Fermentable Sugars from Cassava Pulps Hydrolysate for Ethanol Production

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Abstract
The objective of the study was to determine the effects of diluted acid concentration and reaction time on the production of sugars and on the reaction byproducts. The milled cassava pulps were hydrolysed by 0.01-0.25 M sulfuric acid with the steam at temperatures of 135°C under pressure of 15 pound/inch² for 60 min. The concentrations of cassava pulps were varied from 0.3-15% w/v for acid hydrolysis. The results showed that reducing sugars were maximal produced by 2.5% cassava pulps hydrolysed by 0.025 M sulfuric acid (w/v) producing the highest yield. Batch fermentation by Saccharomyces cerevisiae showed the cassava pulps hydrolysate by 0.025 M sulfuric acid used as a dramatic substrate containing reducing sugar 10.85 g/l after incubation at 30°C with 150 rpm in shaking incubator and resulted in the formation of 4.93 g/l ethanol after 18 h. This study suggested that diluted sulfuric acid was the efficient catalyst for hydrolysis to produce sugars from cassava pulps to be the carbon source for yeast fermentation to produce ethanol.

Introduction
Bioethanol produced from non-food lignocellulosic waste products as wood chips and straw or non-food crops as willow could be an environmentally-friendly alternative (Wyman and Goodman, 1993). Lignocellulose consists of three major constituents: cellulose, hemicellulose and lignin, where hemi-cellulose accounts for up to 33% (dry weight) of the fibres. Conversion of the xylose fraction of hemicellulose (which constitutes up to 85% of hemicellulose) is required to make bioethanol production sustainable. Cassava pulps compose of protein, lipid, fiber, ash, carbohydrate and moisture content; 3.39, 0.24, 15.26, 2.65, 66.22 and 12.21, respectively (Chotineranat, 1996). The carbohydrates, cellulose and hemicellulose are intimately associated with lignin in the plant cell wall. The pentoses might be readily available, but are often found in polymeric chains as xylan, arabinogalactans, arabinans, and mannans. Also, the individual sugars might be methylated or acetylated (Biely, 1985) which can affect the availability. The hexoses can be fermented to ethanol by yeast, whereas the pentoses, can be fermented to ethanol, acetate, lactate, CO₂ and H₂ through the pentose-phosphate pathway with fructose-6-phosphate, glyceraldehyde-3-phosphate and pyruvate as intermediates (Larsen et al., 1997). The objective of these studies was to assess the ethanol production by yeast fermentation of sugar produced by diluted acid hydrolysis of cassava pulps.

Materials and Methods
The cassava pulp hydrolysate was obtained by acid hydrolysis of milled cassava pulp (average size of 45-637 μm), hydrolysed under the following conditions: cassava pulp mass, 0.3-15%; volume of acid-solution, temperature, 90-135°C; reaction time, 15-90 min under pressure 15 lb/inch²; concentration of diluted acids, sulphuric acid, hydrochloric acid and acetic acid 0.01-0.25M. After the hydrolysis, the liquid fraction was determined for sugar compositions and 5-hydroxymethylfurfural acetic acid were determined by high-performance liquid chromatography (HPLC) using a HP1082B chromatography with a Bio-Rad HPX87H column. 5-Hydroxymethylfurfural were also determined by HPLC using a RP18HP column (Garrote et al.,1999)neutralized to pH 7.0 using NaOH. For fermentation process, the hydrolysate was filtered through 0.2 μ filter to sterile the liquid fraction. The treated hydrolysate was further supplemented with nutrients. Fermentations runs inoculated with Saccharomyces cerevisiae were carried out in 125 ml Erlenmeyer flasks containing 50 ml of this medium incubated in a rotary shaker for 20 h at 30°C and 150 rpm. The experiments were made in triplicate and the average values are reported.
**Figure 1** % Reducing sugar yield from 2.5% cassava pulps were hydrolysed by 0.025 M sulfuric acid and 0.025 M hydrochloric acid at 90-135°C for 90 min.

**Figure 2** % Reducing sugar yield from 2.5% cassava pulps were hydrolysed by 0.025 M sulfuric acid and 0.025 M hydrochloric acid at 135°C for 15-90 min.
Figure 3 % Reducing sugar yield from 0.3-15% cassava pulps were hydrolysed by 0.025M sulfuric acid at 135°C for 90 min.

From Fig.1-3, 2.5% cassava pulps in diluted acids were hydrolysed under varying conditions and then determined % reducing sugar yield was determined. The results show that optimal % reducing sugar yields could be obtained at 69.96, 60.3 and 29.58% from hydrolysis at 135°C for 90 min with 0.025 M sulfuric acid, 0.025 M hydrochloric acid and 0.25 M acetic acid%, respectively. Thus, dilute sulfuric produced highest yield of reducing sugar. On the other hand, acetic acid could produce as much reducing sugars as did dilute sulfuric acid and hydrochloric acid, but was needed at the concentration that was much higher than the other two acids. So, the diluted sulfuric acid seemed to be suitable for cassava pulps hydrolysis.

Figure 4 % Reducing sugar yield from 2.5% cassava pulps were hydrolysed by various concentration of diluted acid at 135°C for 90 min.

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Table 1  %yield of sugars and by products in acid hydrolysate determined by HPLC

<table>
<thead>
<tr>
<th>Acid hydrolysis</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Rhamnose</th>
<th>5-HMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfuric acid</td>
<td>41.34</td>
<td>9.5</td>
<td>2.11</td>
<td>0.16</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>36.40</td>
<td>-</td>
<td>-</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 2  Ethanol production by acid hydrolysate fermented by *Saccharomyces cerevisiae*

<table>
<thead>
<tr>
<th>Substrate hydrolysis</th>
<th>Reducing sugars (g/l)</th>
<th>Ethanol (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr.</td>
<td>20 hr.</td>
</tr>
<tr>
<td>Acid hydrolysis</td>
<td>12.41 ± 0.25</td>
<td>0.54 ± 0.02</td>
</tr>
</tbody>
</table>

Results and Discussion

The first step of the present study consisted of the preparation of the cassava pulp hydrolysate. As can be seen in Table 1, the hydrolysis conditions provided a hydrolysate with a high concentration of glucose (41.34%) and a low concentration of xylose (9.5%) and rhamnose (2.11%) for diluted sulfuric acid hydrolysis. The diluted hydrochloric acid hydrolysis conditions provided a hydrolysate with a lower concentration of glucose (36.40%) but xylose was not found and low amount of 5-HMF found in both diluted acid hydrolysates. The main objectives of the present study were to investigate the possibility of ethanol production from cassava pulp from acid hydrolysis to evaluate the potential substrates in ethanol production. The results showed that *S. cerevisiae* was good in fermentation at 30°C using acid hydrolysate. The results showed that cassava pulp could be used to be carbon source for ethanol production. The reducing sugar conversions 46.78% acid hydrolysate. Although, the ethanol yields were considered rather low, 0.23 g ethanol/g cassava pulp added for enzymatic and acid hydrolysate, respectively, they were comparable with data obtained in the literature varied between 0.11 and 0.4 g/g.

Conclusion

The results showed that three diluted acids; sulfuric acid, hydrochloric acid and acetic acid; were evaluated for the acid hydrolysis for the production of reducing sugars from cassava pulps. 0.025 M sulfuric acid was found to yield significantly more reducing sugars than hydrochloric acid and acetic acid. From HPLC determination of sugars in the acid hydrolysate showed high contents of glucose with low amount of 5-HMF. When the hydrolysate was used as carbon source it could be produce high concentration of ethanol so it is possibly used as the carbon source of yeast fermentation for ethanol production.

References

Chotineranat, S.1996 Production of reducing sugars for cassava pulps by enzyme and ultrafiltration M.Sc. (Food Technology) Thesis Chulalongkorn University, Bangkok.