Energy, Plant Cell Wall Deconstruction & Glycoscience

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NRC Workshop on the Future of Glycosciences
Washington, DC
January 12, 2012
Twice in history, major changes in the resources used by humanity have transformed day-to-day life and societal organization.

<table>
<thead>
<tr>
<th>Revolution Type</th>
<th>Duration</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunting &amp; Gathering</td>
<td>Millenia</td>
<td>50 million</td>
</tr>
<tr>
<td>Neolithic Revolution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preindustrial Agricultural</td>
<td>Several centuries</td>
<td>750 million</td>
</tr>
<tr>
<td>Industrial Revolution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presustainable Industrial</td>
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</tbody>
</table>

~10,000 BC... 1750 AD...

Population: 50 million

Today: There are abundant indications that a third revolution is required

<table>
<thead>
<tr>
<th>Revolution</th>
<th>Population</th>
<th>Duration</th>
<th>Scale of societal integration/potential collapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural Revolution</td>
<td>50 million</td>
<td>Millennia</td>
<td>Small groups</td>
</tr>
<tr>
<td>Preindustrial Agricultural</td>
<td>750 million</td>
<td>Several centuries</td>
<td>Farms/villages</td>
</tr>
<tr>
<td>Presustainable Industrial</td>
<td>~7 billion</td>
<td>&lt; a century</td>
<td>Cities/countries</td>
</tr>
<tr>
<td>Sustainable Industrial</td>
<td></td>
<td></td>
<td>Global</td>
</tr>
</tbody>
</table>

The sustainability revolution: More people, less time, higher risk

The defining challenge of our time

Biomass – 23% primary energy supply (largest) in IEA 2050” Bluemap” Scenario

Central and essential role in a sustainable world: The only foreseeable source of food, organic fuels, and organic materials

Liquid preferable phase of matter for transport. Likely → 1/3 to 2/3 transportation energy requirements indefinitely, particularly aviation, long-haul trucking, shipping
# Feedstock has a Pervasive Impact on Biofuel Performance Metrics

<table>
<thead>
<tr>
<th></th>
<th>Land Efficiency (GJ/ha/yr)</th>
<th>Land Availability (in light of other uses)</th>
<th>Feedstock Cost ($/GJ)</th>
<th>Cost-Effective Conversion</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Row Crops</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perennial Cellulosics</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Algae</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

1. Land efficient terrestrial biomass fostered by: a) Plants optimized for growth rather than easily-processed components (sugar, starch, oil), b) perennials, c) C4 mechanism.

2. Increasing evidence suggests that land availability issues can addressed gracefully for cellulosic biomass, although this does not guarantee they will be.

3. $60/dry ton cellulosic biomass = $4/GJ = $23/barrel oil.

4. Very low ghg emissions, many desirable effects of incorporating perennials into agricultural landscapes, indirect land use impacts could be negative but are avoidable.

5. Broad range of rankings possible depending on how the process is configured.
The recalcitrance of cellulosic biomass 
(considered here in relation to biological processing)

- Cellulosic biomass
- Reactive Intermediates e.g. sugars
- Fuels

- Most costly
- Greatest potential for R&D-driven cost reduction
- Advances necessary & sufficient to create cellulosic biofuels industry, generically enabling
The Recalcitrance of Cellulosic Biomass

Result of plants evolving to resist attack by the elements, microbes and their enzymes

Barrier to accessing a leading renewable energy source, mediates critical step in carbon cycle

Manifested at *surfaces* where enzymes, microbes, and plant cell walls interact

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**Enzymatic Plant Cell Wall Deconstruction**
(Multiple proteins, complexed or discretely-acting)

**Microbial Plant Cell Wall Deconstruction**
(Also multiple proteins)

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Both plant cell wall and microbe surfaces are composed primarily of carbohydrate

Glycoscience: Central role in determining, understanding, and alleviating plant recalcitrance
Plant Cell Wall Solubilization, Recalcitrance and Glycoscience Landscape

**Levels of Aggregation**

- **Enzyme components & functional domains**
  - Intramolecular interactions, enzyme-substrate interactions

- **Enzyme mixtures & complexes**
  - + Enzyme-enzyme, complex-substrate interactions

- **Pure microbial cultures**
  - + Microbe-enzymes-substrate

- **Microbial consortia**
  - + Microbe-microbe interactions, consortium-substrate interactions

**Key Interactions**

**Glycoscience Topics**

- Competition, ecology, & functional characterization of mixed species plant cell wall biofilms
- Impact of the microbial surface/glycocalyx
- Gene expression triggered by binding to insoluble carbohydrates
- Capture of solubilization products by adhered microbes
- Understand how substrate properties impact rates, yields, synergy
- Protein structure, function (including glycosylation)
- Substrate physical & compositional heterogeneity, surface properties
Microbial Plant Cell Wall Deconstruction: Evidence that Live Microbes are More Effective Than Enzymes Acting in the Absence of a Physical Association with Cells

Model Substrates

<table>
<thead>
<tr>
<th>Cell-free, enzymatic</th>
<th>Cellulase Specific Solubilization Rate</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. reesei</td>
<td>0.60</td>
<td>1.0</td>
</tr>
<tr>
<td>C. thermocellum cellulase</td>
<td>2.5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microbial</th>
<th>Cellulase Specific Solubilization Rate</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. thermocellum</td>
<td>11 to 20</td>
<td></td>
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Enzyme-Microbe Synergy
Cellulase specific rates 2.7- to 4.7-fold higher when mediated by metabolically active cells with cellulosome on the cell surface as compared to purified cellulosome acting in the absence of cells.

Mechanism not alleviation of inhibition by bulk phase hydrolysis products, likely involves surface phenomena.

Lignocellulosic Substrates. Several microbial cultures including C. thermocellum achieve ~ 5-fold higher solubilization yields than commercial cellulase preparations on switchgrass under controlled conditions (J. Paye, unpublished), although this is not the case for cell-free C. thermocellum cellulase preparations (M. Himmel).
Enzymatic Plant Cell Wall Deconstruction

- Hydrolysis mediated by CE complexes
- Enzymes (several) both bound & free
- Cells may or may not be present

Microbial Plant Cell Wall Deconstruction

- Hydrolysis mediated mainly by CEM complexes
- Enzymes both bound & free
- Cells both bound & free

Cellulose, C

Cellulase enzyme(s), E

Microbes, M (non-cellulolytic)

Microbes, M (cellulolytic)
**Enzymatic Plant Cell Wall Deconstruction**

- Hydrolysis mediated by CE complexes
- Enzymes (several) both bound & free
- Cells may or may not be present

**Microbial plant cell wall deconstruction**

- Hydrolysis mediated mainly by CEM complexes
- Enzymes both bound & free
- Cells both bound & free

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Yeast SSF (added cellulase, not shown), Cotton
Dumitrache & Wofaardt, Ryerson U

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C. thermocellum, Cotton
Dumitrache & Wolfaardt
Microbial Plant Cell Wall Deconstruction: A Surface Phenomenon

“Craters” on filter paper colonized by cellulolytic bacteria.


Jennifer Morrell-Falvey, ORNL.

Progressive colonization of cotton fibers by *C. thermocellum*

A. Dumitrache & G. Wolfaardt, Ryerson U.
Enzyme-Microbe Synergy: In Search of an Explanation

**Enzymatic cellulose solubilization**

Structuring of water near the cellulose surface creates a local environment for plant cell wall deconstruction different from the bulk liquid.

**Microbial cellulose solubilization**

The cell surface further modifies the local environment.

“It is hypothesized that the structured layers of water might present a barrier to the approach of cellulase enzymes... and might inhibit the escape of soluble products, contributing to the slow rates of hydrolysis observed experimentally.”

*Mathews et al., 2006.*

Evolution has had over a billion years to optimize microbial surfaces

Presumed objectives: Effective solubilization, compete for space, capture solubilization products
Glycoscience: Central role in determining, understanding, and alleviating plant recalcitrance

*Increased understanding*

**New, sustainable industrial platform**

**Multiple products:** Ethanol, other fuels, chemicals, feed, materials (e.g. carbon fiber)

**Multiple processes:** Solid and liquid phase fermentation, microbial fuel cells, integration with catalytic and thermochemical processing

**Better understanding of the carbon cycle & integration of human activities into it**