Hydrothermal pretreatment of biomass for ethanol fermentation

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First and second generation ethanol

First generation
• Sugar, Starch, grain
• Easy fermentation to bioethanol
• High price raw material
• Competition with foods

Second generation
• Lignocellulosic residues (wood, straw) and other agricultural residues
• Advanced technology is needed
Saccharification of lignocellulosics

Lignocellulosics

Cellulose
Hemicellulose
Lignin

Pretreatment

Enzymatic hydrolysis

Ethanol fermentation

Ethanol

CH₂OH

H

OH

OH

OH

OH

CH₂OH

H

OH

OH

OH

OH

Glucose

CH₂OH

H

OH

OH

OH

HO

CH₂OH

Enzymatic hydrolysis
# Various pretreatment for saccharification

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Concept</th>
<th>Disadvantage</th>
<th>Author (year) Previous study</th>
</tr>
</thead>
</table>
| Concentrated sulfuric acid | Promote hydrolysis with concentrated sulfuric acid | ・Decomposition of glucose by acid  
 ・High cost to use acids | Gupta R et al. (2009) |
| Dilute sulfuric acid | Promote hydrolysis with dilute sulfuric acid | ・High cost to treat byproducts  
 ・Reactor corrosion | Root et al. (1959) |
| Steam explosion | After heating up in steam, suddenly reduce the pressure | ・Low glucose yield | DeLong (1981) |
| Pulverization | Decrease the crystallinity of cellulose | ・Large amount of energy needed. | Sidiras and Koukios (1989) |
| Hydrothermal | Hemicellulose is dissolved in water by high temperature and pressure. Reduction of crystallinity of cellulose. | ・Low cost  
 ・Low glucose yield | Mok and Antal (1994) |
Inhibitor byproducts for fermentation

Lignocellulosic biomass → Cellulosic component

Hydrothermal Pretreatment

Enzymatic hydrolysis

Monosaccharide

Ethanol fermentation

Ethanol

Inhibitors
Fermentation inhibitors

Yeast *Saccharomyces cerevisiae*

\[
\begin{align*}
\text{Aerobic} & \quad \leftrightarrow \quad \text{Cell growth} \\
\text{Anaerobic} & \quad \leftrightarrow \quad \text{Ethanol fermentation}
\end{align*}
\]

Fermentation inhibitors are produced during hydrothermal pretreatment, which affects the activities of the yeast

\[
\text{Inhibitors} \quad \leftrightarrow \quad \text{Formic acid, Acetic acid, Furfural, 5-HMF}
\]
To commercialize the process, reaction characteristics as well as inhibitor effect should be clarified.

This evaluation has not been reported so far.

Purpose of this study

The purpose of this study is to determine the reaction characteristics and inhibitor effect quantitatively.
Experimental for hydrothermal pretreatment

- **Raw material**
  - Rubber wood residues

- **Hydrothermal pretreatment**

- **Filtration**

- **Liquid fraction**
  - Glucose product

- **Solid fraction**
  - Enzymatic hydrolysis
  - Glucose product

**Enzymatic hydrolysis**

- **Reactant**
  - 1 g

- **Buffer fluid**
  - 60 mL

- **10 g/L cellulase solution**
  - 5 mL

**Autoclave reactor**

The working volume of the pretreatment vessel was 96 mL. The pretreatment agitator was set at 500 rpm.

**Cellulase from Aspergillus niger** powder, ≥0.3 units/mg solid

The flasks were shaken at 250 rpm at 37°C. HPLC with SUGAR K S-802 (Shodex) column operated at 60°C with 0.8mL/min flow of water as an eluate. The detector was a refractive index.
## Experimental conditions

<table>
<thead>
<tr>
<th>Temperature</th>
<th>130, 150, 170, 190, 200, 210, 240, 260 and 280 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubber wood powder</td>
<td>7 g</td>
</tr>
<tr>
<td>De-ionized water</td>
<td>63 g</td>
</tr>
</tbody>
</table>

### Temperature history for different target temperatures.

![Temperature history graph](image)
Rubber wood residue

37% of market share export of the world are from Thailand

CHEMICAL CHARACTERISTICS OF RUBBER WOOD RESIDUE

<table>
<thead>
<tr>
<th>Composition</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemicelluloses</td>
<td>29</td>
</tr>
<tr>
<td>lignin</td>
<td>28</td>
</tr>
<tr>
<td>cellulose</td>
<td>39</td>
</tr>
<tr>
<td>ash</td>
<td>4</td>
</tr>
</tbody>
</table>

• United States Department of Agriculture, “Forage fiber analyses (Apparatus, reagents, procedures, and some applications)”, Agriculture Handbook, 379 (1970)
Time dependence of amount of glucose generated from solid residue treated temperature 130–280°C, 10 wt% of concentration of raw material, treatment time 0 min. (HC denote the solid sample from hydrothermal pretreatment used with enzymatic hydrolysis)

The samples after hydrothermal pretreatment at temperature on 130 (A), 140 (B), 150 (C), 170 (D), and 190°C (E)
Reaction modeling

\[ k = A \exp\left(-\frac{\Delta E}{RT}\right) \]

<table>
<thead>
<tr>
<th>C</th>
<th>Cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>C*</td>
<td>Cellulose hydrolyzed by cellulase after pretreatment</td>
</tr>
<tr>
<td>G</td>
<td>Glucose</td>
</tr>
<tr>
<td>D</td>
<td>Decomposition products of glucose</td>
</tr>
</tbody>
</table>
Reaction rate parameters

\[
\frac{d[C]}{dt} = -k_1[C] - k_2[C]
\]

\[
\frac{d[C^*]}{dt} = k_1[C] - k_3[C^*]
\]

\[
\frac{d[G]}{dt} = k_3[C^*] + k_2[C] - k_4[G]
\]

\[
\frac{d[D]}{dt} = k_4[G]
\]

Reaction rate parameters

<table>
<thead>
<tr>
<th>Preexponential factor [1/s]</th>
<th>Activation energy [kJ/mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_1)</td>
<td>(1.87 \times 10^5)</td>
</tr>
<tr>
<td>(k_2)</td>
<td>(2.02 \times 10^7)</td>
</tr>
<tr>
<td>(k_3)</td>
<td>(1.80 \times 10^{18})</td>
</tr>
<tr>
<td>(k_4)</td>
<td>(2.88 \times 10^2)</td>
</tr>
</tbody>
</table>
Comparison with other feedstocks
Model for the reactions in hydrothermal pretreatment reactor was proposed.

The reaction parameter in the hydrothermal reactor for rubber wood was successfully decided.

Reaction characteristics differs from feedstock to feedstock.
Experiment for inhibitor effect clarification

- 5 wt% YPD 10 mL
- Yeast 0.2 mL
- Fermentation inhibitor

14 mL vial

- rotary shaker, 30 °C
- incubator

- Glucose and ethanol concentration by HPLC
- OD at 600 nm
## Experimental conditions

<table>
<thead>
<tr>
<th>Yeast</th>
<th>S. cerevisiae *</th>
</tr>
</thead>
<tbody>
<tr>
<td>YPD medium (5.0 wt%)</td>
<td>10 mL</td>
</tr>
<tr>
<td>Preculture</td>
<td>0.2 mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inhibitor concentration</th>
<th>0-45 mM</th>
<th>0-45 mM</th>
<th>0-45 mM</th>
<th>0-15 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid, Furfural</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HMF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measuring time</td>
<td>36 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubation temperature</td>
<td>30 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sigma-Aldrich (Type II)
Cell growth model

\[
\frac{dX}{dt} = \mu X
\]

\[
\frac{dS}{dt} = -k \frac{dX}{dt}
\]

(Monod equation)

\[
\mu = \mu_{\text{max}} \frac{S}{S + K}
\]

(1) Lag phase (2) Exponential growth phase
(3) Resting phase

\[
(t - \tau) = \frac{1}{\mu_{\text{max}}} \left\{ (1 + \frac{K}{kX_0 + S_0}) \ln \left( \frac{X}{X_0} \right) - \frac{K}{kX_0 + S_0} \ln \left| 1 + \frac{k}{S_0} (X_0 - X) \right| \right\}
\]

\(X\): Cell concentration
\(X_0\): Initial Cell concentration
\(S\): Culture medium concentration
\(S_0\): Initial culture medium concentration
\(t\): Incubation time
\(\mu_{\text{max}}\): Maximum growth rate
\(K\): Half medium concentration rate
\(k\):
\(\tau\): Lag phase time
Inhibitor effect on cell growth

Formic acid

Acetic acid
Inhibitor effect on cell growth

**Furfural**

**5-HMF**
Monod parameter change by inhibitors

- Formic acid
- Acetic acid
- Furfural
- 5-HMF

**Graphs:**

1. **μmax [1/h] vs. Concentration [mM]**
   - Symbols: ▲, □, △, ○

2. **K/S₀ [-] vs. Concentration [mM]**
   - Symbols: ▲, □, △, ○

3. **k/S₀ [-] vs. Concentration [mM]**
   - Symbols: ▲, □, △, ○

4. **τ [h] vs. Concentration [mM]**
   - Symbols: ▲, □, △, ○
Inhibitor effect on ethanol fermentation

<table>
<thead>
<tr>
<th></th>
<th>0 mM</th>
<th>15 mM</th>
<th>30 mM</th>
<th>45 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td><img src="image" alt="Ethanol Graph" /></td>
<td><img src="image" alt="Ethanol Graph" /></td>
<td><img src="image" alt="Ethanol Graph" /></td>
<td><img src="image" alt="Ethanol Graph" /></td>
</tr>
<tr>
<td>Glucose</td>
<td><img src="image" alt="Glucose Graph" /></td>
<td><img src="image" alt="Glucose Graph" /></td>
<td><img src="image" alt="Glucose Graph" /></td>
<td><img src="image" alt="Glucose Graph" /></td>
</tr>
</tbody>
</table>

Formic acid

Acetic acid
Inhibitor effect on ethanol fermentation

<table>
<thead>
<tr>
<th></th>
<th>0 mM</th>
<th>15 mM</th>
<th>30 mM</th>
<th>45 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furfural</td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>5-HMF</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Concentration [mol/L] vs Time [h]

Furfural

5-HMF
Conclusions (inhibitor effect)

• The inhibitors used in this study slows cell growth and final yeast concentration. Effect on parameters were observed ($\mu_{\text{max}}$, $k$, $\tau$).

• The inhibitors used in this study except acetic acid decreases glucose consumption rate and ethanol production rate for ethanol fermentation.

• Acetic acid affects cell growth but does not affect ethanol production.
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