Cellulosic Biofuels: Do We Have to? Can We?

Presented to:
The Second Annual TIMBR Conference on Cellulosic Biofuels

Presented by:
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April 30, 2010
Oak Ridge National Laboratory evolved from the Manhattan Project

ORNL in 1943
The Clinton Pile was the world’s first continuously operated nuclear reactor
Today, ORNL is DOE’s largest science and energy laboratory

- $1.65B budget
- 4,500 employees
- 4,000 research guests annually
- $500 million invested in modernization

- Nation’s largest concentration of open source materials research
- World’s most intense pulsed neutron source and a world-class research reactor

- World’s most powerful open scientific computing facility
- Nation’s most diverse energy portfolio
- Managing the billion-dollar U.S. ITER project
Delivering science and technology: We lead major R&D programs for DOE and other customers

Energy technologies
- Materials at the nanoscale

Ultrascale computing
- Neutron sciences

Climate
- Nuclear energy

Bioenergy
- National security
Warming of the climate system is unequivocal

- Global atmospheric concentrations of greenhouse gases have increased markedly as a result of human activities since 1750
- Hot extremes, heat waves, and heavy precipitation events will continue to become more frequent
- Global temperature and sea level will continue to rise for at least a millennium

“The costs of stabilizing the climate are significant but manageable”
Where was the Carbon going in 2006? (Canadell et al., PNAS 2007)

800 GtC or 380 ppm

<table>
<thead>
<tr>
<th>Source</th>
<th>Change</th>
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<tbody>
<tr>
<td>Fossil Fuel &amp; Cement</td>
<td>+7.6 Gt C/year</td>
</tr>
<tr>
<td>Land Use Change</td>
<td>+1.5 Gt C/year</td>
</tr>
<tr>
<td>Ocean Uptake</td>
<td>-2.2 Gt C/year</td>
</tr>
<tr>
<td>Terrestrial Uptake</td>
<td>-2.8 Gt C/year</td>
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+4.1 GtC/yr or +1.9 ppm/year
If we fail to act, climate could have potentially devastating effects

Days above 100°F

Recent Past, 1961-1979

Higher Emissions Scenario, 2080-2099

Much of the U.S. would go from 0 - 10 days above 100°F to 45 to 70 days per year above 100°F

Source: NOAA U.S. Global Change Research Program
We focus on accomplishments in four Thrust Areas

Earth System Modeling
- High resolution
- Decadal-scale projections
- Uncertainty quantification
- Improved process representation

Data Integration, Dissemination & Informatics
- Adaptable and accessible information systems to integrate diverse data from observations and model results (gridded, point, images, etc.)

Terrestrial Ecosystem and Carbon Cycle Science
- Remotely-sensed data
- Biogeochemical cycles: (e.g., C, N, P, H₂O)
- Greenhouse gas emissions from all sources

Impacts, Adaptation, and Vulnerability Analyses
- Link ESMs with observations through advanced data systems
- Deploy rapidly to assess mitigation and adaption options

Climate change science
- Observation
- Experiments
- Computing
- Model development

Scales:
- Global scale
- National scale
- Regional scale
- Landscape scale
- Local scale
High Resolution Earth System Modeling: A Necessary Core Capability

Objectives and Impact

• **Strategy**: Develop predictive global simulation capabilities for addressing climate change consequences

• **Driver**: Higher fidelity simulations with improved predictive skill on decadal time scales on regional space scales

Mesoscale-resolved column integrated water vapor
Jaguar XT5 simulation

Eddy-resolved sea surface temperature
Jaguar XT5 simulation

Net ecosystem exchange of CO₂
Free Air CO2 Enrichment (FACE) experiments help improve models

- Increased productivity not maintained
  - Storage in wood increased initially
  - Fine root production later
  - Steady decline in forest NPP may be related to changes in N economy of stand

- Harvesting is ongoing
Increased NPP mostly attributable to greater fine-root production

Fine roots die quickly, so they do not contribute to C storage in the plant... but they can increase soil C

Understanding belowground processes and their response to climate change is an important research priority

Jastrow et al. 2005. GCB 11, 2057–2064
Where is Our Energy Coming From
U.S. Energy Supply Sources

Total = 99.305 Quadrillion BTU

Most renewable energy goes to producing electricity

2008 US Energy Consumption

Note: Sum of Components may not equal 100% due to independent rounding.
Use of Biofuels small but increasing

2008 Transportation Energy Sources

- Petroleum: 95%
- Natural Gas: 2%
- Renewables: 3%

Source: Energy Information Administration, Office of Coal, Nuclear, Electric and Alternate Fuels
The Biomass Economy

Enable sustainable biomass technologies for fuels, chemicals, and other products
BioEnergy Science Center

An Integrated Strategy to Understand Biomass Recalcitrance

BESC: A multi-institutional DOE-funded center

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
- This requires an integrated multidisciplinary approach

<table>
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<tr>
<th>Time frame</th>
<th>Planned</th>
<th>Actual</th>
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<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>
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</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
BESC leverages high-performance computing and neutron capabilities

BESC shares samples and insights with another BER project to develop lignocellulosic biomass-relevant analyses using neutrons and simulation.
Building simulation models of plant cell walls

Length scale

1 nm
Atomic
Model compounds: force field of lignin

Molecular
Lignin

Supra-molecular
Lignin and cellulose

1 μm
Cellular (future)
Switchgrass stem cross section
Genetic block in lignin biosynthesis in switchgrass increases biofuel yields

Phenylalanine → PAL

Agrobacterium-mediated transformation of switchgrass

HOOC

Phenylalanine → PAL

HOOC

C4H

HOOC

Caffeoyl

CCoAOMT

HOOC

CAD

HOH2C

4-Coumaroyl CoA

HOH2C

Coniferaldehyde

HCT

HOH2C

5-Hydroxyconiferaldehyde

CAD

HOH2C

Sinapaldehyde

COMT

HOH2C

Sinapyl alcohol

X. Fu and Z. Wang (Noble), J. Mielzen (ORNL), support from USDA/DOE

Ethanol yield

Wild-type switchgrass

Noble Foundation transgenic switchgrass

25% more ethanol
Mining variation to identify key genes in biomass composition and sugar release

Collected ~1300 samples for *Populus* association and activation-tag study

High-throughput screening pipeline
- Create genetic marker map to identify allelic variation
- Identify marker trait association

Sugar release assay

Establish common gardens for association and activation-tag populations with thousands of plants

Cell wall biosynthesis database

- Existing collections (N = 500; 1–2 trees/site)
- New collections (N = 580; 140–160 trees/site)
High-throughput characterization pipeline for the recalcitrance phenotype

Screening thousands 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
High-throughput screening to analyze natural *Populus* trees

- Screening of 1200 natural *Populus* trees
- Hot water as pretreatment only
- Sugar release varies from 25% to >90% of theoretical value

**Environmental vs genetic?**
Detailed analysis of specific samples inform cell-wall chemistry and structure

AFM of switchgrass showing cellulose microfibrils

Bio-ultraCAT: 3-D density of *Populus* cell walls

Immuno-localization using wall antibodies on switchgrass

Mass spectrometry for key metabolites

Fractionation and chromatography

NMR for cellulose crystallinity

2D $^1$H-NMR sees altered bonds in polysaccharides and lignin in biomass

Chemistry
CBP Organism Development Strategies and Related Fundamentals

Native Strategy
Metabolic engineering to improve product yields, tolerance e.g., in C. thermocellum

Recombinant Strategy
Heterologous enzyme expression to enable cell wall fermentation, e.g., cellulose utilization in yeast T. saccharolyticum

APPLIED OBJECTIVE:
Industrial strains able to produce desired product at high yield without added enzymes

Fundamentals of Microbial Cellulose Utilization

Underlying Fundamental Issue:
Understand cellulose hydrolysis at a microbial rather than enzymatic level

Microbes with good substrate utilization properties (cellulase production, utilization of hydrolysis products)

Microbes with good product-producing properties (high product yields, tolerance)
Cellulase expression in Mascoma yeast (robust C5/C6 fermenting) vs time

- >3000-fold improvement in expression levels over 20 months
- Reduces commercial cellulase addition by more than half

**CBP organism development yeast**

<table>
<thead>
<tr>
<th>Year</th>
<th>Cellulase (mg/g DCW)</th>
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<tr>
<td>1992</td>
<td>0.62</td>
</tr>
<tr>
<td>March 2007</td>
<td>0.03</td>
</tr>
<tr>
<td>March 2008</td>
<td>13.0</td>
</tr>
<tr>
<td>October 2008</td>
<td>24.0</td>
</tr>
<tr>
<td>December 2008</td>
<td>100.0</td>
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* T. reesei cellulase in yeast (Reinikainen et al.)
* T. reesei cellulase in Mascoma yeast
* Proprietary cellulase in Mascoma yeast
* Cellulase expression in Mascoma yeast
Enzymatic and microbial hydrolysis
A fundamentally different relationship between microbes and cellulose

**Enzymatic hydrolysis (classical approach)**
- Hydrolysis mediated by CE complexes
- Enzymes (several) both bound and free
- Cells may or may not be present

**Microbial hydrolysis (CBP)**
- Hydrolysis mediated mainly by CEM complexes
- Enzymes both bound and free
- Cells both bound and free

Yeast, enzymes with biomass (Dumitrache and Wolfaardt)

C. thermocellum on poplar (Morrell-Falvey and Raman, ORNL)
Cellulosome of *C. thermocellum*

- **cellulases and xylanases**
- **scaffoldin**
- **cohesin**
- **CBD**
- **Cellulose**
- **dockerin**
- **cohesin**

Cell
Cellulosome changes in \textit{C. thermocellum} on different biomass substrates

- Pretreated Switchgrass
- Cellobiose
- Amorphous Cellulose
- Avicel - $^{14}$N
- Avicel - $^{15}$N
- Avicel-Pectin
- Avicel-Xylan
- Avicel-Pectin-Xylan

\textbullet \ \textit{C. thermocellum} alters its cellulosome catalytic composition depending upon the growth substrate
\textbullet \ We identified and experimentally verified 16 “new” cellulosome components
\textbullet \ Insights aid in constructing designer cellulosomes with tailored enzyme composition for industrial ethanol production

Understanding cellulosome structure/function

- Assembled 7 domain enzymes
- Ready for computer simulations

*C. thermocellum* CbhA

<table>
<thead>
<tr>
<th>CBM4</th>
<th>IG</th>
<th>GH9</th>
<th>FN3₁</th>
<th>FN3₂</th>
<th>CBM3b</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="CBM4 NREL 2009" /></td>
<td><img src="image" alt="Ig-GH9 PDB" /></td>
<td><img src="image" alt="Fn3₁–Fn3₂ NREL 2009" /></td>
<td><img src="image" alt="CBM3b Bayer et al., 2009 (unpublished)" /></td>
<td><img src="image" alt="Dockerin PDB" /></td>
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</tbody>
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**CbhA** is a critical enzyme

New structures solved at NREL
CBP fundamental cell binding on substrate

• *A. thermophilum*
  – No cellulosome
  – Multi-enzyme complex?

• *C. thermocellum*
  – Cellulosome
  – Binding via cellulose binding domains

**Dynamic binding?**

- Free CBH-active enzymes adsorbed on cellulose via CBM
- Sugar released upon hydrolysis of cellulose
- Protein-mediated cell-substrate interactions?
Computational microscope: Assay and analysis framework

Knowledgebase
- Metabolic pathways
- Ontologies

Integrate experimental data with external reference sets

LIMS
- Assays
  - Experimental conditions
  - Phenotypes
  - Gene expression
  - Mass spec
  - Protein interaction
  - 2D gels
  - Metabolites/concentrations
  - Proteins

Infer significant processes

Navigate pathways

Pathways

Infer significant processes

Gene structure
- Operons

Experimental conditions
- Treatments
- Imaging
- Genotypes
- Mutants
- SNPs
- Genome sequence
- Orthologs

Gene expression
- Mass spec
- Protein interaction
- 2D gels
- Metabolites/concentrations
- Proteins

Protein expression

Proteins

2D gels

Phenotypes
- SNPs
- Genome sequence
- Orthologs

BESC
BioEnergy Science Center
BESC will revolutionize how biomass is processed and converted.

- Modified Plants
- Switchgrass mutant (25% more Ethanol and therefore accessible sugar)

Biomass Modification

Consolidated Bioprocessing

- Reduced or No Pretreatment
- No Separation
- CBP Microbes
- Next Generation Biofuel

CBP yeast development significant reduction in cellulose addition

Synergy

Advanced New Process

Butanol Isobutanol Hydrocarbons
Industrial partners facilitate strategic commercialization
Translating discoveries to the scientific community

- 159 scientific publications
  - 33% of publications include external collaborators at non-BESC Institutions
- BESC publications have already been cited 262 times in peer-reviewed journals
- Several publications in top-tier journals
  - *Nature Biotechnology*, 2008, Lynd et al., How biotech can transform biofuels
  - *PNAS*, 2008, Shaw et al., Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield
  - *Nature Nanotechnology*, 2010, Tetard et al., New modes of subsurface atomic force microscopy through nanomechanical coupling
- 17 inventions disclosed (under evaluation by BESC Commercialization Council)
Influencing next generation of scientists

- National Geographic, The Jason Project, filmed and generated an educational module on bioenergy with BESC researchers
  - This module is available from www.jason.org

- Created an interactive biofuels outreach lesson for students in Grades 3-8
  - Piloted more than 220 lessons which reached over 6,000 students
  - Partnered with the Creative Discovery Museum
  - Available on www.bioenergycenter.org

- Piloted ten Biofuels Family Science Nights with an average attendance of 250 people each
Thank you

BESC is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science