Enzyme Mimetics for Bioprocessing Agricultural Residues

Yulin Lu, Nathan S. Mosier
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• Jon Wilker
• Wilfred Vermerris

• Reid Formo
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Integrated, Multidisciplinary Approach to Bioenergy Production

- Pre-treatment
  - Mike Ladisch
  - ABE

- Cellulose hydrolysis
  - Nate Mosier
  - ABE

- Ethanol fermentation
  - Nancy Ho

- Biohydrogen fermentation
  - John Patterson
  - Animal Science

- Ethanol recovery
  - Mike Ladisch
  - ABE

- Biomass genetics
  - Wilfred Vermerris
  - ABE & Agronomy

Laboratory of Renewable Resources Engineering
Timeline of Hydrolysis Technologies

- **1950**: Acid hydrolysis, Glucose-to-ethanol, Hemicellulose sugars to disposal
- **1970**: Enzyme production, Enzyme hydrolysis, Glucose-to-ethanol, Hemicellulose sugars to disposal
- **1980**: Enzyme production, Enzyme hydrolysis, Glucose-to-ethanol, Hemicellulose sugars-to-ethanol
- **Today**: Enzyme production, Enzyme hydrolysis, Glucose-to-ethanol, Hemicellulose sugars-to-ethanol
- **Tomorrow?**

### Trend

**History—Enzymes vs. Acid**

- Enzyme Technology
- Dilute Acid
- Power (Dilute Acid)
- Expon. (Enzyme Technology)

Experimentally-Verified Cases

Research targets only

- 1970
- 1975
- 1980
- 1985
- 1990
- 1995
- 2000

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Bioethanol – Moving Into the MarketPlace, US DOE, National Renewal Energy Laboratory, Jul 2001;
Characteristics of Leading Hydrolysis Technologies

<table>
<thead>
<tr>
<th>Dilute Acid Hydrolysis</th>
<th>Vs.</th>
<th>Enzymatic Hydrolysis</th>
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<tbody>
<tr>
<td><strong>Superiority</strong></td>
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<td><strong>Superiority</strong></td>
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<tr>
<td>– Relatively low cost</td>
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<td>– High specificity/selectivity</td>
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<td>– Simple operational</td>
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<td>– No degradation products</td>
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<tr>
<td>requirements</td>
<td></td>
<td>– Mild hydrolysis conditions</td>
</tr>
<tr>
<td><strong>Drawbacks</strong></td>
<td></td>
<td><strong>Drawbacks</strong></td>
</tr>
<tr>
<td>– Cost of neutralization or acid recycle</td>
<td></td>
<td>– High specificity</td>
</tr>
<tr>
<td>– Environmental problems associated with salt disposal</td>
<td></td>
<td>– High cost for enzyme production</td>
</tr>
<tr>
<td>– Substantial sugar degradation products</td>
<td></td>
<td>– Difficulties in recovery of the enzymes</td>
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</tbody>
</table>
Enzyme Mimetic
Combine the Best of Both Worlds

- Based on understanding enzyme structure/function
- Organic chemical (not protein)
- Less expensive than enzymes
- Only need a small amount
- Less product degradation than sulfuric acid
Prototype BioMimetics Systems

- Block Copolymer Catalysts (Kenichiro Arai, 1979)

Results Confirmed Hypothesis:

1. Hydrolysis rate enhancement because of "Proton Concentration Effect"; (5-fold rate increase the best case)
2. Michaelis-Menten Type Kinetics

Cellulolytic Enzyme Systems

Features of Cellulolytic Enzyme Systems

- Catalytic Domain
  - Hydrolyzes cellulose chain
- Cellulose Binding Modules (CBMs)
  - Bind to the cellulose surface
  - Bring catalytic domain close to substrate


The Protein cellobiohydrolase I (CBH1) from [www.csc.fi/chem/gallery.phtml](http://www.csc.fi/chem/gallery.phtml)
BioMimetics Hypothesis

Organic Macromolecule Designed As Cellulase Mimetic

- CBM Mimetic (CBMM) Candidates
  - Hydrogen bonding with cellulose
  - Hydrophobic interaction
    (D. Kilburn & C. Haynes)

- Catalytic Domain Mimetic (CDM) Candidates
  - Organic acids that hydrolyze cellulose with minimal product degradation

Mosier NS, Enzyme Mimetics for Cellulose Hydrolysis, Ph.D. Thesis, Purdue University, 2003;
Modeling Acid Catalyzed Cellulose Hydrolysis

\[ k = k^o \times e^{-\frac{E_a}{RT}} \]  
Arrhenius Equation

\[ k = k^{o'} \times \left[ H^+ \right]^m \times e^{-\frac{E_a}{RT}} \]  
Modified Arrhenius Equation

Cellobiose Hydrolysis

Kinetic Constants at 160°C

\[ [H^+] \text{ Measured at 20°C} \]

\[ R^2 = 0.9855 \]

1. Mosier, NS; et al. Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. Biotechnology and Bioengineering. 79(6), 610-618, 2002;
Glucose Degradation

Kinetic Constants at 160°C
[H+] Measured at 20°C

- Carboxylic Acids
- Sulfuric Acid
- Control

1. Mosier, NS; et al. Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. Biotechnology and Bioengineering. 79(6), 610-618, 2002;
Kinetic Constants at 135, 145, 160, and 175°C

50 mM Sulfuric Acid

\[ E_a = 118 \pm 37.5 \text{ KJ/gmol} \]
\[ k^0' = 1.80 \times 10^{-11} \pm 1.11 \times 10^{-11} \text{ sec}^{-1} \]

50 mM Maleic Acid

\[ E_a = 72.6 \pm 22.5 \text{ KJ/gmol} \]
\[ k^0' = 3.62 \times 10^{-5} \pm 1.28 \times 10^{-5} \text{ sec}^{-1} \]
Maleic Acid

pKa’s 1.9
4.4
Glucose from Hydrolysis of Microcrystalline Cellulose (Avicel®)

2. Mosier, NS; etc. Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. Biotechnology and Bioengineering. 79(6), 610-618, 2002;
Results

- For a given Brønsted acid: HA
- Hydrolysis catalyzed by H⁺
- Glucose degradation catalyzed by A⁻
- SO₄²⁻ catalyzes glucose degradation
- COO⁻ does not catalyze glucose degradation more than water alone
Maleic Acid for Prehydrolysis

- Dilute acid pretreatment for enhance enzyme digestion
- Selectively generate xylose from hemicellulose with reduced degradation
- Improve subsequent enzyme hydrolysis similar to dilute sulfuric acid
Hydrolysis of Sorghum Stover

175 C, 15 min.

20 FPU/g, 24 hrs

Glucose Content (g/L)

Non-Pretreated
50mM Maleic Acid
50mM Sulfuric Acid

N27-1  N27-2  N27-3  bmr27-1  bmr27-2  bmr27-3
Hydrolysis of Sorghum Stover Hemicellulose

175 C, 15 min.
20 FPU/g, 24 hrs

Xylose Content (g/L)

- Non-Pretreated
- 50 mM Maleic Acid
- 50 mM Sulfuric Acid

N27-1  N27-2  N27-3  bmr27-1  bmr27-2  bmr27-3
Pretreatment of Sorghum Stover

175 C, 15 min.

Furfural Content (g/L)

- 50mM Maleic Acid
- 50mM Sulfuric Acid

N27-1, N27-2, N27-3, bmr27-1, bmr27-2, bmr27-3
Conclusions

• Maleic acid has higher selectivity than sulfuric acid for cellulose and hemicellulose hydrolysis
• Maleic acid can pre-hydrolyze/pretreat biomass similar to sulfuric acid
• Future Work - Cellulose binding domain mimetics may improve hydrolysis kinetics through proton concentration at cellulose surface
Thank You