Enhancement of Cellulose Saccharification Kinetics Using an Ionic Liquid

Ananth Dadi, Sasidhar Varanasi, Constance Schall
Department of Chemical & Environmental Engineering
University of Toledo, USA
Ethanol from Biomass

Biomass Source: Lignocellulose

- Pretreatment
  - Cellulose
  - Hemicellulose
  - Lignin

Saccharification Reaction: Hydrolysis to Glucose

- Cellulose
- Xylose
- Lignin Processing

Glucose Fermentation

Ethanol for Fuel

Hydrolysis: critical step
- Enzymatic hydrolysis
- Acid hydrolysis
## Cellulose Saccharification

### Acid catalyzed hydrolysis

**Advantages**
- inexpensive catalyst
- modest reaction rates

**Disadvantages**
- degrades glucose (inhibits fermentation)
- corrosive

### Enzymatic hydrolysis using *cellulases*

**Advantages**
- selective for glucose
- non-corrosive

**Disadvantages**
- slow reaction rate
- high cost of enzyme and difficult recovery
Enzyme Hydrolysis

- Enzyme molecule
- Cellulose fibril
- Amorphous cellulose

Slow hydrolysis

Rapid hydrolysis
Cellulose Dissolution in Ionic Liquids

- Ionic liquids (IL) are salts that melt at temperatures near ambient.
- Solvent properties can be “tuned” through the cation/anion selection.
- BmimCl has been found to dissolve cellulose*

1-n-butyl-3-methyl imidazolium chloride

Cellulose Regeneration

Cellulose

Ionic liquid

Anti-solvent
Cellulose Regeneration
XRD of Regenerated Cellulose

(A) untreated Avicel
(B) water
(C) methanol
(D) acetonitrile
(E) ethanol
FTIR of Regenerated Cellulose

A is untreated cellulose (Avicell, PH101)
B is regenerated cellulose (anti-solvent: water)
Hydrolysis Experiments

• Enzyme hydrolysis
  – Worthington *T. reesei* cellulase, 150 to 340 FPU/g glucan
  – Celluclast 1.5L, 50 FPU/g glucan; with/without Novozyme 188, 0-166 CBU/g glucan

• Substrate
  – Untreated and IL-regenerated Avicel PH101

• Sugar assay
  – Total soluble reducing sugars (DNS assay)
  – Glucose formation, glucose hexokinase assay
Avicel samples incubated at 130° C for 10 minutes in BmimCl.

Anti-solvent:
- (□) ethanol
- (●) deionized water
- (▲) methanol
- (►) untreated Avicel

17 mg/ml Avicel hydrolyzed with *T. reesei* cellulase activity of 170 FPU/g glucan
Cellulose Hydrolysis

Glucose formation rate

Avicel samples incubated at 130° C for 10 minutes in BmimCl.

Anti-solvent:
- ( ) ethanol
- ( ) deionized water
- ( ) methanol
- ( ) untreated

17 mg/ml Avicel hydrolyzed with *T. reesei* cellulase activity of 170 FPU/g glucan
Initial Hydrolysis Rate

![Graph showing initial hydrolysis rate with time (min) on the x-axis and total reducing sugar released (mg/ml) on the y-axis. The graph includes data points and a trend line.]
# Initial Rate of Formation of Soluble sugars

<table>
<thead>
<tr>
<th>Anti-solvent</th>
<th>Initial rate (mg ml(^{-1})min(^{-1}))</th>
<th>Rate Enhancement*</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>0.6473</td>
<td>52</td>
</tr>
<tr>
<td>methanol</td>
<td>0.6823</td>
<td>55</td>
</tr>
<tr>
<td>ethanol</td>
<td>0.6473</td>
<td>53</td>
</tr>
<tr>
<td>untreated</td>
<td>0.0125</td>
<td>-</td>
</tr>
</tbody>
</table>

*Rate enhancement = initial rate regenerated cellulose / untreated cellulose
Total Sugars vs. Glucose

The graphs depict the relationship between total sugars and glucose over time. The x-axis represents time in hours (0-24), and the y-axis represents the amount of sugar released (mg/ml). The graphs show a gradual increase in sugar release over time, with slight variations indicated by data points.
## Initial Rate of Formation of Soluble Sugars

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Initial Rate (mg/ml min)</th>
<th>Rate Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated Cellulose</td>
<td>Regen. Cellulose</td>
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<tr>
<td><strong>Cellulase</strong></td>
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<td></td>
</tr>
<tr>
<td>FPU</td>
<td>β-glucosidase CBU</td>
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<tr>
<td>25</td>
<td>0</td>
<td>0.0004</td>
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<tr>
<td>25</td>
<td>83</td>
<td>0.0004</td>
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<tr>
<td>50</td>
<td>0</td>
<td>0.0043</td>
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<tr>
<td>50</td>
<td>83</td>
<td>0.0044</td>
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<tr>
<td>100</td>
<td>0</td>
<td>0.0110</td>
</tr>
<tr>
<td>100</td>
<td>83</td>
<td>0.0140</td>
</tr>
</tbody>
</table>

* Rate enhancement = initial rate regenerated cellulose / untreated cellulose
Avicel samples incubated at 130° C for:

- (□) 10 min
- (○) 30 min
- (△) 1 hour
- (▼) 3 hours
- (►) untreated

Precipitated with water

15 mg/ml Avicel hydrolyzed with *T. reesei* cellulase activity of 150 FPU/g glucan
Cellulose Hydrolysis
Effect of dissolution temperature

Avicel samples incubated for 2 h at:

- (square) 130° C
- (circle) 140° C
- (triangle) 150° C
- (up triangle) untreated

Precipitated with water

17 mg/ml Avicel hydrolyzed with *T. reesei* cellulase activity of 340 FPU/g glucan
Summary & Conclusions

• Simple dissolution of cellulose in the ionic liquid followed by rapid precipitation with anti-solvent is required: incubation time and temperature in IL does not affect hydrolysis.

• Regeneration appears to increase access of cellulose to endo- and exo-glucanases, resulting in significantly improved release rate of soluble sugars.

• Addition of β-glucosidase resulted in higher enzymatic hydrolysis rates.
Summary & Conclusions

- Cellulose regenerated from ionic liquid solution is essentially amorphous.

- Regenerated cellulose exhibited up to a 90 fold increase in hydrolysis rates.
Process Schematic

- Alcohol (fresh feed and recycle)
- Cellulose
- Ionic Liquid
- Pretreatment Tank
- Cellulose Precipitator
- Centrifuge
- Flash Distillation
- Ionic liquid recycle

Cellulose to Hydrolysis Reactor
Alcohol recycle
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Cellulose Hydrolysis
Effect of $\beta$-glucosidase addition

Avicel samples incubated at 130 °C for 10 min in BmimCl and precipitated with water

(Δ) 166 CBU/g glucan
(○) 83 CBU/g glucan
(□) no added $\beta$-glucosidase

untreated Avicel hydrolysis in closed symbols

15 mg/ml Avicel hydrolyzed with T. reesei cellulase activity of 50 FPU