The Separation of Solids from the Liquefied Mash of Cassava Tuber and Continuous Saccharification by Immobilized Glucoamylase

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Summary. The solid material in liquefied mash of cassava tuber was very efficiently separated by a mixture of Trichoderma cellulase and Aspergillus niger pectinase. The solid content of the residue after the treatment and centrifugation decreased from 29.5% to 7.0%. The transparent digested solution from cassava tuber after centrifugation was continuously saccharified by glucoamylase immobilized in a gel which was prepared using polyvinylpyrrolidone and γ-ray irradiation. The addition of 50 ppm of sulfite ion completely prevented microbial contamination during the 18 days of operation. The final DE (dextrose equivalent), glucose content and disaccharide content in the hydrolyzate were 98, 94.4 and 3.3%, respectively.

Introduction

The production of ethanol for use as an automobile gasoline substitute has great potential in Brazil, the United States and a number of tropical countries (Marion 1979). Sugar cane, corn or cassava are the raw materials for ethanol production.

Rapid technological advances are also being made in the production of ethanol from molasses and sugar cane juice. Two examples being the continuous fermentation system using immobilized growing yeast (Kierstan and Bucke 1977) and the short-term fermentation system using a yeast recycling process (Inoue 1981). However, such systems are inappropriate for the production of ethanol from starchy raw material since they do not provide efficient separation of the solid material from the mash. Initially, more efficient separation procedures for potential use in new fermentation systems were therefore investigated using cassava tubers. A second step used direct saccharification of the digested cassava solution after separation of the solids using immobilized glucoamylase. A saccharification process in which microbial contamination was eliminated was especially investigated.

Materials and Methods

Materials. Dry cassava cubes from Manihot utilissima POHL were imported from Lampung, Indonesia. They contained 9.4% moisture, 79.7% starch, 1.1% crude protein, 2.4% ash and 7.4% other components.

Three kinds of cellulase, 3 kinds of pectinase and 2 kinds of glucoamylase preparations were used. Their origin, commercial names, producers and abbreviations are shown in Table 1.

Assay of the Enzyme Protein. Enzyme Protein was determined by Lowry’s method (Lowry et al. 1951).

Assay of Ionic Species. Metal ions were measured with an atomic adsorption spectrophotometer. Chloride, sulfate, phosphate and cyanide ions were determined by analytical methods described in “Japanese Industrial Standard”.

Assay of the Reducing Sugar, Total Sugar and Glucose Content. These assays were performed according to Maeda et al. (1979).

Assay of the Enzyme Activities

Avicelase. One ml of the enzyme solution was added to 9 ml of 0.1 M acetate buffer (pH 5.0) containing 100 mg of microcrystalline cellulose suspension and the mixture was reciprocally shaken at 130 strokes/min at 40 °C for 2 h. The enzyme reaction was stopped by adding 10 ml of “Somogyi” copper solution (modified method of Kobayashi 1954) to assay the reducing value. The hydrolysis curves are shown in Fig. 1. The activity was calculated from the slope of the second linear part of the curve.

CMCase. One ml of the enzyme solution was added to a substrate solution containing 5 g of 1% CMC solution (pH 5.0) and 4 ml of
Table 1. List of enzyme preparations employed

<table>
<thead>
<tr>
<th>No.</th>
<th>Kind of enzyme</th>
<th>Origin</th>
<th>Commercial name and producer</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cellulase</td>
<td><em>Trichoderma pseudokoningii</em></td>
<td>Sumyzyme C, Shin-Nihon Kagaku Co.</td>
<td>C-TP-S</td>
</tr>
<tr>
<td>2</td>
<td>Cellulase</td>
<td><em>Trichoderma reseei</em></td>
<td>Celluclast, Novo Industry Co.</td>
<td>C-TR-N</td>
</tr>
<tr>
<td>3</td>
<td>Cellulase</td>
<td><em>Aspergillus niger</em></td>
<td>Sumyzyme AC, Shin-Nihon Kagaku Co.</td>
<td>C-AN-S</td>
</tr>
<tr>
<td>4</td>
<td>Pectinase</td>
<td><em>Aspergillus niger</em></td>
<td>Sumyzyme AP2, Shin-Nihon Kagaku Co.</td>
<td>P-AN-S</td>
</tr>
<tr>
<td>5</td>
<td>Pectinase</td>
<td><em>Aspergillus genus</em></td>
<td>Ultrazyme, Novo Industry Co.</td>
<td>P-AN-N</td>
</tr>
<tr>
<td>6</td>
<td>Pectinase</td>
<td><em>Aspergillus genus</em></td>
<td>Igazyme, Swiss Ferment Co.</td>
<td>P-AG-S</td>
</tr>
<tr>
<td>7</td>
<td>Glucoamylase</td>
<td><em>Rhizopus delmer</em></td>
<td>Sumyzyme, Shin-Nihon Kagaku Co.</td>
<td>G-RD-S</td>
</tr>
<tr>
<td>8</td>
<td>Glucoamylase</td>
<td><em>Aspergillus niger</em></td>
<td>Amyloglucosidase 150 L, Novo Industry Co.</td>
<td>G-AN-N</td>
</tr>
</tbody>
</table>

**Fig. 1.** Hydrolysis of avicel by a cellulase preparation from *Aspergillus niger*. Three and 0.1 mg of the enzyme protein were used in each assay.

0.1 M acetate buffer (pH 5.0) and the mixture was then incubated at 40 °C for 10 min with shaking. The enzyme reaction was stopped by adding the copper solution and the reducing value was assayed. The activity was calculated from the slope of the initial linear part of the curve.

**Xylanase and Glucoamylase.** One ml of the enzyme solution was added to a mixture of 5 ml of 2% xylene or 2% dextrine (DE 10) solution and 4 ml of 0.1 M acetate buffer (pH 5.0 or pH 4.5) and the mixture was then incubated unshaken at 40 °C for 10 min. The enzyme reaction was stopped by addition of the copper solution.

**Assay of the Activity for Immobilized Glucoamylase Gel**

A desired amount of the gel, 1 ml of distilled water and 4 ml of 0.1 M acetate buffer (pH 4.5) were put into a 100-ml Erlenmeyer flask. After the contents of the flask had been incubated at 40 °C for 5 min, 5 ml of 2% dextrine (DE 10) solution at 40 °C was added to the flask to start the enzyme reaction. The flask was reciprocally shaken at 130 strokes/min and 40 °C for 10 min during the course of the enzyme reaction. The copper solution was added to stop the reaction and the reducing value assayed.

All enzyme activities are defined in international standard units.

**Preparation of Liquefied Cassava Solution for a Bench-Scale Experiment**

One kg of cassava cubes was put into 3 l of tap water and homogenized with a "Polytron" type of blender (model PT, 2,000). Thermostable α-amylase (0.5 ml; Termamyl 60 L, Novo Industry Co.) was thoroughly mixed with the homogenized material and a small amount of the mixture was gradually added to 1.7 l of hot water; the temperature being maintained at 75–85 °C for liquefaction. The above procedure took about 20 min. The mixture was then kept for 160 min at 85–95 °C. After confirming that addition of iodine gave a red colour at the final stage, liquefaction was stopped by adjusting to pH 4.5–5.0.

To separate the solid material, a desired amount of the enzyme preparation or the enzyme mixture was added to the liquefied material and the mixture was incubated at 40 °C for 17.5 h with mechanical mixing. The enzyme reaction was stopped by heating to 80 °C after bringing the volume to 5.3 l and the digested material centrifuged and filtered. The recovery of the supernatant was checked.

**Preparation of Immobilized Glucoamylase Gel**

Crude glucoamylase solution from *Aspergillus niger* was used after dialysis. The activity and the protein content were 3,080 units (40 °C, pH 4.5) and 57.2 mg/ml, respectively. The gel was prepared according to Maeda (1975). Ten grams of polyvinyl-