Approach of Cassava Waste Pretreatments for Fuel Ethanol Production in Thailand

Teerapatr Srinorakutara*, Lerdluk Kaewvimol and La-aied Saengow

Cassava waste can be utilized to produce ethanol due to its containing cellulose and hemi-cellulose at levels of 24.99 and 6.67 % (w/w) respectively. A cassava pretreatment method using enzymatic and acid hydrolysis for converting starch into fermentable sugars was investigated. The cassava waste was hydrolyzed by using 0.2-5.0 M sulfuric acid (H_2SO_4) at a temperature of 60-120^oC for 30 min. It was found that the maximum reducing sugar obtained at 6.1% (w/v) using a cassava waste to acid ratio of 1:2 (w/v) at 0.6 M H_2SO_4 and 120^oC. While the maximum reducing sugar obtained in this study was at 6.2% (w/v), using a mixture of cellulase and pectinase at pH 4.5, 28^oC for 1 hr followed by α-amylase at pH 5.5, 100^oC for 2 hrs and finally glucoamylase at pH 4.5, 60^oC for 24 hrs. This is the optimum condition for cassava waste pretreatment using enzymatic hydrolysis. It was also found that the maximum ethanol production using *Saccharomyces cerevisiae* TISTR 5596 from enzymatic hydrolyzed sugar at initial reducing sugar 8.92% (w/v) was 3.62% (w/v) at 24 hrs of fermentation, corresponding to a 91% theoretical yield.

Key words: Cassava waste, cellulosic pretreatment, ethanol production and *Saccharomyces cerevisiae*

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การย่อยกากมันสำปะหลังเพื่อผลิตเชื้อเพลิงเอทานอลในประเทศไทย

ธีรภัทร ศรีนรคุตร เลิศลักษณ์ แก้ววิมล และละเอียด แซ่โง้ว (2549)
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กากมันสำปะหลัง ประกอบด้วยเซลลูโลส และเฮมิเซลลูโลส ร้อยละ 24.99 และ 6.67 โดยน้ำหนักแห้ง ตามลำดับ สามารถนำกากมันสำปะหลังมาใช้ประโยชน์เพื่อการผลิตเอทานอลได้โดยผ่านขั้นตอนการ pretreatment ด้วยการไฮโดรไลซ์โดยใช้กรดหรือเอนไซม์เพื่อเปลี่ยนแป้งให้เป็นน้ำตาล ในการศึกษาการไฮโดรไลซ์ด้วยกรดจะใช้กรดซัลฟูริกความเข้มข้น 0.2-5.0 โมลาร์ ทำการย่อยที่อุณหภูมิ 60-120^{O}C เป็นเวลา 30 นาที พบว่าใช้กรดซัลฟูริกความเข้มข้น 0.6 โมลาร์ ที่อุณหภูมิการย่อย 120^{O}C อัตราส่วนกากมันสำปะหลังต่อกรดเท่ากับ 1:2 จะได้น้ำตาลรีดิวซ์สูงสุด ร้อยละ 6.1 โดยน้ำหนักต่อปริมาตร ในขณะที่การใช้วิธีไฮโดรไลซ์ด้วยเอนไซม์ผสม พบว่าการใช้เอนไซม์ผสมระหว่างเซลลูเลสและเพคติเนส ที่อุณหภูมิ 28^{O}C และค่า pH 4.5 เป็นเวลา 1 ชั่วโมง ตามด้วยเอนไซม์แอลฟาอะไมเลส ย่อยที่อุณหภูมิ 100^{O}C และค่า pH 5.5 เป็นเวลา 2 ชั่วโมง และเอนไซม์กลูโคอะไมเลส ที่อุณหภูมิ 60^{O}C และค่า pH 4.5 เป็นเวลา 24 ชั่วโมง เป็นสภาวะที่เหมาะสมที่สุด ซึ่งได้น้ำตาลรีดิวซ์ ร้อยละ 6.2 โดยน้ำหนักต่อปริมาตร และการนำน้ำตาลที่ได้จากการย่อยกากมันสำปะหลังด้วยเอนไซม์ ปริมาณน้ำตาลรีดิวซ์ ร้อยละ 8.92 โดยน้ำหนักต่อปริมาตร ไปหมักกับเชื้อยีสต์ *Saccharomyces cerevisiae* TISTR5596 ในถังหมักขนาด 10 ลิตร จะได้ปริมาณเอทานอลสูงสุด ร้อยละ 3.62 โดยน้ำหนักต่อปริมาตร ในชั่วโมงที่ 24 คิดเป็นประสิทธิภาพการหมักร้อยละ 91

คำสำคัญ กากมันสำปะหลัง การย่อยเซลลูโลส การผลิตเอทานอล *Saccharomyces cerevisiae*

INTRODUCTION

Cassava is a major raw material used in many industries in Thailand. Production of MSG (Monosodium glutamate) and other amino acids, sweeteners, ethanol, makes use of cassava. The cassava wastes left over from these production processes are abundant and still contain a high amount of starch content. Most cassava wastes can be used as animal feed due to its high content of protein and other nutrients which are necessary for animal growth. In addition, cassava wastes can be used to produce ethanol. Use of cassava waste as raw material in ethanol production not only reduces waste material created from the cassava starch industry, but also lowers the cost of ethanol production.[1]

Two technologies used to convert cellulose and hemicellulose to fuel ethanol are acid and enzymatic hydrolyses. The most common is acid hydrolysis.[2]

Acidic hydrolysis is an effective method used for raw material pretreatment in ethanol production. In previous research, a high concentration of acid has been used to treat waste materials that contain cellulose. Although acids are powerful agents used for biomass hydrolysis, concentrated acids are toxic, erosive and hazardous. Handling high concentrations of acid requires reactors that are resistant to erosion in raw material pretreatment. Diluted acid hydrolysis has been successfully developed for pretreatment of cellulose materials. Diluted sulfuric acid (H_2SO_4) can achieve significant results. Agu *et al.*[3] reported on the effect of a combined heat treatment and acid hydrolysis study on cassava grated waste (CGW) biomass. At 120^{o}C for 30 min and using a high concentration of H_2SO_4 (1-5M) hydrolysis was achieved but with excessive charring or dehydration reactions. A 60% process efficiency was achieved with 0.3 M H_2SO_4 with ethanol yield at 3.5%(v/v) after yeast fermentation. Acid hydrolysis of cellulosic pyrolysate to glucose and its fermentation to ethanol were investigated by Zhisheng and Hongxun.[4,5] The maximum glucose yield of 17.4% was obtained by hydrolysis with 0.2 mol/l H_2SO_4 using autoclave at 121^{o}C for 20 min. The fermentation by *Saccharomyces cerevisiae* of a hydrolysate medium containing 95.8% glucose, resulted in about a 47% increase in ethanol production as compared to its present strain, which was fermented using commercial glucose**.** The reducing sugar was measured at 69.96% from 2.5% cassava pulp at 135^{o}C after 90 min.[6]

Another method of hydrolysis is enzymatic hydrolysis. Enzymes are naturally occurring plant proteins that cause certain chemical reaction to occur. However, for enzymes to work, they must obtain access to the molecules to be hydrolyzed. Eva-Lena Jakobsson[7] reported that an enzymatic pretreatment at 190^{o}C and 10 min resulted in the best over all yield for glucose. A hundred grams of wheat straw yielded 42.6 g glucose and 22 g xylose.

In the present paper, the optimum condition for acid and enzymatic hydrolyses of cassava waste was investigated.

Table 1. The chemical composition of cassava waste.

Composition	Cassava waste %(w/w)		
	Sample 1	Sample 2	Sample 3
Moisture	78.16	79.50	82.74
Protein	1.82	2.03	2.31
Fat	0.09	0.20	0.16
Ash	1.61	2.38	2.05
Fiber	10.61	14.35	14.56
Starch	69.90	61.84	64.36

Note: Cyanide content in cassava waste = 154 mg/kg dry weight and cyanide content after acid hydrolysis = 4.54 mg/kg dry weight

MATERIALS AND METHODS

Sample collection

Cassava waste was tendered by Sanguan Wongse industries company limited, Nakornratchasrima, Thailand in a frozen form. The samples were thawed to room temperature before performing the experiment.

Acid hydrolysis

Cassava waste (50 g) was hydrolyzed with 100 ml (1:2 w/v) of various concentrations of H_2SO_4 at 60-120^oC. The hydrolysates were separated to obtain any suspended or unhydrolysated materials. Each sample was neutralized by 2 M NaOH solution for analytical processing.

Enzymatic hydrolysis

Cassava wastes (8, 10, and 12 g) were hydrolyzed with various enzymes (*i.e.* α-amylase, glucoamylase, cellulase and pectinase) which were generous gifts from Genencor International Company. Several hydrolysis conditions, including cellulase 15 unit per gram cassava at pH 4.8, 40^oC for 20 min, pectinase 4.7 unit per gram cassava at pH 3.5, 20^oC for 30 min, mixture of cellulase and pectinase at pH 4.5, 28^oC for 1 hr, α-amylase 24.02 unit per gram cassava at pH 5.5, 100^oC for 2 hrs, and glucoamylase 0.66 unit per gram cassava at pH 4.5, 60^oC for 24 hrs, were tested. The hydrolysates were separated by centrifuge at 9,000 rpm for analysis.[8]

Analytical method

The AOAC method[9] was employed in the determination of the composition of cassava waste. The main components were starch, protein, fiber, ash, fat and moisture.

The reducing sugar content in the hydrolysates was determined quantitatively by using the Nelson Somogyi method as outlined by Agu *et al.*[3] After mixing the samples with the assay reagent, the absorbance was measured at 520 nm against the appropriate blank solution by using Spectrophotometer model Uvikon-xs. The amount of reducing sugar released was colorimetrically determined. A calibration curve was obtained using D-glucose as standard.

Cell number was determined by pore plate in yeast malt agar and a haemacytometer.[8]

Ethanol concentration was measured by using and ebulliometer.

Ethanol Production

Reducing sugar obtained from hydrolysis of cassava waste was fermented using *Saccharomyces cerevisiae TISTR 5596* in 10L fermenter at 30^oC for 60 hrs. Samples of fermented broth were collected every 6 hrs for cell number, reducing sugar, pH, and ethanol concentration analysis.

RESULTS AND DISCUSSIONS

Composition of cassava waste

Cassava waste was analyzed for its composition as shown in Table 1. The major component, starch, was approximately 65.37%. The second highest component was fiber at 13.17%. The composition of the cellulose in cassava waste is presented in Table 2. It can be seen from Table 2 that holo-cellulose and alpha-cellulose, which are composed of C6 and C5 sugars respectively, account for the majority of cellulose found. For ethanol production from cassava waste, C6 and C5 sugars were studied.

Table 2. The cellulosic composition of cassava waste.

	Composition (%w/w)
Holo-cellulose	100.00
- Alpha-cellulose	73.31
- Pentosan	26.69

Acid hydrolysis of cassava waste

In our first step, cassava wastes were hydrolyzed in a 60^oC waterbath for 30 min. Other samples were autoclaved at 100^oC, 110^oC, and 120^oC for 30 min at various H_2SO_4 concentrations (0.2-5.0 M). The oligomers and cellulosic materials in cassava waste were converted into glucose. The results showed cassava waste in a 60^oC waterbath for 30 min provided a lower sugar content than cassava waste hydrolyzed by autoclaving at 100^oC, 110^oC and 120^oC. The maximum reducing sugar at 6.09% (w/v) recovered from cassava waste was detected after pretreatment with 0.6 M H_2SO_4 for 120^oC for 30 min (Figure 1).

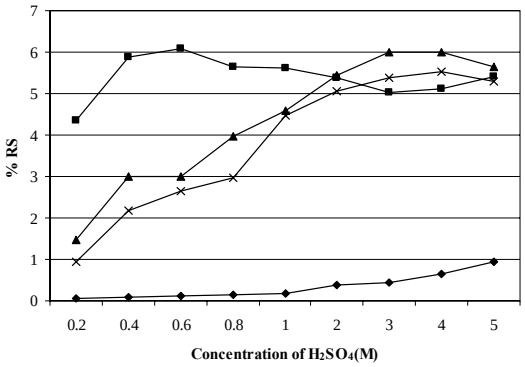

Figure 1. Effect of acid concentration on hydrolysis of cassava waste to reducing sugar (RS) at various temperature for 30 min.(♦) 60°C; (×) 100°C; (▲) 110°C; (■) 120°C.

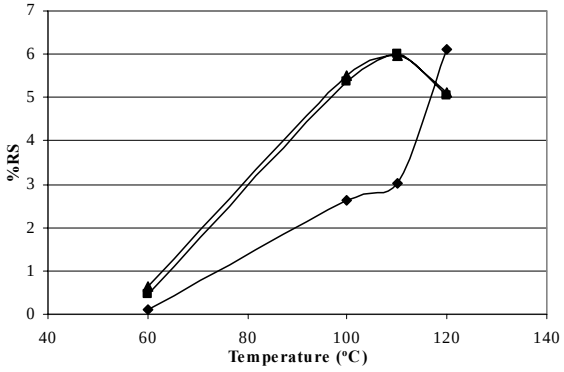

Figure 2. Percentage of reducing sugar at various temperatures of acid hydrolysis at (♦) 0.6 M; (■) 3 M; (▲) 4 M H_2SO_4

At higher concentrations of H_2SO_4 than 0.6 M, the reducing sugar was lower than 6.09%. This suggests that less reducing sugar in the solution may be derived from dehydrating or oxidizing by sulfuric acid on hydrolytic products. The solution obtained in this study also showed various colors. Similar results of acid hydrolysis of CGW biomass has been reported by Agu *et al.*[3] At high acid concentration used for CGW biomass hydrolysis, a lot of charring or browning or dehydrating reactions occurred to a varying degree. Other chemical reactions reported in previous studies include the formation of furfural from xylose.[10] Furfural was reported to inhibit activities of some glycolytic enzymes, particularly dehydrogenases in *S.cerevisiae* for ethanol production.[11]

After comparison of acid concentration and temperature for hydrolysis, time for acid hydrolysis was investigated. The effect of time for hydrolysis was studied at the maximum reducing sugar of each temperature.

The increase in temperature from 60°C to 110°C resulted in an increased amount of reducing sugar from 0.5 to 6.1%w/v particularly when using 3.0 and 4.0 M H_2SO_4. The reducing sugar decreased about 1%w/v when increasing temperature from 110°C to 120°C. However, the cassava waste pretreated using 0.6 M H_2SO_4 gave an increase in sugar level from 0.2 to 6.2%w/v when performing the experiment at all temperatures from 60°C to 120°C (Figure 2).

Figure 3 shows the consequence of acid hydrolysis time at 0.6 M H_2SO_4, 120°C on reducing sugar content. The reducing sugar content in the early phase of hydrolysis rapidly increased, but after 30 min the reducing sugar contents were almost constant. It was previously found[6] that the highest content of reducing sugar was also produced from an acid hydrolysis of 2.5 w/v cassava pulps at 135°C and 90 min reaction time. However, increasing hydrolysis time and temperature may increase the processing costs.

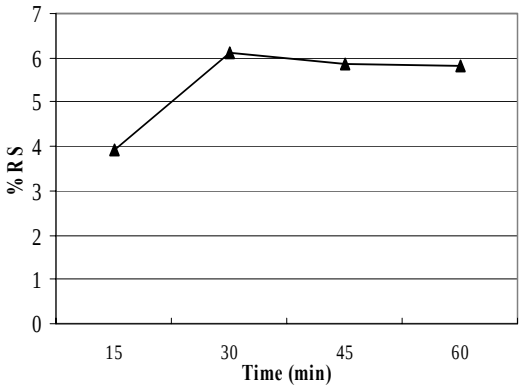

Figure 3. Percentage of reducing sugar from 0.6 M H_2SO_4 120°C at various time of acid hydrolysis.

Enzymatic hydrolysis of cassava waste

The cassava waste was hydrolyzed using four enzymes, cellulase, pectinase, α-amylase and glucoamylase and the hydroysis produced the maximum reducing sugar (Table 3). This was similar to the result of Supavadee.[12] The reducing sugar obtained using the mixture of all four enzymes was higher than when using mixtures of three enzymes (cellulase, α-amylase and glucoamylase or pectinase, α-aulase and glucoamylase).

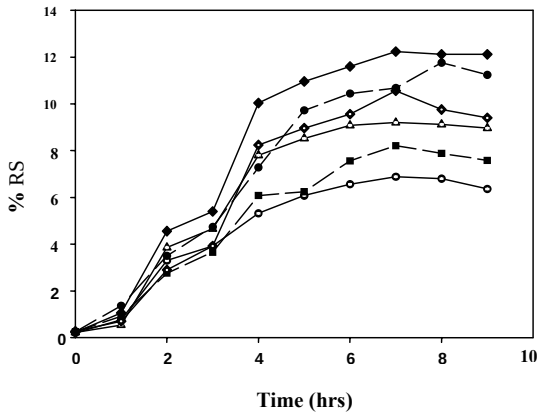

Figure 4. The amount of reducing sugar at different concentrations of carbohydrate by four enzyme treatment. (o) 7%; (■) 8%; (Δ) 9%; (◇) 10%; (◆) 11%; (●) 12%.

Figure 4 shows the effect of carbohydrate concentration on reducing sugar production. The reducing sugar in the early phase of hydrolysis rapidly increased and mostly showed maximum level at 7 hrs except for 12% carbohydrate showing a maximum level at 8 hrs. After 7-8 hrs of hydrolysis, the reducing sugar decreased. The maximum level of reducing sugar obtained at 11% of carbohydrate concentration at 7 hours is shown in Figure 5.

For the same amount of cassava waste, % reducing sugar by acid hydrolysis gave the same level as enzyme hydrolysis, *i.e.* about 6.2% reducing sugar. However, we used enzyme pretreatment for the ethanol production process because of the unwanted browning reaction of the product and the higher cost of acid hydrolysis.

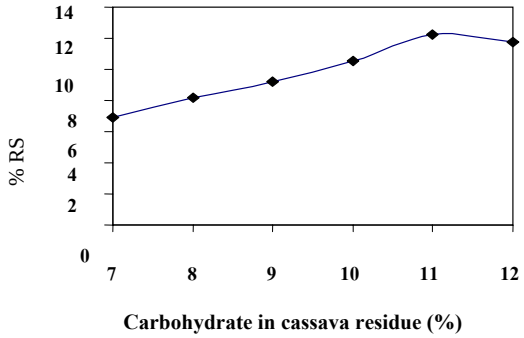

Figure 5. The maximum reducing sugar at different amounts of carbohydrate.

Table 3. Effect of different enzymatic treatments on percentage of reducing sugar.

Cassava residue (g, dry weight)	%Reducing sugar concentration by different enzymes						
	aA Glu	Ce aA Glu	Ce	Pe aA Glu	Pe	Ce Pe aA Glu	Ce Pe
8	2.91	3.11	0.13	3.26	0.19	3.39	0.32
10	3.16	3.68	0.21	3.35	0.26	3.86	0.43
12	4.23	4.74	0.25	4.53	0.30	4.98	0.52

Note: aA: a-amylase, Glu: Glucoamylase, Ce: Cellulase, and Pe: Pectinase.

Ethanol production

Ethanol production derived from enzymatic hydrolysate containing 8.92% (w/v) reducing sugar was done in 10L fermenter using *S. cerevisae* TISTR 5596. The maximum ethanol production obtained at 24 hours of fermentation was 3.62% (w/v), corresponding to 91% of theoretical yield and after that the level of ethanol remained constant (Figure 6).

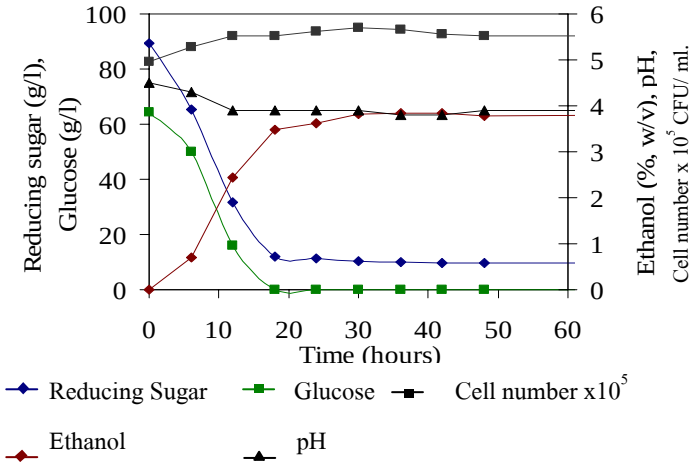

Figure 6. Ethanol production from enzymatically pretreated cassava waste by using *S.cerevisae* TISTR 5596 in 10L fermenter.

The results showed a shorter production time when compared with the results of Theppanya[13] who reported that 4.21%w/v ethanol production from cassava waste hydrolysate at 54 hours.

Ethanol production derived from acid hydrolysate and enzymatic hydrolysate and also from microorganism hydrolysate (not earlier mentioned) have been under investigation. Among the three hydrolysis methods, the use of microorganisms is probably the best one to pursue for fuel ethanol production in Thailand because it is not necessary to import expensive enzymes, and the microorganism hydrolysis method is friendly to the environment.

CONCLUSIONS

Dilute sulfuric acid was used to pretreat cassava waste in the production of reducing sugar. This study shows that the hydrolysate at 0.6 M H_2SO_4, at 120°C for 30 min gave the maximum reducing sugar at 6.1% (w/v). For a shorter hydrolysis time, a higher acid concentration must be used.

The use of the mixture of cellulase and pectinase at pH 4.5, at 28°C for 1 hr followed by α-amylase at pH 5.5, at 100°C for 2 hrs and finally glucoamylase at pH 4.5, at 60°C for 24 hrs was the optimum condition for enzymatic cassava waste pretreatment in this study and also gave the maximum reducing sugar at 6.2% (w/v).

The maximum ethanol production obtained from enzymatic hydrolysate containing 8.92% (w/v) reducing sugar using *S. cerevisae* TISTR 5596 in 10L fermenter at 24 hrs was 3.62% (w/v), corresponding to 91% of theoretical yield.

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