Production of Glucose from the Hydrolysis of Cassava Residue using Bacteria Isolates from Thai Higher Termites

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Abstract—The possibility of using cassava residue containing 49.66% starch, 21.47% cellulose, 12.97% hemicellulose, and 21.86% lignin as a raw material to produce glucose using enzymatic hydrolysis was investigated. In the experiment, each reactor contained the cassava residue, bacteria cells, and production medium. The effects of particles size (40 mesh and 60 mesh) and strains of bacteria (A002 and M015) isolated from Thai higher termites, *Microcerotermes* sp., on the glucose concentration at 37°C were focused. High performance liquid chromatography (HPLC) with a refractive index detector was used to determine the quantity of glucose. The maximum glucose concentration obtained at 37°C using strain A002 and 60 mesh of the cassava residue was 1.51 g/L at 10 h.

Keywords—Hydrolysis, termites, glucose, cassava

I. INTRODUCTION

DURING the last decades, shortages of petroleum and climate change have increased the interest in renewable energy in many countries. Conversion of abundant lignocellulosic biomass to biofuels as transportation fuels presents a viable option for improving energy security and reducing greenhouse emission [1]. Lignocellulosic biomass, a carbon neutral resource, mainly consists of three components: cellulose, hemicellulose and lignin, together with some extractives and minerals [2].

Lignocellulosic biomass comprising forestry, agricultural, and agro-industrial wastes are abundant, renewable, and inexpensive energy sources. Such wastes include a variety of materials such as sawdust, poplar trees, sugarcane bagasse, waste paper, brewer's spent grains, switchgrass, and straws, stems, stalks, leaves, husks, shells and peels from cereals like rice, wheat, corn, sorghum and barley, among others. Lignocellulose wastes are accumulated every year in large quantities, causing environmental problems. However, due to their chemical composition based on sugars and other compounds of interest, they could be utilized for the production of a number of value added products, such as ethanol, food additives, organic acids, enzymes, and others. Therefore, besides the environmental problems caused by their accumulation, the non-use of these materials constitutes a loss of potentially valuable sources [3].

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Cellulose and hemicellulose are the largest fraction of the plant cell wall of agricultural residues such as straw from wheat, corn, rice soy and cotton, sugarcane bagasse, and cassava residue.

Cassava residue, a potential candidate for bioethanol production, is a solid fibrous dry by-product of cassava-processing industry. The dry residue has a composition of starch (56-60%), cellulose (15-18%), hemicellulose (4-5%), lignin (2-3%), protein (1.5-2.0%), pentosans (2%), and reducing sugars (0.4-0.5%). Because of its low content of cellulose and hemicellulose and high starch content, cassava residue could be considered as a cellulo-starch by-product. And due to the rich organic nature and low ash content, cassava residue could offer numerous advantages in comparison to other crop residues such straw of rice and wheat. Compared with sugarcane bagasse, cassava offers advantages, as it does not require any pretreatment and can be easily attacked by micro-organisms [4,5].

Enzymatic hydrolysis of cellulose is carried out by cellulase enzymes, which are highly specific. Products of the hydrolysis are usually reducing sugars including glucose. This method has many advantages compared to chemical hydrolysis such as low utility cost, higher sugar yields, and no corrosion problem [6,7].

Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: 1) endoglucanase (EG, endo 1,4-D-glucanohydrolase, or EC 3.2.1.4.); 2) exoglucanase or cellobiohydrolase (CBH, 1,4-β-D-glucan cellobiohydrolase, or EC 3.2.1.91.); 3) β-glucosidase (EC 3.2.1.21) [6]. These cellulases can be produced by bacteria in higher termite's gut. obtained strains with a high specific activity for the cellulases [8].

The purpose of this research was to study and optimize the production of glucose through enzymatic hydrolysis of cassava residue. Effects of particle size of cassava residue, types of bacteria, and operation temperature were investigated.

II. EXPERIMENTAL

A. Materials

Cassava residue was from Sapthip Co., Ltd., Thailand. carboxymethyl cellulose (CMC) was purchased from Fluka, Sigma-Aldrich Co., Inc., Singapore. Malt extract was from Lab Scan Analytical Sciences, Thailand. Yeast extract was from Bio Springer, France. Sodium hydroxide (NaOH) was from Merck KGaA, Germany. Standard sugars (glucose, xylose, arabinose, mannose and galactose) for HPLC analysis, from Merck KGaA, Germany.

B. Studied Conditions

The studied parameters were particle size of cassava residue (40 mesh and 60 mesh), and bacteria strains(A002 and M015) at 37°C

C. Preparation of Cassava Residue and Composition Analysis

Cassava residue was dried at 105 °C and stored in sealed plastic bags. Then, the dried cassava residue was milled to small particle sizes and sieved to sizes between 40 to 60 mesh.

D. Preparation of Bacteria Cells for Microbial Hydrolysis

For the preparation of bacteria cells, an innoculum was prepared by transfering a loop of colonies into a 250 ml Erlenmayer flask containing 50 ml of 65 modified DSMZ broth medium 2 with pH of 7.2. The culture was incubated at 37 °C in a shaking incubator at 180 rpm for 12 h. Then, 50 ml of the prepared inoculum was transferred into a 500 ml bottle with a screw cap containing 450 ml of the production medium (65 modified DSMZ broth medium 2, pH 7.2) and incubated again at 37 °C in a shaking incubator at 180 rpm for 12 h. After that, the cells were harvested by centrifugation (8,000 rpm, 4 °C for 10 min)[9].

E. Microbial Hydrolysis

For the hydrolysis, the reactor was added with the production medium (65 modified DSMZ broth medium 2 without CMC, pH 7.2) and cassava residue powder, which was autoclaved under clean conditions. The reactor contained 0.10-0.11 g cassava residue powders, 0.3-0.4 g bacteria cells, and 1 mL of the production medium. The reactor was placed in a shaking incubator at 37°C and was shaken at 180 rpm. All the samples were taken out every hour for 24 h.

F. Determination of Glucose Concentrations

Glucose was analyzed by a high performance liquid chromatography (HPLC) with an organic acid column (aminex HPX-87P). Distilled water was used as the mobile phase at a flow rate of 0.6 ml/min. The column temperature was fixed at 80 °C.

III. RESULTS AND DISCUSSION

A. Biochemical Constituents of Cassava Fibrous Residue

The results of elemental and chemical composition of cassava residue are shown in Tables I and II, respectively. The major components of cassava residue are carbon, followed by oxygen and hydrogen. And cellulose is the major component of fiber of this raw material.

TABLE I
ELEMENTAL COMPOSITION OF THE CASSAVA RESIDUE

Elemental composition	wt%, dry basis
Carbon	39.66
Hydrogen	5.38
Nitrogen	1.52
Oxygen	53.36
Sulfur	0.08

TABLE II
CHEMICAL COMPOSITION OF CASSAVA RESIDUE

Chemical composition	wt%, dry basis
Moisture	4.94
Protein	13.31
Fat	1.18
Fiber	23.30
Ash	12.60
Starch	49.66
ADF	43.33
NDF	56.29
Cellulose	21.47
Hemicellulose	12.97
Lignin	21.86

B. Enzymatic Hydrolysis

The casava residue was hydrolyzed for 24 h at 37 °C by two effective isolates (strain A 002 and M 018). The two isolates were obtained from Thai higher termites *Microcerotermes* sp., and found to be effective for cellulose hydrolysis [8].

1. Effects of Cassava Residue Particle Size on the Produced Sugar Concentration

The optimum particle size of cassava residue for the enzymatic hydrolysis was investigated under fixed concentration of substrate between 1.0-1.1 g/L. As shown in Figure 1, using the 60 mesh size of cassava residue and bacteria strain A 002 slightly increase the amount of glucose concentration and decrease in an hour later. Then, it sharply increases to the maximum, about 1.51 g/L, at 10 h before gradually decreasing. With the 40 mesh size of cassava residue, although the glucose evolution has similar pattern with the 60 mesh size, the evolution is faster. Effects of the cassava residue particle size using strain M 015 are shown in Figure 2. Both particle sizes of raw material result in the gradual increase in the amount of glucose concentration untill it reaches the maximum concentration, 1.10 g/L for 60 mesh and 0.87 g/L for 40 mesh size of cassava residue.
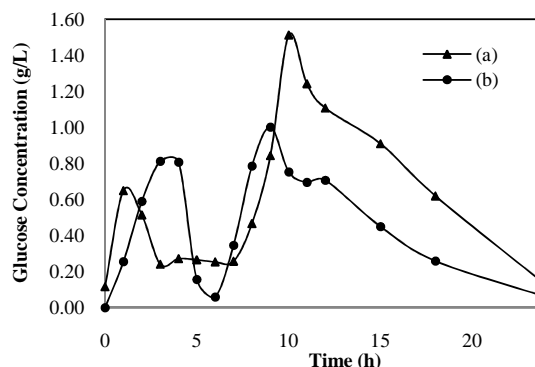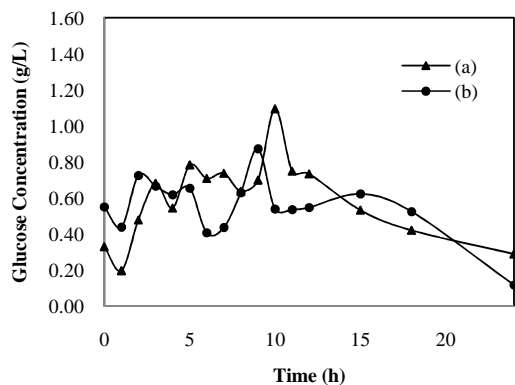

Fig. 1 Effects of particle size for 60 mesh (a) and 40 mesh (b) of casava residue on the glucose concentration produced from the hydrolysis of casava residue at 37 °C using the bacterial strain A 002.

Fig. 2 Effects of particle size for 60 mesh (a) and 40 mesh (b) of casava residue on the glucose concentration produced from the hydrolysis of casava residue at 37 °C using the bacterial strain M015.

With both strain A 002 and strain M015, the particle size clearly affects the enzymatic hydrolysis of cassava residue. At 37 °C, the smaller particle, which has a higher specific surface area than the larger particles, can be hydrolyzed faster than the larger particle raw material. The obtained results are in agreement with the results from Yeh et al. [10], who reported that a particle size in the submicron scale caused a significant increase in the digestibility of cellulose.

2. Effects of Bacterial Strains on the Produced Glucose Concentration

The performance of both strain A 002 and strain M 015 on the enzymatic hydrolysis of cassava residue was investigated. Comparing between results from both strains at 60 mesh size of casava residue, shown in Figure 3, strain M 015 almost shows higher amount of glucose concentration than strain A015 during the first 7 h and seems to maintain the glucose concentration over 18 h. This result is due to strain M 015 has a higher specific endoglucanase activity to create more free chain-ends of cellulose than strain A 002 [8].
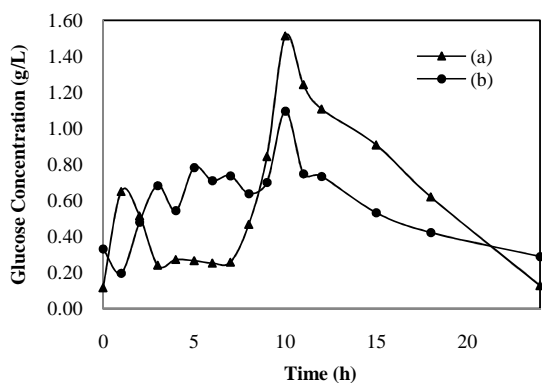

Fig. 3 Effects of bacteria strains A002 (a) and M015 (b) for 60 mesh of casava residue on the glucose concentration produced from the hydrolysis of casava residue at 37 °C
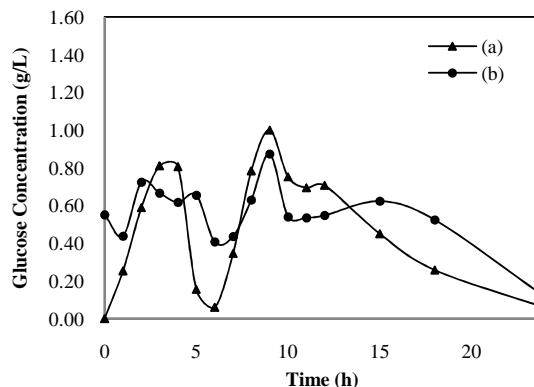

Fig. 4 Effects of bacteria strains A002 (a) and M015 (b) for 40 mesh of casava residue on the glucose concentration produced from the hydrolysis of casava residue at 37 °C

However, strain A 002 shows higher amount of glucose concentration for both of 60 mesh and 40 mesh size of cassava residue, about 1.51 g/L and 1.00 g/L, respectively as shown in Figures 3 and 4. This is because of its β-glucosidase activity. This activity is an ability to convert free chain-ends of cellulose to glocose.

C. Structure of Enzymatically Hydrolyzed Cassava Residue Sample

The morphological changes of cassava residue charac-teristics due to the enzymatic hydrolysis can be seen from the scanning electron micrographs at 1,000 magnifications. Figure 5a shows the smooth surface of cassava residue before hydrolysis, and the morphologies of 60 mesh size of cassava residue, which were hydrolyzed by bacteria strain A 002 and strain M 015 at 37°C, are shown in Figure 5b and Figure 5c, respectively. After considering the last two figures, the morphology of using strain A002 as a bacteria cell changes more significantly, consistent with the results from Taechapoempol et al. [8], which shows the higher activity of the strain A 002.
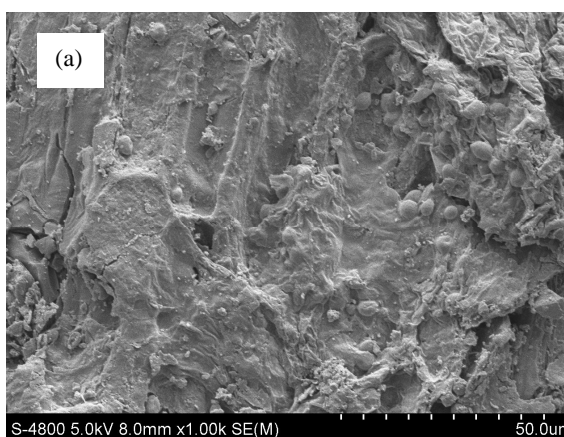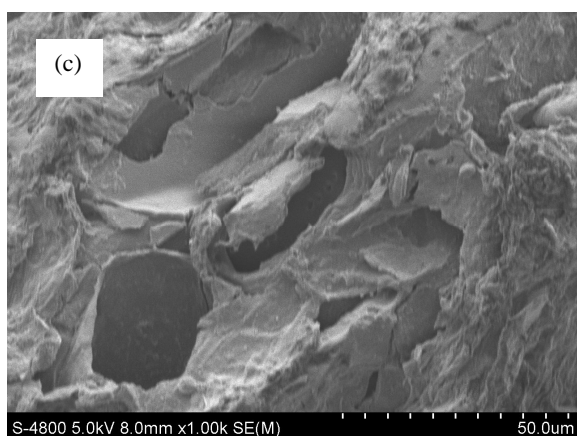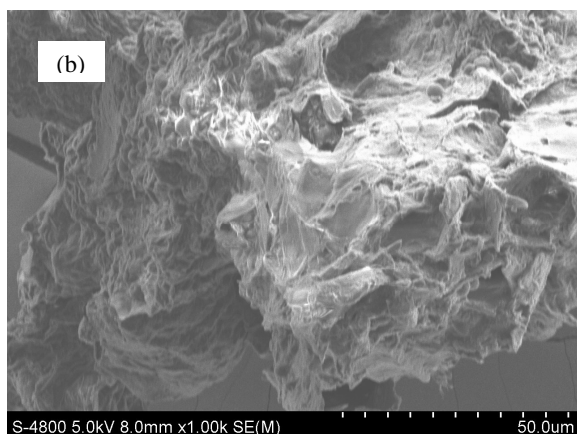
Fig. 5 Scanning electron micrographs of the 60 mesh size cassava residue surface (a) before hydrolysis (b) after hydrolysis at 37 °C with the strain A 002 and (c) after hydrolysis at 37 °C with the strain M 015

IV. CONCLUSIONS

In this research, the two effective isolates (strain A 002 and strain M 015), *Microcerotermes* sp., from Thai higher termites were used to determine their hydrolysis activity of cassava residue at 37 °C. The optimum condition for maximum glucose concentration was 1.51 g/L at 10 h using 60 mesh sizes of cassava residue and strain A 002 as a bacteria cell at 37 °C.

ACKNOWLEDGMENTS

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