Southern Pine Based Biorefinery Center

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Project Overview

Technical hurdle to be overcome?

- Optimal material science for pyrolysis and pretreatment reactors
- Viable generation of green/biodiesel
- Optimized bioethanol generation integrated with pulp/paper/modified cellulosics pathways
- Integration into a modern SW kraft pulp mill operation
Quad Chart Overview

**Timeline**

- Project start date
  - Oct. 1, 2010

- Project end date
  - Sept. 30, 2012

- Percent complete: 12%

**Project Development**

- Research studies have been initiated and on track
- Project Scope is following program deliverables
- Completion of deliverables projected for Sept/30/2012.

**Project Participants**

Co-PI: Dr. P. Singh, MSE/GT
Industrial kick-off meeting

- Project managed by deliverables
- Initial testing facilities been ordered
- Exploratory research studies on pine conversion technologies initiated
Approach

- Develop on the GA Tech campus an integrated southern pine wood to biofuels/biomaterials processing facility that will test advanced integrated wood processing exploratory technologies at the bench scale.

**Exploratory research program is structured to evaluate key chemical/enzymatic conversion processes needed to convert southern pine to biofuels and novel biomaterials in a pulp mill.**

Research program

**Focus on Process Chemistry of Conversion & Material Science of Conversion Reactors**
Technical Accomplishments/Progress/Results

Tasks Completed

- Task A: Collect GA pine woodchips, residues and bark
  - Feedstock for laboratory studies for next duration of two year project

- Task A.1: Determine biomass constituents

<table>
<thead>
<tr>
<th>% Component</th>
<th>Pine wood chips</th>
<th>Pine wood bark</th>
<th>Pine wood residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holocellulose</td>
<td>68.5</td>
<td>50.9</td>
<td>65.9</td>
</tr>
<tr>
<td>α-cellulose</td>
<td>45.4</td>
<td>29.3</td>
<td>42.1</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>12.2</td>
<td>13.7</td>
<td>12.5</td>
</tr>
<tr>
<td>Lignin</td>
<td>28.0</td>
<td>46.3</td>
<td>30.4</td>
</tr>
<tr>
<td>Acid insoluble lignin</td>
<td>27.3</td>
<td>45.5</td>
<td>29.4</td>
</tr>
<tr>
<td>Acid soluble lignin</td>
<td>0.69</td>
<td>0.81</td>
<td>1.02</td>
</tr>
<tr>
<td>Ash</td>
<td>0.27</td>
<td>0.91</td>
<td>0.81</td>
</tr>
<tr>
<td>Extractives in DCM</td>
<td>2.5</td>
<td>3.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Tannin</td>
<td>–</td>
<td>11.6</td>
<td>–</td>
</tr>
</tbody>
</table>

-Basics constituents info needed for all studies
Technical Accomplishments/Progress/Results

Task A.2: Structural analysis of pine - ongoing

Cellulose

Crystallinity & DP – key parameter for enzymatic deconstruction of cellulose for bioethanol

Lignin

Data needed for upgrading Lignin studies for Green Diesel/biodiesel
Technical Accomplishments/ Progress/Results

Task A.2: Structural analysis of pine - ongoing

Tannins

Structural components may contribute to corrosion & inhibit biofuel production by biological route
Technical Accomplishments/Progress/Results

Task C.2: Microbial upgrading lignin and pyrolysis oil from pine residue - ongoing

Single cell oils produced by heterotrophic microorganisms

Oleaginous species under growth restricting conditions (high C:N ratio) are capable to produce large quantities of TAG.

Using agricultural wastes to grow two high yielding strains *Rhodococcus opacus* PD630 and *Gordonia sp* DG, the latter can produce TAGs up to 14.45 mg L\(^{-1}\) d\(^{-1}\) on orange waste while earlier converted carob waste with 59.27 mg L\(^{-1}\) d\(^{-1}\) productivity.
Technical Accomplishments/Progress/Results

Task C.2: Microbial upgrading lignin and pyrolysis oil from pine residue - ongoing

Microorganisms Under Study
1. Rhodococcus opacus DSM 1069 (DSM 1069)
2. Rhodococcus opacus PD 630 (PD 630)

R. opacus DSM 1069 growth on different substrates as measured by optical density (OD) at 600 nm.

\( p \)-hydroxy benzoate Na\(^+\) salt (PHA); 4-hydroxy-3-methoxy benzoate (vanillic acid) (VanA)
Ultrasonicated lignin (UE)
Technical Accomplishments/Progress/Results
Task C.2: Microbial upgrading lignin and pyrolysis oil from pine residue - ongoing

Microorganisms Under Study
1. Rhodococcus opacus DSM 1069 (DSM 1069)
2. Rhodococcus opacus PD 630 (PD 630)

Both strains are growing on lignin like structures
Need determine TAG production, my require reduction in lignin DP
Technical Accomplishments/Progress/Results
Task C.GN.2 Pine residue and bark pyrolysis - ongoing

Pine residue pyrolyzed at 550 °C with 0.5 L/min nitrogen flow into the tube
- Pyrolysis oil 45%
- Char 40%
- Non-condensable: 15%
Structure characterization ongoing

Examining role of lignin in pyrolysis

Lignin yields a light/heavy oil
Preferred temp 550-600 °C
Technical Accomplishments/Progress/Results

Task C.GN.2 Pine residue and bark pyrolysis - ongoing

Light oil is primarily water
Change in pyrolysis temp increases catechols & reduces aliphatic hydroxyls
Technical Accomplishments/Progress/Results

Task C.GN.2 Pine residue and bark pyrolysis - ongoing

Ongoing Studies:
- Improved reactor design
- Catalysis pyrolysis reactions
Technical Accomplishments
Progress/Results

Task D.2: Cellulosic derivatives – ongoing

Objective: Develop new high-valued cellulosic co-products based on cellulosic whiskers
Technical Accomplishments
Progress/Results

Task D.2: Cellulosic derivatives – ongoing
Objective: Develop new high-valued cellulosic co-products based on cellulosic whiskers

AFM image of 0.5 wt% suspensions (a) whisker (b) DAC (c) DAC-MA (d) DAC-BA

- Testing physical properties
- Composite applications
Relevance

Fossil Energy Ratio (FER) = Energy Delivered to Customer/Energy Used

- Extract Hemicelluloses
- Wood Residues

2-5 billion gallons Ethanol

- Pretreatment fermentation
- Wood Pyrolysis
- Microbial Fermentation
- Process to manufacture Liquid Fuels and Materials

Pyrolysis/Microbial Fermentation
100 million barrels

Black Liquor & Residuals

Steam, Power & Chemicals

Manufacturing

→ Pulp
SW 55 million tons

The *Forest* Biorefinery – Production

[Graphs and data visualizations related to energy and biorefinery processes]
The BioEnergy Science Center
Brief Overview
BioEnergy Science Center

**BESC**: A multi-institutional DOE-funded center dedicated to understanding and modifying plant biomass recalcitrance

- Samuel Roberts Noble Foundation
- National Renewable Energy Laboratory
- Brookhaven National Laboratory
- University of California–Riverside
- Cornell University
- Washington State University
- University of Minnesota
- North Carolina State University
- Virginia Polytechnic Institute
- University of California–Los Angeles
- Oak Ridge National Laboratory
- University of Georgia
- University of Tennessee
- Dartmouth College
- Georgia Institute of Technology
- West Virginia University
- ArborGen, LLC
- Ceres, Incorporated
- Mascoma Corporation
- Verenium Corporation

322 people in 20 institutions
Access to the sugars in lignocellulosic biomass is the current critical barrier

- Overcoming this barrier will cut processing costs significantly and be used in most conversion processes

<table>
<thead>
<tr>
<th>Time frame</th>
<th>Planned</th>
<th>Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified plants to field trials</td>
<td>Year 5</td>
<td>Year 4</td>
</tr>
<tr>
<td>New or improved microbes to development</td>
<td>Year 4–5</td>
<td>Year 3–4</td>
</tr>
<tr>
<td>Analysis and screening technologies</td>
<td>Year 3 on</td>
<td>Year 2 on</td>
</tr>
</tbody>
</table>
A Two-pronged Approach to Increase the Accessibility of Biomass Sugars

Modify the plant cell wall structure to increase accessibility

Improve combined microbial approaches that release sugars and ferment into fuels

Both utilize rapid screening for relevant traits followed by detailed analysis of selected samples
Comparative impacts of R&D on biomass processing cost

<table>
<thead>
<tr>
<th>A1</th>
<th>Increase hydrolysis yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>Halve cellulase loading</td>
</tr>
<tr>
<td>A3</td>
<td>Eliminate pretreatment</td>
</tr>
<tr>
<td>A4</td>
<td>Consolidate bioprocessing</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B1</th>
<th>Simultaneous C5 and C6 use</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2</td>
<td>Increased fermentation yield</td>
</tr>
<tr>
<td>B3</td>
<td>Increased ethanol titer</td>
</tr>
</tbody>
</table>

A: Conversion of biomass into available sugars
B: Conversion of sugars into biofuels

Without overcoming biomass recalcitrance (A), cellulosic biofuels will be more expensive than corn biofuels. Improved sugar conversion (B) is not enough.

BESC Will Revolutionize How Biomass is Processed

Baseline, Multi-step Cellulosic Ethanol Production

Native Plants → Pretreated Biomass → Solid/Liquid Separation → Cellulose Enzymes → Enzyme Hydrolysis → Hexose Fermentation → Biofuel

Pentose Sugars → Pentose Fermentation → Biofuel

Biomass Modification

Modified Plants

Consolidated Bioprocessing

Reduced or No Pretreatment → Biomass

No Separation → CBP Microbes → Biofuel
**Targeted cell wall synthesis approach:**

- Test known putative recalcitrance genes in via *Populus* and switchgrass transgenics (TP)
- Basic research to identify unknown genes and decipher how they effect recalcitrance

**Discovery-based natural variation approach:**

- Identify natural variation in recalcitrance
- Identify gene responsible
- Test via *Populus* and switchgrass transgenics (TP)
- Activation tagging
HTP Characterization Pipeline for the Recalcitrance Phenotype

• Screening of 1000’s of samples

Composition analytical pyrolysis, IR, confirmed by wet chemistry → Pre-treatment new method with dilute acid and steam → Enzyme digestibility sugar release with enzyme cocktail

Detailed chemical and structural analyses of specific samples
Mining Variation to Identify Key Genes in Biomass Composition and Sugar Release

Collected ~1300 samples for *Populus* Association and Activation-tag Study

**HTS Pipeline**

- Sugar Release Assay
- Analytical Pyrolysis

Create Genetic Marker Map to identify allelic variation

Identify Marker Trait Association

Establish common gardens for association and activation tag populations with 1000s of plants
Association Study – Composition Data

- The association samples display extreme variation in lignin, S/G ratio, and sugar content
- Extreme phenotypes have been characterized for detailed pretreatment and sugar release assays
- All sampled genotypes are being replicated and will be established in a common garden experiment
**Populus Association Study**

- Tested for enhanced sugar release characteristics through pretreatment and enzymatic hydrolysis
  - Hot water pretreatments at 160 and 180°C
- HTP pretreatment and co-hydrolysis in 96 well-plates
- Preliminary observations:
  - Sugar yield increases with S/G ratio
  - Lignin content has minimal effect
  - Some outlier poplar samples exhibit very high sugar release
- Characterization pipeline works

**Lignin content [%]**

**Pretreatment conditions:**
- 180°C, 18Min
- 160°C, 68Min
- Standard BESC poplar
- Theoretical sugar yield

*Studer, Wyman et al.*
Establishment of a transformation protocol for switchgrass
Genetic block in lignin biosynthesis in switchgrass increases biofuel yields

Phenylalanine → PAL

Agrobacterium-mediated transformation of switchgrass

C4H → 4-coumaric acid

4CL → 4-coumaroyl CoA

CCR → 4-coumaraldehyde

CAD → 4-coumaroyl alcohol

HCT → 4-coumaroyl shikimic acid or quinic acid

C3H → caffeoyl shikimic acid or quinic acid

HCT → caffeoyl CoA

CCoAOMT → feruloyl CoA

CCR → coniferyl alcohol

CAD → 5-hydroxyconiferyl alcohol

F5H → 5-hydroxyconiferaldehyde

COMT → sinapaldehyde

5-hydroxyconiferaldehyde → sinapaldehyde

COMT → sinapyl alcohol

3-hydroxyconiferaldehyde → sinapaldehyde

CCoAOMT → feruloyl CoA

CCR → coniferyl alcohol

Cad → 5-hydroxyconiferyl alcohol

F5H → 5-hydroxyconiferaldehyde

COMT → sinapaldehyde

5-hydroxyconiferaldehyde → sinapaldehyde

The Samuel Roberts Noble Foundation

Understanding the Structure/Function of the Cellulosome - CbhA is a Critical Enzyme

C. thermocellum CbhA

New structures solved at NREL

Assembled 7 domain enzyme
• Ready for computer simulations
New tools from “Systems Biology” offer confidence for conversion success

- Pretreated Switchgrass
- Cellobiose
- Amorphous Cellulose
- Avicel - ¹⁴N
- Avicel - ¹⁵N
- Avicel-Pectin
- Avicel-Xylan
- Avicel-Pectin-Xylan

- *C. thermocellum* alters its cellulosome catalytic composition depending upon the growth biomass substrate
- We identified and experimentally verified 16 “new” cellulosome components

BESC Will Revolutionize How Biomass is Processed and Converted

Biomass Modification

Consolidated Bioprocessing

Modified Plants

COMT Switchgrass mutant (25% more Ethanol and therefore accessible sugar)

Reduced or No Pretreatment

Biomass

No Separation

CBP Microbes

Next Generation Biofuel

Butanol, Isobutanol, Hydrocarbons

Synergy

Advanced New Process
Industrial partners facilitate strategic commercialization

BOD: Commercial Representatives

PiperJaffray

CBP

Enzymes

MASCOMA

Verenium

Switchgrass

Populus

Ceres

Commercial Affiliates

Battelle Ventures

Mendel

Thank You!