140°C released the highest concentrations of xylose. Future work will be focused on determining the effect of temperature on xylose recovery of pure sweetgum wood. Once the optimum pretreatment conditions are established, sweetgum wood will be contaminated with bark and other understory species.

Figure 1. The effect of loading of the reactor on the xylose recovery from sweetgum pure wood pretreated with 1% dilute sulfuric acid at 140°C for 10, 30, 60, 90 and 120 min. Optimum results were obtained using bottom loading of the sample for 90 min treatment.
MICROWAVE ASSISTED PYROLYSIS OF MICROALGAE FOR RENEWABLE BIO-OILS

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Microalgae recently received much attention in the field of biofuels production due to its numerous advantages, including high biomass production, high lipids content, CO\(_2\) sequestration and potential for wastewater treatment. However, most of the algae utilization research is concentrated on biodiesel production with algal lipids through conventional transesterification reaction. There are few reports on bio-oils production from pyrolysis of microalgae. Microwave assisted pyrolysis (MAP) provides many advantages over conventional pyrolysis techniques. The objective of the present study was to evaluate the technical feasibility of microwave assisted pyrolysis of microalgae.

The pyrolysis reaction was carried out in a microwave cavity oven by mixing 30 g microalgae powder with 30 g char in a quartz flask, which was placed inside the microwave cavity. Power levels ranged from 500 to 1250 W at the microwave frequency of 2450 MHz. Liquid product from MAP was a mixture of an oil phase and a water phase, which separated automatically. Bio-oil yield reached 29.8% under optimal conditions. Bio-oil was analyzed with an elemental analyzer, GC-MS, FTIR spectroscopy and thermogravimetric analysis. As identified with GC-MS, compounds in the bio-oils included: chain hydrocarbons, aromatics, long chain fatty acids, esters and nitriles. In terms of hydrocarbon variety and quantity, bio-oil from pyrolysis of algal biomass was better than from cellulosic biomass.
ISOLATION AND TRANSFECTION OF PROTOPLASTS FROM *Brachypodium distachyon* FOR SUBSEQUENT STUDIES OF CELL WALL BIOGENESIS MACHINERY

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Development of cleaner, cheaper and more reliable alternative energy sources such as biofuels is necessary to meet the increasing demand for energy production and environmental sustainability. The biofuel that is expected to be used around the globe is cellulosic ethanol. In this regard, temperate grass *Brachypodium distachyon* is in use for studying on how to modify grass crops for biofuel feedstock production. However, functional genomics tools have not been developed in *Brachypodium* and its cell wall biogenesis pathway is not understood. Since protoplasts regenerate their cell walls, gene inactivation via double strand (ds) RNA interference (RNAi) in protoplasts emerges as a rapid approach for discovery of genes involved in cell wall biogenesis.

Here we report the first protocol for high efficiency protoplast isolation from leaves of *Brachypodium*. In our method, 0.2 g of leaf tissue from 14 day old seedlings yielded $5 \times 10^6$-$10^7$ protoplasts. Furthermore, we transiently expressed GFP, the green fluorescence protein from *Aequorea victoria*, in *Brachypodium* protoplasts using a modified PEG mediated transfection procedure. As determined by fluorescent microscopy, GFP mediated fluorescence was observed after 24 hr of transfection with an efficiency greater than 50%.

Leaf derived *Brachypodium* protoplasts can be used to study a variety of cellular processes ranging from subcellular localization to functional analysis of RNAi knockdown genes, with future applications in a high throughput format. Because of the recent disclosure of the *Brachypodium* genome, development of the protoplast isolation and transfection procedures are timely and will provide novel tools for the expanding *Brachypodium* research community. Details of these procedures and the suitability of *Brachypodium* protoplasts for RNAi mediated gene silencing and functional analysis of the components of cell wall biogenesis machinery will be discussed.
EFFECT OF PHYTASE ADDITION ON GERM AND PERICARP FIBER RECOVERY FROM THE E-MILL PROCESS

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Phytase addition was evaluated on germ and pericarp fiber recovery in the E-Mill process. The E-Mill process involves soaking corn, grinding and incubation with starch hydrolyzing enzymes. Germ and pericarp fiber are separated and the remaining endosperm fraction is fermented. Endosperm fiber is recovered after completion of fermentation (Singh et al 2005). Phytases are enzymes that hydrolyze phytic acid into inorganic phosphate and inositol. In the dry grind process, phytases can be used to reduce slurry viscosity, increase alpha-amylase activity, and reduce phytic acid content of distillers dried grains with solubles (DDGS) (Shetty et al 2008). Since corn germ is the major repository of phytic acid, addition of phytases to the E-Mill process could affect coproduct (germ and pericarp fiber) yields and quality (residual starch contents).

The E-Mill process was modified with an additional phytase incubation step prior to incubation with starch hydrolyzing enzymes. Slurry specific gravities prior to fermentation were measured with and without phytase incubation. Germ and pericarp fiber yields were compared for processes with and without phytase treatment. Germ oil, protein and residual starch contents were determined. Pericarp fiber was analyzed for residual starch content. Slurry specific gravities with and without phytase addition were 1.076 sp. gr. (10.2 ± 0.53 °Baume) and 1.067 sp. gr. (9.13 ± 0.64 °Baume), respectively. Phytase treatments had no effect on coproduct yields. Germ oil contents were higher with phytase incubation (40.9%) than without phytase addition (39.1%). Phytase treatment resulted in germ with higher protein content (20.0%) compared to germ with no phytase addition (19.2%). Phytase treatment resulted in lower residual starch contents in germ and pericarp fiber (12.2 and 19.9%, respectively) compared to germ and pericarp fiber without phytase addition (18.1 and 27.4%, respectively).
LITERATURE CITED


MASS BALANCE CLOSURE OF XYLOSE Oligomers DURING TOTAL XYLOSE CONTENT ANALYSIS

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Xylose oligomers, chains of xylose molecules linked with β-1,4-bonds with the degree of polymerization greater than two, are intermediate products of xylan depolymerization into xylose monomer. The study of xylose oligomers depolymerization kinetics is critical in describing hemicellulose depolymerization, since the results will help determine the depolymerization processing conditions such that the formation of undesired byproducts are minimized, while the desired xylose monomers are maximized. Quantification of xylose oligomers is important to allow for accurate tracking of the oligomer concentration during various stages of xylose oligomers depolymerization. Common methods of oligomers quantification involve chromatographic quantification based on the calibration curve of individual oligomers, which are limited to the commercial availability of xylose oligomers up to xyloheptaose (DP6) and NREL laboratory analytical procedure of total sugar content analysis (Sluiter et al 2006) which do not account for the loss of xylose into byproducts. Objectives were to perform mass balance closure of xylose during the total sugar hydrolysis and to determine reaction rate of xylose degradation.

Tested material consisted of xylose standard purchased from Sigma Aldrich (St. Louis, MO). Duplicate samples of 10 mL were loaded into 25 mL test tubes with xylose concentration of 2 to 20 mg/mL. To obtain 4% acid concentration, a 228 µL volume of 98% w/w sulfuric acid was added to each sample. Test tubes were closed with caps and placed in an autoclave (Getinge Castle® 122MA Gravity Steam Sterilizer, Getinge USA, Inc, Rochester, NY) at 121°C for 30, 60 and 120 min. After acid hydrolysis, samples were cooled under tap water for 30 min. Recovered sample volumes and pH were measured and divided into two samples. One sample was analyzed directly by HPLC without pH adjustment for furfural. The second sample was neutralized with calcium carbonate to pH 6 to 8 before analyzed by HPLC for xylose concentration. HPLC analysis of furfural was conducted using a Waters 2695 HPLC system (Waters Corporation, Milford, MA) equipped with an Aminex HPX-87H column (55°C) and Micro-Guard cation H (Bio-rad, Hercules, CA) refill cartridges guard column with 0.005 M aqueuous sulfuric acid as eluent at a flow rate of 0.6 mL/min. Detection was obtained with a Waters 2996 Photodiode Array Detector set at 280 nm. HPLC analysis of xylose was conducted using a Waters 2695
HPLC system equipped with a SP0810 column (85°C) and SP-G precolumn (Shodex, Kawasaki, Japan) with water as eluent at a flow rate of 0.2 mL/min, with detection via a Waters 2414 refractive index detector.

Xylose degradation followed first order kinetics, with an average rate constant of 0.0019 mg/mL/min (Table 1). Values reported were 2 to 10 times lower than previously reported (Kumar and Wyman 2008); the lower rate could be attributed to the lower pretreatment temperature (121°C) while previously reported values were determined at 160°C. Furfural does not account completely for degraded xylose for extended hydrolysis time, such as 120 min, as some solid precipitation was observed. For the NREL recommended procedure of 60 min of xylose hydrolysis, 90% of xylose remained after hydrolysis.

Table 1. The percentage of hydrolyzed xylose recovered, mass balance closure and the calculated reaction rate of xylose.

<table>
<thead>
<tr>
<th>Hydrolysis time (min)</th>
<th>Hydrolyzed xylose recovery # (%)</th>
<th>Mass balance closure of xylose (%)</th>
<th>1st order reaction rate constant, k1* (mg/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>94.7 ± 3.9</td>
<td>100.1 ± 4.0</td>
<td>0.0018 ± 0.0014</td>
</tr>
<tr>
<td>60</td>
<td>90.3 ± 2.1</td>
<td>98.8 ± 3.6</td>
<td>0.0017 ± 0.0004</td>
</tr>
<tr>
<td>120</td>
<td>78.8 ± 7.1</td>
<td>92.0 ± 9.1</td>
<td>0.0020 ± 0.0007</td>
</tr>
<tr>
<td>Average</td>
<td>87.9 ± 8.3</td>
<td>96.9 ± 6.9</td>
<td>0.0019 ± 0.0009</td>
</tr>
</tbody>
</table>

* k1 = ln (X0/X) / t, where X0 and X are the initial and final concentration of xylose, respectively, and t is the hydrolysis time.

# According to the NREL calculation method which did account for any dilution to the samples made prior to HPLC analysis, including the addition of sulfuric acid to adjust pH.

LITERATURE CITED


PRODUCTION OF ASTAXANTHIN FROM CELLULOSIC BIOMASS SUGARS BY MUTANTS OF THE YEAST *Phaffia rhodozyma*

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Astaxanthin is a carotenoid of high value to aquaculture, nutraceutical and pharmaceutical industries. Three mutant strains of the astaxanthin producing yeast *Phaffia rhodozyma*, which were derived from the parent strain ATCC 24202 (UCD 67-210) and designated JTM166, JTM185 and SSM19, were tested for their capability of utilizing the major sugars, including glucose, xylose and arabinose, that can be generated from cellulosic biomass for astaxanthin production. While all 3 strains were capable of metabolizing these sugars, individually and in mixtures, JTM 185 had the greatest sugar utilization and astaxanthin production. The kinetics of sugar utilization were studied in fermenters using mixtures of glucose, xylose and arabinose at varied concentrations. Glucose was utilized preferentially, followed by xylose and arabinose. The greatest astaxanthin production per total sugar consumption (0.21 mg/g) was observed when glucose was supplied in low levels relative to xylose and arabinose; although the final astaxanthin concentration (8.3 mg/L) was lower than when glucose was supplied at higher concentrations. Hydrolysates produced from sugarcane bagasse and barley straw pretreated by the soaking in aqueous ammonia (SAA) method and hydrolyzed with the commercial cellulase preparation, Accellerase™ 1000, were used for astaxanthin production by the mutant strain JTM185. The organism was determined to be capable of metabolizing all sugars present in the hydrolysates from both biomass sources, and produced similar amounts of astaxanthin from both hydrolysates, although this amount was lower when compared to yields using synthetic sugars.
A process was developed to fractionate and isolate the hemicellulose B component of corn fiber generated by corn wet milling. The process consisted of pretreatment by soaking in aqueous ammonia (SAA) followed by enzymatic cellulose hydrolysis, during which the hemicellulose B was solubilized by cleavage into xylo-oligosaccharides and recovered by precipitation with ethanol. The pretreatment step resulted in a high retention of major sugars. Glucan digestibility of the pretreated biomass was 85% after 72 hr of hydrolysis with cellulase. Mass balance accounted for 87, 91 and 90% of the initial glucan, xylan and arabinan, respectively. The hemicellulose B was hydrolyzed by a cocktail of enzymes that consisted of β-glucosidase, pectinase, xylanase and ferulic acid esterase (FAE). Xylanase alone was ineffective, with yields of less than 2% of xylose and arabinose. The greatest xylose and arabinose yields, 44 and 53%, respectively, were obtained by the combination of pectinase and FAE. Glucose solutions from hydrolysis of the corn fiber cellulose and starch were used to produce a corn mash, which was fermented to produce ethanol. The result, compared to water mashing, was a 2% (v/v) increase in ethanol titer. The overall fermentation efficiency was increased by 7% when the hydrolysis solutions were used.
SYNGAS FERMENTATION TO BIOFUEL: 
EVALUATION OF HYDROGEN MASS TRANSFER AND 
A CORRELATION BETWEEN MYOGLOBIN PROTEIN 
BIOASSAY AND GAS CHROMATOGRAPHY METHOD FOR 
CARBON MONOXIDE DETERMINATION

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Syngas (a gas mixture of CO and H2) can be converted into biofuel, such as ethanol, by anaerobic microbes. One major drawback of syngas fermentation is the mass transfer limitation of the sparingly soluble gases in the aqueous phase. In this study, volumetric mass transfer coefficients ($k_{La}$) for H2 and a correlation between myoglobin-protein bioassay and the head space gas analysis by gas chromatography (GC) for CO concentration in the aqueous phase were examined in an air lift reactor coupled with a 20 μm bulb diffuser. The mass transfer experiments were conducted at gas flow rates of 1 to 5 Lmin⁻¹. Gas samples were taken from the head space at regular intervals for 140 sec and analyzed by the GC (thermal conductivity detector (TCD)). H2 concentration in aqueous phase was obtained by Henry’s law. The highest $k_{La}$ of 72.36 h⁻¹ was observed at 5 Lmin⁻¹ gas flow, while the minimum was 16.92 h⁻¹ at 1 Lmin⁻¹. Aqueous phase CO concentrations determined using GC-TCD and myoglobin-protein bioassay methods were correlated ($R^2 = 0.963$). We confirmed that the myoglobin-protein method, which is much simpler, relatively faster and cheaper method than the GC analysis, can be used as a reliable method of determining CO concentrations in the aqueous phase. This is the first study in which the correlation between the gas phase CO (GC-TCD) and liquid phase CO (myoglobin-protein bioassay) concentration was developed and verified.
INTEGRATION OF SUCCINIC ACID AND ETHANOL PRODUCTION WITH POTENTIAL APPLICATION IN A CORN OR BARLEY BIOREFINERY

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Production of succinic acid from glucose by *Escherichia coli* strain AFP184 was studied in a batch fermenter. The bases used for pH control included sodium hydroxide (NaOH), potassium hydroxide (KOH), ammonium hydroxide (NH4OH) and sodium carbonate (Na2CO3). The yield of succinic acid with and without carbon dioxide (CO2) supplied by an adjacent ethanol fermenter using either corn or barley as feedstock was examined. The CO2 gas from the ethanol fermenter was sparged directly into the liquid media in the succinic acid fermenter without any pretreatment.

Without CO2 supplement, the highest succinic acid yield was observed with Na2CO3, followed by NH4OH, and lowest with the other two bases. When CO2 produced during ethanol fermentation was sparged into the media in the succinic acid fermenter, no improvement of succinic acid yield was observed with Na2CO3. However, several fold increases in succinic acid yield were observed with the other bases, with NH4OH resulting in the highest yield increase. The yield of succinic acid with CO2 supplement from the ethanol fermenter when NH4OH was used for pH control was equal to that obtained when Na2CO3 was used, with or without CO2 supplementation. The benefit of sparging CO2 from ethanol fermentation on the yield of succinic acid demonstrated the feasibility of integration of succinic acid fermentation with ethanol fermentation in a biorefinery for production of fuels and industrial chemicals.
STRESS HARDENED BACTERIAL CELLS FOR BIOTECHNOLOGY: EXPLOITING EVOLUTIONARY MECHANISM FOR ROBUST BIOPROCESSING TECHNOLOGIES

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In nature, nutrient limitation and natural selection force bacterial cells to continually push their limits of efficient metabolic activity to survive in a stressful and competitive environment. Beneficial genetic alterations due to various external stresses shape the course of evolution giving rise to robust bacterial ecosystems that can survive in the face of a dynamic, unpredictable environment. The focus of our research program is to incorporate these beneficial genetic changes into industrially relevant microorganisms to build a level of robustness into bioprocesses comparable to that seen in nature. Escherichia coli K-12 cells subject to nutritional stresses modify their metabolic activities to consume available nutrients present and thus proliferate.

Cell cultures exposed to previous periods of nutritional stresses tend to prevail over naïve cells coming from the same genotype. This phenomenon is known as the growth advantage in stationary phase (GASP) and is thought to be a result of rpoS mutations which have occurred in the stress hardened cells due to the nutritional stress it has seen. The nutrition starved cells scavenge and recycle amino acids from proteins, carbohydrates and remains of the other cells. These mutations give rise to a phenotype which expresses sufficient amounts of gene products thereby increasing the fitness of the cell during its stationary phase. The ability of the rpoS mutant cell to catabolize amino acids gives it an edge over other mutant cells. This mutation can be harnessed to create stronger, contamination resistant cell lines which can produce products such as protein based pharmaceuticals or industrial enzymes with fewer input costs.
LITERATURE CITED


INNOVATIVE BIOREFINERY CONCEPT FOR SUGAR BASED ETHANOL INDUSTRIES: PRODUCTION OF PROTEIN RICH FUNGAL BIOMASS ON VINASSE AS AN AQUACULTURE FOOD INGREDIENT

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A biorefinery approach is important for a long term sustainability of biofuel industries. These industries produce significant amounts of low value residues. Value added processing of residues provides an opportunity of additional revenue generation. Sugarcane is a major feedstock for ethanol production in Brazil and potentially in Hawaii. However, sugarcane to ethanol plants generate low value liquid stream, vinasse, a leftover after ethanol recovery. Vinasse has high organic content and other nutrients and poses a serious disposal threat to an environment. We examined the potential of producing protein rich edible fungus, *Rhizopus microspores*, var. *oligosporus*, on vinasse. Prolific fungal growth was optimized as pellets at pH 5.0 and 30°C on sterile vinasse with nutrient (nitrogen and phosphorus) supplementation.

The highest specific fungal biomass yield of 0.21 (g biomass increase/g initial biomass · g soluble chemical oxygen demand (SCOD) removed) was observed with biomass yield of 4.28 g biomass increase/g initial biomass. Organic and inorganic matters were reduced by 42.0% SCOD, 24.1% nitrogen (as total Kjeldahl nitrogen (TKN)), 25.8% phosphorus (as PO$_4^{3-}$), 34.3% potassium (K) and 24.4% total dissolved solids. Treated effluent could be recycled as a process water or land applied. Fungal biomass had a crude protein content of 45.6% with high amounts of arginine and threonine.

Although methionine and tryptophan contents were low, fungal biomass could be cofed with commercial protein sources, eg, soybean meal, to maintain the amino acid requirement for aquaculture foods. Application of fungal technology not only provides the opportunity for producing the food grade fungal biomass as a protein source for animals but also provides an opportunity for water reclamation. We envision the process could be used to produce high grade fungal protein, which may be utilized as a dietary supplement for human beings.
PRODUCTION OF ETHANOL
FROM KANLOW SWITCHGRASS BY THERMOTOLERANT
Kluyveromyces marxianus IMB3 USING SIMULTANEOUS
SACCHARIFICATION AND FERMENTATION

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A simultaneous saccharification and fermentation (SSF) of pretreated Kanlow
switchgrass was performed using a thermotolerant yeast Kluyveromyces marxianus IMB3. Pretreatment to improve the accessibility of cellulose to hydrolytic enzymes was done using hydrothermolysis at 200°C for 10 min. Pretreated solids had a glucan content of 58.2%. The SSF process was conducted at three temperatures, 37, 41 and 45°C, in triplicate and agitated for 7 days at 130 RPM. The initial pH was 5.5 and a 50 mM citrate buffer was used to minimize pH decline. Pretreated switchgrass solids containing 4 g of glucan were added to each 250 mL baffled flasks along with 10 mL of yeast peptone medium (YP) and 5 mL of 1M citrate buffer. Accelerase\(^\text{®} 1500\) cellulase enzyme was used at a loading of 0.25 ml/g glucan (9 FPU/g glucan) for hydrolysis of the solid substrate. In addition, an experiment was conducted with three loadings of Accelerase\(^\text{®} 1500\) (0.1, 0.3 and 0.5 ml/g glucan) and a temperature of 45°C. Ethanol and sugar concentrations were determined to compare ethanol titer, productivity and yield.

The highest ethanol concentration was 15.2 g/L, corresponding to 67% of the theoretical yield, in flasks maintained at 45°C after 7 days. There was no difference between 41 and 45°C. A loading of 0.5 ml enzyme/g glucan resulted in the highest ethanol yield (61% of theoretical, 14.2 g/L) in the enzyme loading study. However, ethanol yields observed using Accelerase\(^\text{®} 1500\) were not as high as those observed using Fibrilase cellulase at a loading of 0.25 ml/g glucan under similar conditions (Faga et al 2010). Costs of these two enzymes are not known by the authors and could not be compared on a constant cost basis.
LITERATURE CITED

LIFE CYCLE ANALYSIS OF CELLULOSIC ETHANOL FROM WHEAT STRAW IN THE US PACIFIC NORTHWEST

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Wheat straw is a nonfood, cellulosic biomass resource which is plentiful in the Pacific Northwest (Graf and Koehler, 2000). Cellulosic ethanol process models for producing ethanol from wheat straw using acid hydrolysis and steam explosion pretreatment processes were developed using SuperPro Designer software. Life cycle analysis for Pacific Northwest wheat production was conducted using the GREET modeling software. Life cycle analyses were compared to lifecycle impacts of conventional gasoline, corn ethanol and generic herbaceous biomass.

This well to wheel life cycle analysis of cellulosic ethanol production showed reductions in fossil fuel use compared to conventional gasoline and corn ethanol. These fossil fuel reductions lead to reductions in CO2 emissions. These results, based on process models utilizing Pacific Northwest wheat straw as a feedstock, were based on current state of the art production technologies and process efficiencies and greenhouse gas reductions will only improve with time.

Both acid hydrolysis and steam explosion pretreatment process models resulted in: 61% less fossil energy used to move a vehicle one mile compared to gasoline, 46% less fossil energy used to move a vehicle one mile compared to corn ethanol, 70% reduction in CO2 emissions per mile compared to gasoline and 55% reduction in CO2 emissions per mile compared to corn ethanol.

A sensitivity analysis was conducted to determine the effect of various input parameters on production costs. Ethanol production cost was sensitive to enzyme usage and cost. In both
scenarios, doubling incentives had the greatest impact in lowering the cost per gallon of ethanol. The unit cost of each parameter was multiplied independently by 0.5 and 2 to yield the resulting sensitivity of the system to that parameter. Doubling the straw cost results in a $0.50 increase in the final cost of ethanol while cutting the cost in half reduced the cost $0.30. Similarly, doubling the incentives drove the cost down over $0.50 while cutting the incentives in half raised the price $0.30.

The combined influences of prime market conditions and government support can propel this emerging technology. Such life cycle analysis can be used to drive energy policy in an environmentally sustainable direction.

LITERATURE CITED

COPRODUCTS FROM CORN PROCESSING:
ENERGY, NUTRIENTS AND WATER

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Coproducts, including fuel ethanol and a range of biobased products, are produced in parallel with a primary product during processing. One fourth to one third of material exiting modern corn processes does not increase in value relative to original corn value (Rausch and Belyea 2006). In addition to market pressures, coproduct value is correlated to two major factors: 1) unpredictable variation in characteristics (Belyea et al 2010) and 2) suitability for the end user. Animal producers require consistent dietary ingredients with known characteristics. However, most coproducts are a result of processing to obtain a primary product, rather than specific animal or human nutritional needs. Because coproducts are an afterthought in bioprocessing design, nutrients are not recovered using methods that result in coproducts ideally designed for ruminant, nonruminant or human foodstuffs.

Research is needed to identify nutritional value of components available in corn process streams and develop new processes that would isolate these nutrients (Rausch et al 2005, 2007). As processing of biological materials to produce food, fuel and industrial products is increased, coproduct value will become more critical, along with water and energy use during processing. There are clear linkages between coproduct value and efficient water and energy use in corn processing (Rausch and Belyea 2006). Unfortunately, coproducts that have low market value require large amounts of capital, energy and water removal. Process research can generate designs that improve coproduct value while reducing energy and water demand. For example, the enzymatic dry grind process creates a germ coproduct with oil content similar to germ from wet milling and a DDGS coproduct with reduced fiber and increased protein contents (Johnston et al 2005, Singh et al 2005). Membrane filtration of process streams in wet milling (Rausch 2002, Thompson et al 2006) and dry grind (Arora et al 2009, 2010b) can generate water for recycle and reduce evaporator fouling (Arora et al 2010a, Wilkins et al 2006).
LITERATURE CITED


WET PROCESSING OF A TROPICAL GRASS, Pennisetum purpureum, FOR ENHANCED SUGAR RELEASE AND COPRODUCT GENERATION

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Pennisetum purpureum, commonly known as banagrass, is a perennial tropical grass that has been naturalized in Hawaii and resembles the former staple crop of the state, sugarcane. Due to its high moisture content, banagrass presents an opportunity for additional high value product generation (alongside biofuel) via wet processing prior to chemical pretreatment of the lignocellulosic biomass. Banagrass, approximately 4 mo old, was harvested and shredded with a Vincent Corporation shredder. Half of the sample was dewatered using a screw press (40 psi backpressure), whereby the extracted juice was used as a substrate for cultivating an edible fungus, Rhizopus microsporus, as a source of protein for fish food supplementation. The shredded biomass was divided into four separate streams: 1) wet, juiced, 2) dry, juiced, 3) wet, unjuiced and 4) dry, unjuiced. Juiced samples referred to banagrass extruded from the screw press; unjuiced samples referred to banagrass collected after shredding. Dried samples were stored at 105°C, while wet samples were stored at 4°C following shredding or dewatering. The four streams were pretreated with dilute sulfuric acid and compared on the basis of sugar release at acid concentrations of 1.0, 2.5 and 5.0%, temperatures of 105, 120 and 135°C, and residence time of 30, 45 and 60 min.

Wet, juiced banagrass released the most soluble sugar (229 mg xylose/g dry banagrass and 142 mg glucose/g dry banagrass) compared to the other streams. An examination into further enhancing sugar release was conducted by applying high power ultrasonication at varying times (10, 20 and 30 sec) and with different boosters of 0.6:1, 1:1 and 2:1 gains. Using a Duncan’s statistical analysis, we found no improvement of sugar release following enzyme hydrolysis. Fungal biomass generated from the collected liquid fraction (juice) of banagrass has promise for utilizing the juice as a fungal protein production substrate.
EFFECT OF CARBON MONOXIDE AND HYDROGEN CONCENTRATION ON HYDROGENASE ACTIVITY AND ETHANOL PRODUCTION IN SYNGAS FERMENTATION BY Clostridia P11

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Thermochemical conversion of biomass to energetic gases and biological conversion of these gases to ethanol is one of the promising technologies for biofuel technology. With the advancement of gasification technology, more specifically the ability of gases to be shifted to specific compositions through chemical reactions prior to fermentation, it becomes important to evaluate how the composition of these gases affects the production of ethanol and ability of these microorganisms to utilize the provided gas mix. Increasing overall efficiency of this biofuel platform involves the optimization of the organism’s use of all energetic gases available, such as carbon monoxide (CO) and hydrogen (H2). H2 utilization can be evaluated by measuring the activity of hydrogenase enzymes present in the microorganism.

We focused on the bacterium Clostridium strain P11 and the effect of three concentrations of CO (20, 30 and 40%) and three concentrations of H2 (10, 20 and 30%) in the gaseous substrate on ethanol production and hydrogenase activity. Hydrogenase activity appeared to be least inhibited by the lowest concentrations of H2 and CO. The highest levels of hydrogenase activity appeared early in the growth phase of the microorganism prior to solventogenesis. At lower concentrations of CO and H2, the activity of the enzyme appeared to recover slightly through the solventogenesis stage. At the lowest level of CO (20%) the maximum ethanol concentration (0.8 g/L for 30% H2) was reached 24 hr later in the fermentation than for higher CO concentrations (30 and 40%) reached maximum ethanol concentration (0.9 g/L for 30% H2). Both depletion of acetic acid and increase in ethanol concentration were seen in only the trial at the lowest concentration of H2 and CO. The highest concentration of ethanol was seen in this bottle (1.4 g/L) after 10 days. No ethanol production was observed when hydrogenase activity was not present.
PROTEASE USE IN A DRY FRACTIONATED CORN ETHANOL PROCESS

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Corn kernel fractionation separates endosperm from germ and pericarp. It improves the economics of corn to ethanol processing by increasing fermentation throughput and generating sellable coproducts (Singh et al 2005). One such fractionation technology, dry fractionation (DF), suffers from loss of germ derived nutrients and amino acids, resulting in poor fermentation kinetics (Murthy et al 2006). In the brewing and fuel ethanol industry, such deficiencies may be addressed by increasing inorganic nitrogen and other nutritional supplements.

We investigated the addition of a commercial protease as an alternative to exogenous nitrogen supplementation. Although proteases have the ability to break down the endosperm protein matrix, we observed that protease pretreatment did not alter the effectiveness of starch degrading enzymes during liquefaction and saccharification. Protease generated free amino nitrogen (FAN) resulted in fermentation being 99% complete in 48 hr, compared to 93% with urea supplementation. Total nitrogen as FAN required to complete fermentation was three times lower than it was for total nitrogen as urea. Cell biomass yields were similar in both FAN and urea supplemented fermentations. Addition of urea to protease generated FAN resulted in similar fermentation performance as with FAN alone, which indicated FAN was assimilated preferentially. A small decline in final ethanol yield (0.3% v/v) at high protease loading (high FAN levels) was attributed partly to lower utilization of maltose. Using model fermentations, we confirmed the deficiency of maltose uptake with excess FAN. In contrast to conventional dry grind process employing separate high temperature liquefaction, a granular starch hydrolyzing enzyme process did not result in reduced ethanol yields, including at excess FAN concentrations.
LITERATURE CITED


EFFECT OF AMMONIA PRETREATMENT ON SWITCHGRASS FOR PRODUCTION OF CELLULASE USING *T. reesei* Rut C-30

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Switchgrass was milled to 3 particle sizes (0.5, 1, and 5 mm avg), pretreated with 15% ammonia hydroxide and compared to nonpretreated switchgrass as control. Shaker flask studies were conducted using *Trichoderma reesei* Rut C-30 for production of cellulases for the selection of best particle size potential. Scaleup tests were conducted using 2, 5 and 10% switchgrass loadings in a 7 L NBS fermenter. Corresponding enzyme activity was analyzed using HPLC and DNS reducing sugar methods. Pretreated samples yielded nearly twice the enzyme activity for all particle sizes at about 7 FPU/ml after 150 hr. Scaleup to 7 L fermenter showed improved results of 11 FPU/ml after 150 hr fermentation using a 5% loading of pretreated switchgrass.

LITERATURE CITED


Biomass Gasification Tar Removal and Syngas Conditioning Using Novel Catalysts

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Gasification is a promising technology for using biomass materials to produce fuels and chemicals. Syngas produced from biomass gasification can be burned in furnaces, boilers, internal combustion engines and microturbines or further converted to liquid transportation fuels and chemicals. However, unwanted impurities, especially condensable tars, are produced along with syngas in the process. Tars need to be removed to avoid damaging and clogging downstream pipes or equipment. They also may deactivate catalysts in the refining process. Several approaches for tar removal, such as physical treatment, thermal cracking, plasma assisted cracking and catalytic reforming, have been reported in the literature. Among these, catalytic reforming is considered the most promising in large scale applications because of its fast reaction rate and reliability and its ability to increase the quantity of useable gases in syngas.

For catalytic reactivity and economic reasons, Ni based catalysts are considered the most promising for tar removal and syngas reforming. Nickel catalysts usually are supported by metal oxides (eg, Al₂O₃ and MgO) or natural materials (eg, dolomite, olivine, activated charcoal and brown coal char). Some of these supports are expensive and the catalyst preparation steps involving impregnation and calcination are time and energy consuming; these factors limit extensive application of Ni based catalysts. As a promising alternative, chars have been reported to be an inexpensive catalyst with fair performance in tar removal and also an excellent adsorbent; however, more severe reaction conditions, such as higher reaction temperature (>850°C), are necessary due to its lower catalytic performance. In this pioneering research, they investigated catalytic performance of a new catalyst called Ni/char, which was made by mechanically mixing char particles and NiO powders. This catalyst is unique in that char serves as an inexpensive support and also a catalyst and that no impregnation or calcination steps are needed in catalyst preparation. The new char/Ni, char/nano-Ni and HTC-char/Ni catalysts were effective to remove more than 99% tars in biomass gasification syngas and increased the concentration of H₂ and CO (Wang et al 2010a, 2010b, 2010c).
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CATALYTIC BIOMASS CONVERSION
AND BIO-OIL REFINING

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Pyrolysis is an efficient thermochemical conversion process for the production of liquid
fuels from a wide variety of solid biomass feedstocks. However, biomass pyrolysis is fraught
with many technical challenges that limit its commercial use. For example, liquid fuels produced
from the fast pyrolysis of biomass are complex in their chemical composition and unstable in
terms of physical composition, properties and combustion characteristics thereby limiting
practical use. To date, researchers have sought solutions to improve the physical and chemical
characteristics of these pyrolytic liquid biofuels with limited success. Our novel strategy is to
overcome the major hurdles facing the commercial development of pyrolysis derived biofuels
and differs markedly from other approaches by use of both a novel microwave assisted catalytic
pyrolysis process and low pressure catalytic upgrading process.

Microwave assisted pyrolysis (MAP) is a robust process and offers many technical
advantages over conventional pyrolysis. MAP does not require a high degree of feedstock
grinding and can handle mixed feedstock, and its conversion products are cleaner (less char
fines) than those from gasification and conventional pyrolysis because MAP does not use
biomass powder and does not require agitation and fluidization. We focused on the use of
catalysts in the conversion steps to improve yield and quality of conversion products and in
postconversion upgrading steps. One objective is to control bio-oil chemical profiles through
catalytic pyrolysis (also termed in situ upgrading). Certain catalysts promoted formation of some
compounds while suppressing others, resulting in a narrow chemical profile of the bio-oil

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produced. For the postconversion upgrading, we developed a continuous flow, fixed bed reactor operated at pressurized or ambient for catalytic refining of bio-oil. During development, nonedible oils were used in the study of activity, selectivity performance and characteristics of catalysts and the device. Liquid fuels with chemical profiles similar to gasoline were obtained. Testing of waste oils, mixtures of nonedible oil and bio-oil, and bio-oil is underway.
PHENOLIC ACIDS, LIPIDS AND PROTEINS
IN CORN FIBER GUM

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An arabinoxylan (hemicellulose B), termed corn fiber gum (CFG), is obtained by the alkaline extraction of corn kernel pericarp and/or endosperm fractions of corn fiber (Yadav et al 2010). Two classes of phytochemicals, hydroxycinnamic acids (p-coumaric and ferulic) and lipids were released, when CFG was hydrolyzed with 1.5N methanolic KOH at 70°C for 1 hr (Moreau and Hicks 2004). The released phenolic acids and lipids were isolated, identified and quantified using HPLC with detection by both UV and a highly sensitive evaporative light scattering detector (ELSD) (Moreau et al 1996).

During the wet milling of corn, two types of corn fiber are produced, coarse fiber which is primarily pericarp, and fine fiber which is from the endosperm. The total phenolic acid content in CFGs from coarse corn fiber (CCF) or pericarp fiber is comparatively higher than that in fine corn fiber (FCF) or endosperm fiber. Two different types of CFG, type-1 and type-2, were prepared from corn fiber (Yadav et al 2010). The CFG-2 from coarse corn fiber was richer in both lipid and protein content than the corresponding CFG-1 from the same source. The amount of both total lipid and protein content in CFG-2 from fine corn fiber was only slightly higher than the corresponding CFG-1 isolated from the same fiber source. The presence of these phenolic acids, lipids and protein in CFG may contribute to its excellent emulsifying properties and may combine to give other chemical, physical and even nutritional properties.
LITERATURE CITED

