Effect of particle size on enzymatic hydrolysis of pretreated miscanthus

VIjay Singh  
*University of Illinois at Urbana-Champaign*

Esha Khullar  
*University of Illinois at Urbana-Champaign*

Bruce Dien  
*University of Illinois at Urbana-Champaign*

Kent Rausch  
*University of Illinois at Urbana-Champaign*

M.E. Tumbleson  
*University of Illinois at Urbana-Champaign*

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Esha Khullar, Bruce S. Dien, Kent D. Rausch, M. E. Tumbleson and Vijay Singh

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Cellulosic Feedstocks for Fuel

- Leading contender for ethanol production
  - Relative abundance
  - Low energy inputs
  - Ability to grow on marginal lands
  - Sustainable production

- Uncertainties
  - Establishment: yields with low fertilizer application, marginal lands
  - Lack of technology
  - Biomass availability, seasonality of crop

- 1.3 billion tons of biomass available
EISA defines **Cellulosic Biofuel** as “renewable fuel derived from any cellulose, hemicellulose, or lignin that is derived from renewable biomass and that has lifecycle greenhouse gas emissions...that are at least 60 percent less than baseline lifecycle greenhouse gas emissions.” The EPA interprets this to include cellulosic-based diesel fuel.

EISA defines **Advanced Biofuel** as “renewable fuel, other than ethanol derived from corn starch, that has lifecycle greenhouse gas emissions...that are at least 50 percent less than baseline lifecycle greenhouse gas emissions.” This includes biomass-based diesel, cellulosic biofuels, and other advanced fuels such as sugarcane-based ethanol.
Different Integrated Biorefineries

Integrated Biorefineries

Biochemical Conversion
- Pretreatment & Conditioning
- Enzymatic Hydrolysis
- Enzyme Production
- Distillation
- Sugars
- Fermentation
- By-Products
- Wastes/Residue

Thermochemical Conversion
- Fast Pyrolysis
- Liquid Bio-oil
- Zeolite Cracking
- Hydrogenolysis
- Gasification
- Syngas
- Fischer Tropsch
- Alcohol Synthesis
- Lipid (Oil) Extraction
- Algal Oil
- Transesterification
- Fractionation

Feedstock Production & Logistics
- Energy crops
- Forest Residue
- Agricultural wastes
- Algae
- Corn

REFINING
- DDGS
- Lignin (for power)
- Ethanol
- Butanol
- Olefins
- Gasoline
- Diesel
- Jet Fuel
The EBI’s multidisciplinary research teams explore total-system solutions to global energy problems.
Miscanthus x giganteus

- Perennial grass
- High yielding (20 to 25 dry ton/hectare)
- Less inputs
Lignocellulose Structure

- Cellulose (40 to 50%)
- Hemicellulose (25 to 35%)
- Lignin (15 to 20%)
- Miscanthus
  - 40% cellulose, 18% hemicellulose, 25% lignin
**Microfibril**
- 36 glucan chains, DP 500 to 14000
- Intra- and inter chain hydrogen bonds

**Macrofibril**
- Ribbon like bundles
- Hemicellulose coats microfibrils, hydrogen bonds with cellulose

Lignin deposited in final stages and enclose microfibrils and polysaccharides
Biochemical Conversion

**Feedstock**
- Production
- Logistics
- Storage
- Handling

**Pretreatment**
- Physical
- Chemical
- Biological

**Enzyme Hydrolysis**
- Cellulase
- Hemi-cellulase
- Other

**Fermentation**
- Engineered microbes
- C5 and C6 sugars

**Product Recovery**
- Ethanol
- Residues
Objective

Effect of particle size on enzymatic hydrolysis of pretreated *Miscanthus*
Experimental Design

- Miscanthus 8 mm
- Particle Size Reduction
  3 different sizes
- Pretreatment
  Hot water, dilute acid or dilute ammonium hydroxide
- Enzyme Hydrolysis
  Cellulases and accessory enzymes
- Sugars
Particle Size Reduction

- Hammer mill
- Sieve sizes: 0.08, 2.0 and 6.0 mm
- Particle size distributions and geometric mean diameters
Pretreatment

- Tubular reactors in a fluidized sand bath
- Reactor fitted with thermocouple for internal temperature measurements
- 10% solids content (d.b.)

**Conditions**

- Hot water: 200°C, 30 min
- Dilute acid: 160°C, 10 min, 1% w/w sulfuric acid
- Dilute ammonium hydroxide: 160°C, 5 min, 5%
- Unpretreated
Enzyme Hydrolysis

- **Enzymes**
  - Accellerase 1500: exoglucanase, endoglucanase, hemicellulase and beta-glucosidase
  - Accellerase BG: beta-glucosidase
  - Accellerase XY: hemicellulase
  - Accellerase XC: endoglucanase and xylanase

- **Conditions**
  - 10% solids content
  - 50°C, 75 rpm
  - 72 hr (samples at 3, 12, 24, 48 and 72 hr)
  - HPLC determination of sugars
Data Analysis

- Full factorial, completely randomized design
- Particle size analyses, pretreatments and enzyme hydrolysis conducted in triplicates
- Glucan, xylan and total polysaccharide conversion (%)
## Results

### Geometric Mean Diameters

<table>
<thead>
<tr>
<th>Sieve Size (mm)</th>
<th>Geometric Mean Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>56.00 ± 0.54 C</td>
</tr>
<tr>
<td>2.00</td>
<td>300.5 ± 4.10 B</td>
</tr>
<tr>
<td>6.00</td>
<td>695.3 ± 69.1 A</td>
</tr>
</tbody>
</table>

![Particle Size Distribution](Image)

- **0.08 mm**
- **2.0 mm**
- **6.0 mm**
Conclusions

- Decreased particle size increased total polysaccharide conversion for all pretreatments.
- Unpretreated Miscanthus; 20 to 60% lower than chemical pretreatments.
- Sample ground using 0.08 mm sieve screen was used for further studies.
Objective

Optimization of hot water pretreatment for *Miscanthus*
Hot Water Pretreatments

- Water at 160 to 240°C
- At high temperatures,
  - water acts as a weak acid
  - solubilizes hemicellulose as oligosaccharides
  - loosens lignin
- Advantages
  - No chemicals; lessen need for expensive reactors, eliminated need for recycling chemicals or catalysts
  - No neutralization
  - Minimizes monosaccharides from hydrolysis; minimizes inhibitor production
Hot Water Pretreatments

- Have been applied to many substrates
  - Corn stover
  - Switchgrass
  - Yellow polar sawdust
  - *Eucalyptus grandis*
  - Corn fiber
  - Sugarcane bagasse
  - Wheat straw
  - Alfalfa fiber
  - Prarie cord grass
- Not for *Miscanthus × giganteus*
Experimental Design

- **Miscanthus 0.08 mm**
- **Hot Water Pretreatment**
  - Temperature: 160, 180 or 200°C
  - Reaction Time: 0, 10, 20 or 30 min
- **Enzyme Hydrolysis**
  - Accellerase 1500, BG, XY and XC
- **Sugars**
Simultaneous Saccharification & Fermentation (SSF)

- Optimized pretreatment conditions used for SSF experiment

- Miscanthus 0.08 mm

- Hot Water Pretreatment
  - 200°C, 10 min

- SSF
  - Optiflow RC2 cellulase
  - Novo 188 cellobiase
  - Multifect pectinase
  - *Saccharomyces cerevisiae* D5A

- Ethanol and sugars
SSF Results

- Final concentrations at 72 hr
  - Ethanol: 2.04% w/v
  - Glucose: 0.050 %w/v
  - Xylose: 0.093 %w/v
- Ethanol yield was 70%
Conclusions

- Optimized conditions were 200°C for 10 min
- At optimal conditions, pretreated washed solids had 77% glucan, 12% xylan and 62% total conversion
- SSF of pretreated solids resulted in 70% ethanol yield
Thanks!