การผลิตเอทานอลจากไฮโดรไลเสตของการย่อยสลายเปลือกมันสำปะหลัง
ด้วยกรดโดยใช้ *Saccharomyces cerevisiae*

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บทคัดย่อ

ความเป็นไปได้เชิงพาณิชย์ของการผลิตเอทานอลจะเพิ่มขึ้น   ถ้าใช้แหล่งน้ำตาลจากการย่อยสลายวัสดุเหลือทิ้งลิกโนเซลลูโลสที่มีราคาถูก เช่น เปลือกมันสำปะหลังราคาถูกและมีอยู่เป็นจำนวนมากในประเทศเขตร้อน ดังนั้น วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาการผลิตเอทานอลจากสารละลายที่ได้จากการย่อยสลายของเปลือกมันด้วยกรดเจือจาง ผลการศึกษาพบว่ากรดซัลฟูริกเจือจางสามารถย่อยสลายเปลือกมันให้ผลได้ของน้ำตาลมากกว่าการใช้กรดไฮโดรคลอริกและกรดแอซีติก ไฮโดรไลเสตเปลือกมันสำปะหลังจากการย่อยโดยกรดซัลฟูริกเจือจางประกอบด้วยน้ำตาลกลูโคสเป็นส่วนมากภายใต้สภาวะที่เหมาะสมดังนี้ ความเข้มข้นกรดซัลฟูริก 0.1 โมลาร์ อุณหภูมิ 135 °ซ และเวลาที่ใช้ในการย่อยสลาย 90 นาที หลังจากการทำให้เป็นกลางด้วยด่าง ไฮโดรไลเสตประกอบด้วยน้ำตาลรีดิวส์และกลูโคสเท่ากับ 60.74 กรัม/100 กรัม และ 37.09 กรัม/100 กรัม เปลือกมัน ตามลำดับ ไฮโดรไลเสตจากเปลือกมันสำปะหลังถูกนำมาเป็นสับสเทรตในอาหารเพาะเลี้ยงเชื้อเพื่อศึกษาคุณสมบัติทางจลนพลศาสตร์การหมักเอทานอลของยีสต์ *Saccharomyces cerevisiae* (5019) โดยศึกษาการหมักเปรียบเทียบระหว่างการใช้ไฮโดรไลเสตและน้ำตาลกลูโคสเป็นสับสเทรตในอาหารเพาะเชื้อกึ่งสังเคราะห์ในขวดทดลองเขย่า ผลการทดลองพบว่า น้ำตาลกลูโคสในอาหารเพาะเชื้อกึ่งสังเคราะห์ถูกใช้หมดไปภายใน 18 ชั่วโมง ให้อัตราผลผลิตเอทานอลเชิงปริมาตร 0.51 กรัม/ลิตร.ชั่วโมง และผลได้เอทานอล 0.43 กรัม/กรัมกลูโคส กรณีที่ใช้ไฮโดรไลเสตเป็นสับสเทรตซึ่งมีน้ำตาลรีดิวส์ 18.42 กรัม/ลิตร และกลูโคส 10.24 กรัม/ลิตร น้ำตาลกลูโคสถูกใช้หมดภายใน 10 ชั่วโมง ให้อัตราผลผลิตเอทานอลเชิงปริมาตร 0.29 กรัม/ลิตร.ชั่วโมง และผลได้เอทานอล 0.27 กรัม/กรัมน้ำตาลรีดิวส์ ผลการทดลองแสดงให้เห็นว่าไฮโดรไลเสตจากเปลือกมันสำปะหลังที่ได้จากการย่อยสลายด้วยกรดซัลฟูริกเจือจางสามารถใช้เป็นสับสเทรตของการหมักเอทานอลโดยยีสต์ได้

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Ethanol Production from Acid Hydrolysate of Cassava Peels using *Saccharomyces cerevisiae*

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Abstract

Economic feasibility of ethanol production can be enhanced if the fermentable sugars can be obtained from the acid hydrolysis of low-cost lignocellulosic wastes. Cassava peels are renewable, cheap and widely available wastes in tropical countries. The aim of this work was to study the ethanol production from cassava peel hydrolysate prepared by dilute-acid hydrolysis. Sulfuric acid was found to be more effective acid for the degradation of cassava peels to fermentable sugars, compared to hydrochloric acid and acetic acid. Acid hydrolysate of cassava peels, comprised mainly of glucose, was obtained after dilute-acid hydrolysis under optimum condition at 135 °C for 90 min. Neutralized hydrolysates containing reducing sugars and glucose ca 60.74 g/100g and 37.09 g/100g cassava peels, respectively, were used as substrates for ethanol production. Cassava peel hydrolysates with high sugar concentration were used as the substrates in the fermentation medium to evaluate the kinetic behavior of *Saccharomyces cerevisiae* (5019) during the fermentation. Experiments were conducted with using glucose semi-synthetic medium in shaking flasks. Glucose was consumed within 18 h of fermentation. The volumetric ethanol productivity of 0.51 g/l.h and ethanol yield of 0.43 g/g were achieved. When reducing sugars from hydrolysates were used the fermentable sugars were consumed within 10 h. The volumetric ethanol productivity of 0.29 g/l.h and ethanol yield of 0.27 g/g were obtained. These results showed that diluted-acid hydrolysis of cassava peels are promising substrates for use in ethanol production.

Keywords : Ethanol / Cassava Peels / Acid Hydrolysate / Fermentation

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1. Introduction

The bioconversion of crops and residues to fuels and chemicals is receiving increased interest due to the perceived need for the reduction of consumption and importation of petroleum fuels. Many of the biomass feedstocks contain significant quantities of lignocellulose, which upon hydrolysis yield sugars. A more complete utilization of biomass can be achieved by controlled hydrolysis of the cellulose and hemicellulose fractions and bacterial fermentation of the pentose and hexose sugars [1]. Chemical hydrolysis, especially acid hydrolysis, is one of a number of a viable technologies being developed as biomass conversion process. Dilute acids can be also used for hydrolysis of agricultural residues. It consists of the hydrolysis of starch, hemicellulose, cellulose and lignin fractions. Sulfuric acid [2], hydrochloric acid [3], HF [4] or acetic acid [5] are commonly employed in acid hydrolysis . The acids release protons that break the heterocyclic ether bonds between the sugar monomers in the polymeric chains formed by the starch, hemicellulose and the cellulose. The breaking of these bonds releases several compounds, mainly sugars such as xylose, glucose and arabinose. Other compounds released are oligomers, furfural and acetic acid [6]. Hydrolysis reactions of sugar polymers in a dilute-acid medium are very complex. The mechanism of the hydrolysis reaction includes: (i) diffusion of protons through the wet lignocellulosic matrix; (ii) protonation of the oxygen of a heterocyclic ether bond between the sugar monomers; (iii) breaking of the ether bond; (iv) generation of a carbocation as intermediate; (v) solvation of the carbocation with water; (vi) regeneration of the proton with cogeneration of the sugar monooligomer or polymer depending on the position of the ether bond; (vii) diffusion of the reaction products in the liquid phase if it is permit for their form and size; (viii) restarting of the second step [6].

After acid hydrolysis, the predominant monosaccharides from lignocellulose are glucose and xylose (about 40% glucose and 20% xylose of dry matter) [7, 8]. The most efficient microorganisms for converting glucose into ethanol are industrial yeast strains of *Saccharomyces cerevisiae* and bacterial strains of *Zymomonas mobilis,* but none of these are able to utilize xylose and arabinose. Recently, recombinant strains of *S. cerevisiae, Z. mobilis,* and *Escherichia coli* have received the genes coding of enzymes for conversion of xylose into ethanol [9]. Cassava is an important source of carbohydrate and cassava peel is the waste material obtained from cassava starch production. However cassava peel consisted of starch, cellulose, hemicellulose and lignin is discarded. Cassava peels, the main by- product from processing tuberous roots of cassava for human consumption, could be used to be the source of fermentable sugars for ethanol production. The aim of this work was to investigate the ethanol production from the solubilized reducing sugars obtained by dilute- acid hydrolysis of cassava peel using *S. cerevisiae.*

2. Materials and Methods

2.1 Sample collection and preparation

Cassava peel containing 20-25% moisture was collected from Kowchangaear factory, Chonburi province. They were dried at 35 °C in a hot-air oven for 4 days, milled, screened to select the fraction of particles with a size of 45-697 µm, homogenized in a single lot and stored until needed.

2.2　Acid hydrolysis of cassava peels

The cassava peel hydrolysates were obtained by acid hydrolysis of dried and milled cassava peel under the following conditions: cassava peel mass, 0.3-15% w/v; temperature, 105-135°C; reaction time, 15-90 min under pressure 15 lb/inc^2; sulphuric acid, hydrochloric acid or acetic acid, 0.01-0.25 M.

2.3　Analysis of samples

After the hydrolysis, the liquid fraction of sugars was measured by HPLC (LDC Model 4100, USA) with refractive index detector (LDC Model 4100, USA). In the hydrolysates, sugars were separated on an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 65°C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.5 ml/min. The inhibitor of hydrolysate, 5-hydroxymethyl-furfural (HMF), was determined by HPLC. It was quantified by a RP18HP column with a linear eluent gradient of methanol (10-90%) at pH 3 [10]. HMF was detected with a UV-detector at 284 nm (UV detector model CTO-2A, Shimadzu, Japan)

2.4　Fermentation conditions

Saccharomyces cerevisiae 5019 was obtained from Bangkok MIRCEN. It was maintained at 4°C on slants of Sabouraud agar. Inocula of *S. cerevisiae* was grown on glucose in Erlenmeyer flasks at 30°C on a rotary shaker at 150 rpm for 24 h. The inoculum medium was composed of 20 g/l glucose, 1 g/l yeast extract, 1 g/l $MgSO_4 \cdot 7H_2O$, 2 g/l $(NH_4)_2SO_4$, and 0.5 g/l KH_2PO_4. Sugar solution was autoclaved separately. The batch fermentations were carried at 30°C on a rotary shaker at 150 rpm for 18 h in 250 ml Erlenmeyer flask. The working volume was 50 ml

of synthetic medium. The synthetic medium composed of 1 g/l yeast extract, 1 g/l $MgSO_4 \cdot 7H_2O$, 2 g/l $(NH_4)_2SO_4$, 0.5 g/l KH_2PO_4 and the carbon source 20 g/l glucose or cassava peel hydrolysate from diluted sulfuric acid hydrolysis.

The hydrolysate was neutralized to pH 7.0 using NaOH for fermentation process, then the hydrolysate was filtered through 0.2 mm membrane. The treated hydrolysate was supplemented with nutrients (1 g/l yeast extract, 1 g/l $MgSO_4 \cdot 7H_2O$, 2 g/l $(NH_4)_2SO_4$, and 0.5 g/l KH_2PO_4). The fermentation was carried out at 30°C for 18 h. The fermentation broths were filtered through a 0.45°m Millipore filter. Ethanol in the samples was determined by gas chromatograph using a 60:80 Carbopack B: 5% Carbowax 20 M glass column. The injector was operated at 200°C. The flame ionization detector (FID) was kept at 200°C. Nitrogen gas was used as carrier gas at a flow rate of 30 ml/min. The temperature was programmed at 120°C for 1.4 min, from 120°C to 240°C at 30°C/min, then held 5 min at 240 °C.

Biomass concentrations were determined by dry cell weight, whereby 3 x 10 ml samples were filtered through a membrane filter, washed twice by distilled water, dried at 104°C for 24 h and weighted.

2.5　Sugar concentration

Sugar concentration was determined by Nelson-Somogyi method [11]. It was used to monitor soluble RS (reducing sugar) formation during the acid hydrolysis of cassava peels. Glucose concentration in hydrolysates was measured by the glucose oxidase-peroxidase method using 2, 2'-azino-di-(3-ethylbenzthiazoline)-6-sulphonate (ABTS) as a chromogen [12].

Reducing sugar yield (%) was represented the amount of g of reducing sugar per 100 g of
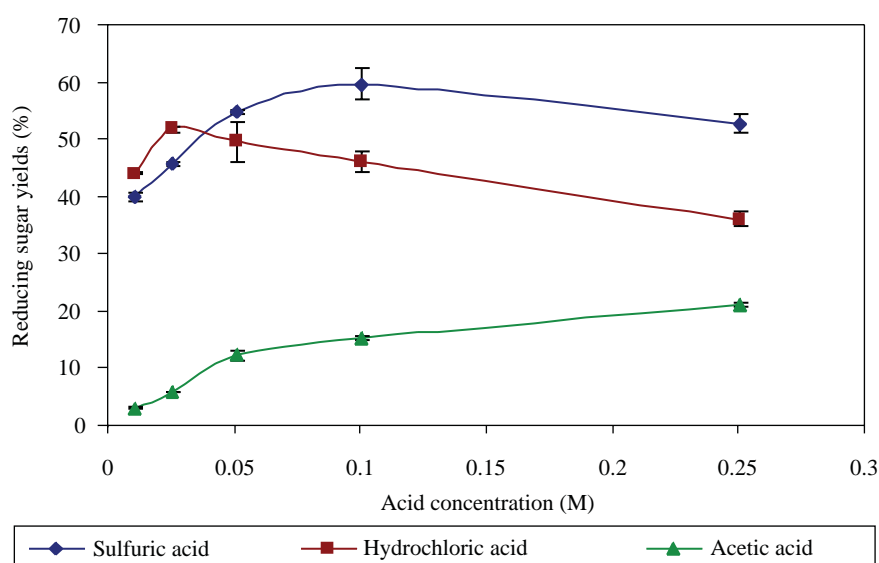
cassava peels.

All determinations were triplicated to estimate mean values and standard deviations.

3.　Results and Discussion

Fig. 1 showed the effect of acid concentrations on reducing sugar yields after acid hydrolyzing of 1.5% cassava peels. About 20-60% of reducing sugar was obtained from cassava peels with a solid to liquid ratio of 1.5% using 0.01-0.25 M sulfuric acid, hydrochloric acid and acetic acid at 135°C for 90 min. In the case of hydrochloric acid, acid concentrations in the slurry had an adverse effect on reducing sugar yields. The conversion of cassava peels to reducing sugar was about 53.1% at 0.025 M hydrochloric acid whereas it was 37.5% at 0.25 M hydrochloric acid. The reducing sugar yields of hydrolysate at 0.1 M sulfuric acid indicated that it was the highest yields under these conditions.

The maximum conversion by sulfuric acid was about 60.7%. It did not enhance reducing sugar yields when concentration of sulfuric acid was increased. Weak acid, acetic acid, exhibited low reactivities under the identical hydrolysis conditions (Fig.1). The reduced reducing sugar concentrations likely resulted from the degradation to degraded products such as furfural. Compared to glucose, xylose is more sensitive to degradation to furfurals, particularly at acid concentrations over 1% and reaction temperatures higher than 120°C [13].

Fig. 1 Effects of various acid concentrations on reducing sugar yields of cassava peel hydrolysates at 135°C for 90 min

Increasing the acid concentration resulted in marginal improvements in cassava peel conversion with sulfuric acid, and a decrease of about 16% when the hydrochloric acid level was raised from 0.025 to 0.25 M. At the higher acid concentrations, a dark colored hydrolysate, along with inversion by-products, was observed, thus suggesting sugars degradation. From Fig.1, cassava peels in different

diluted acids were hydrolysed under varying concentrations and then % reducing sugars was determined. The results showed that optimal % carbohydrate conversion could be obtained at 60.74% from hydrolysis at 135°C for 90 min with 0.1M sulfuric acid. Dilute sulfuric produced high yield of reducing sugars. Thus, the diluted sulfuric acid is suitable for cassava peels hydrolysis to produce reducing sugars.

Torget *et al.* [14] demonstrated that xylose decomposition at 160°C was proportional to acid concentration and reaction time. In this study, with a reaction temperature of l35°C, it should be no significant xylose degradation. Increases in acid concentration from 0.1 to 0.25 M sulfuric acid and 0.025 to 0.25 M hydrochloric acid, reduced hy drolysis conversion about 10%.

The yield of reducing sugar decreased as acid concentration increased more than 0.025 M hydrochloric acid and 0.1 M sulfuric acid. However, maximum conversion was achieved somewhat more rapidly using sulfuric acid than with hydrochloric acid. The results were not in agreement with the results obtained by Abraham *et al.* [15], which the hydrolysis efficiency with sulfuric acid was 50% less than that with hydrochloric acid at the same parameter values in the range of 0.2-1.0 M acid concentration.

Fig. 2 showed the conversion of cassava peels by sulfuric acid and hydrochloric acid at 135°C. Hydrolysis occurred rapidly (within 15 min after heating treatment) at 0.1 M sulfuric acid and 0.025 M hydrochloric acid produced 60 and 50 % conversion rate at 90 min, respectively. The results showed that the optimum hydrolysis time was 90 min.

The conversion ratios for lower acid levels could be increased to some extent by increasing the hydrolysis time. However, it would tend to make the uneconomical process, due to the high reactor capital cost. High yields at short residence times could only be achieved by using high-temperature dilute acid hydrolysis.
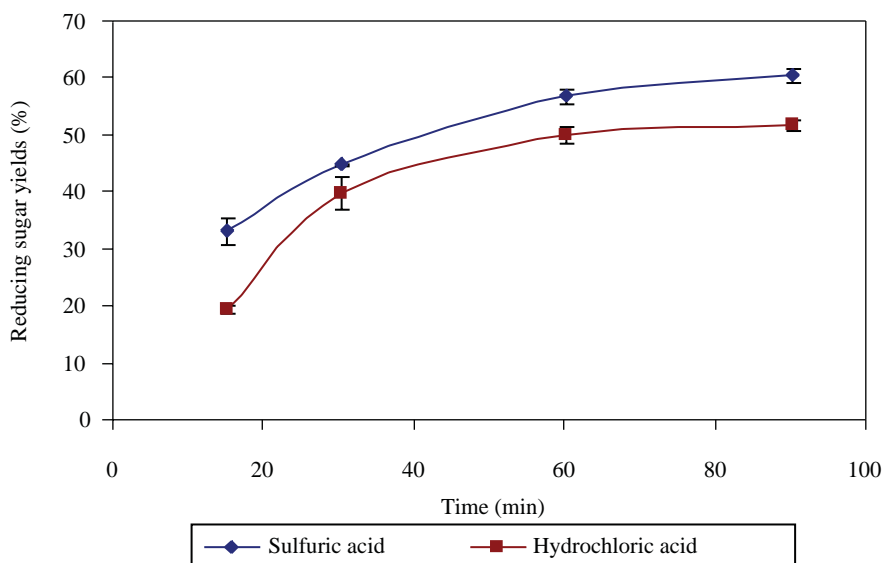
Fig. 2 Effect of reaction time on reducing sugar yields of cassava peel hydrolysates using 0.1 M sulfuric acid and 0.025 M hydrochloric acid.

Fig. 3 showed the temperature had an important effect on the reaction rate. Sugar conversions of 20.15 % and 42.32 % were obtained within 90 min at 105°C and 121°C, respectively. At higher temperatures, the maximum percentage of reducing sugars was higher.
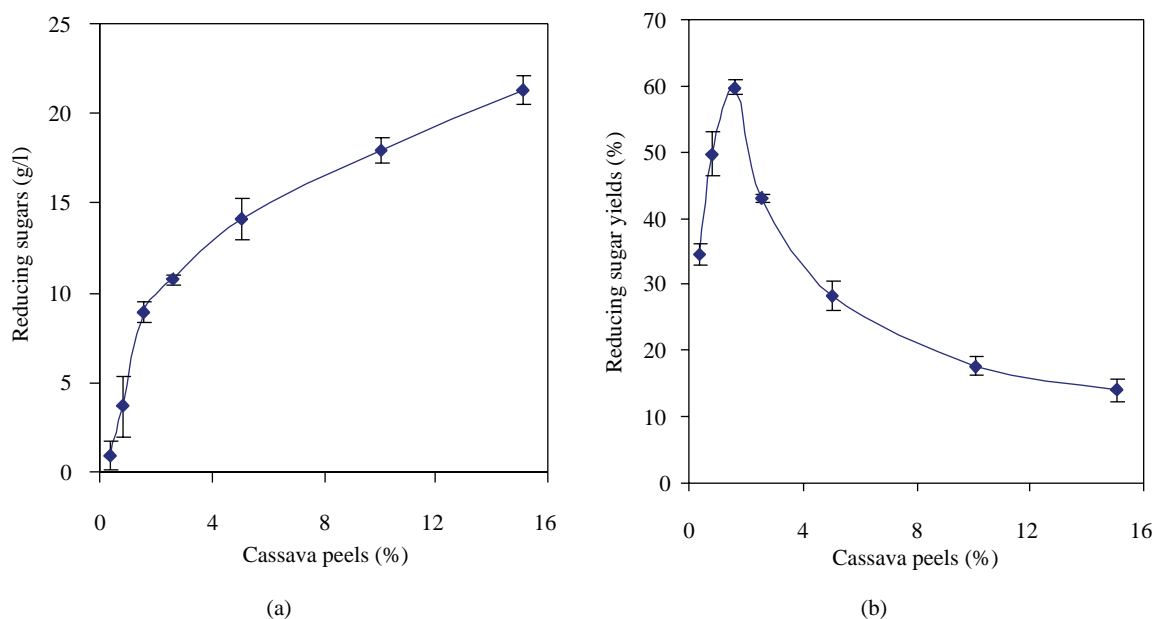
The results for acid hydrolysis of cassava peels indicated that the effect of acid concentration on reducing sugar yields was more critical at the higher temperature due to the high degradation rate of carbohydrate.
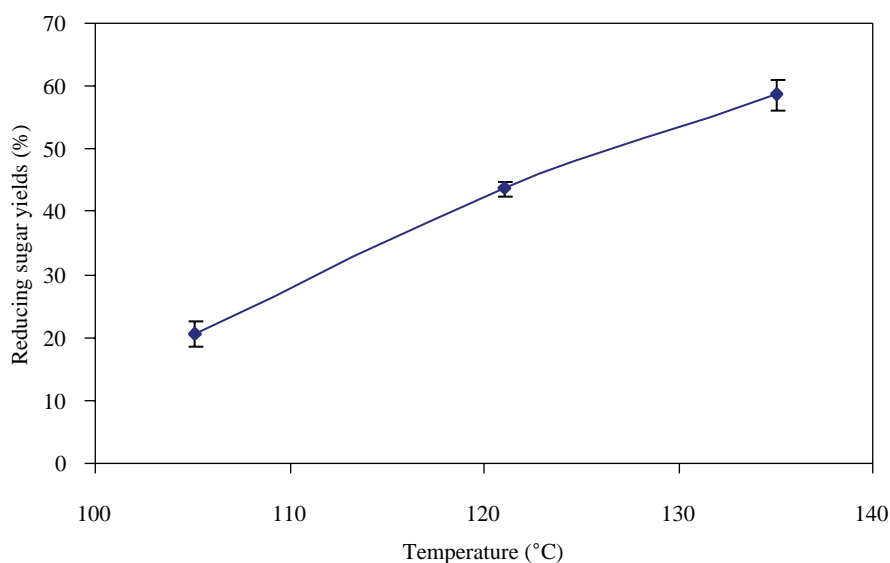
Fig. 3 Effect of temperature on reducing sugar yields of cassava peel hydrolysates using 0.1 M sulfuric acid.

Fig. 4 showed the effect of substrate concentrations for materials on parameters of hydrolysis with 0.1 M sulfuric acid. Reducing sugars increased with increasing substrate concentration (Fig. 4a) whereas reducing sugar yields decreased with increasing substrate concentration after 1.5% of cassava peels (Fig. 4b). The results showed that the highest reducing sugar yields were 60.74% at 1.5% of cassava peels. Under these conditions, reducing sugars were 8.9 g/l.

(a)

(b)

Fig. 4 Effect of cassava peel concentrations on the acid hydrolysis
of 0.1 M sulfuric acid at 135 °C for 90 min.

The sugar yields obtained from biomass hydrolysis at the optimum acid concentrations, temperature, and hydrolysis time are shown in Table 1. The hydrolysis conditions provided a hydrolysate with a high concentration of glucose (37.09 g/100 g) and a low concentration of xylose and rhamnose. The presence of hydrolysis by-product such as HMF was also observed.

Since filtrate from acid hydrolysis contains reducing sugars and monosaccharides, experiments for glucose was used as a model substrate. The semi-synthetic medium and cassava peel hydrolysate were adjusted to pH 6.0. Ethanol fermentation was incubated for 18 h by *S. cerevisiae*. The filtrate contained reducing sugars (18.42 g/l), glucose (10.24 g/l) and HMF (0.04 g/l). HMF is the product of a typical Maillard reaction involving monosaccharides [16]. It consists of a furan ring, formyl group at position C2, and hydroxymethyl group at position C5. The compound is a common product of these two reactions. It is formed from 3-deoxyhexosulose, the dehydration product derived from 1,2 enolization of glucose and fructose [17]. HMF is considered as the essential decomposition product of hexoses, especially when the pH is low. In this experiment, HMF concentration in the hydrolysate was lower than the threshold considered inhibitory for microbial metabolism (<1 g l^{-1}) [18], and the compound does not seem to affect fermentation at concentrations below 1.0 g l^{-1}.

Table 1 Compositions of the cassava peel hydrolysate obtained under the optimum conditions of 0.1 M sulfuric acid hydrolysis.

| Compositions	Concentrations (g/100 g cassava peels)[a]
Reducing sugars	60.74 ± 2.84
Glucose	37.09 ± 3.15
Xylose	4.79 ± 0.31
Rhamnose	4.05 ± 0.18
HMF	0.14 ± 0.06

[a]The values based on the concentration of three times.

Fig. 5 and Fig. 6 showed the fermentation of glucose and fermentation of cassava peel hydrolysate by *S. cerevisiae*, respectively. Ethanol was produced and no lag phases were observed for both cultivations. The fermentation of glucose for ethanol production was higher than that of cassava peel hydrolysate. However, the fermentation of cassava peel hydrolysate for ethanol production was observed after 2 hours of incubation. Ethanol fermentation of glucose was lower than that of cassava peel hydrolysate about 1.59 times. The results from the experiments with incubation of *S. cerevisiae* in cassava peel hydrolysate were presented in Fig. 6. Though the glucose concentrations in the filtrate were very low (Fig. 6), ethanol production could be take place after 2 hours of incubation. After 8 hours of incubation, the highest alcohol concentrations (4.22-4.41 g/l) were observed. The results suggested a more efficient substrate to cell mass conversion. Cell growth after depletion of the glucose suggested the presence of other sugars in the medium.
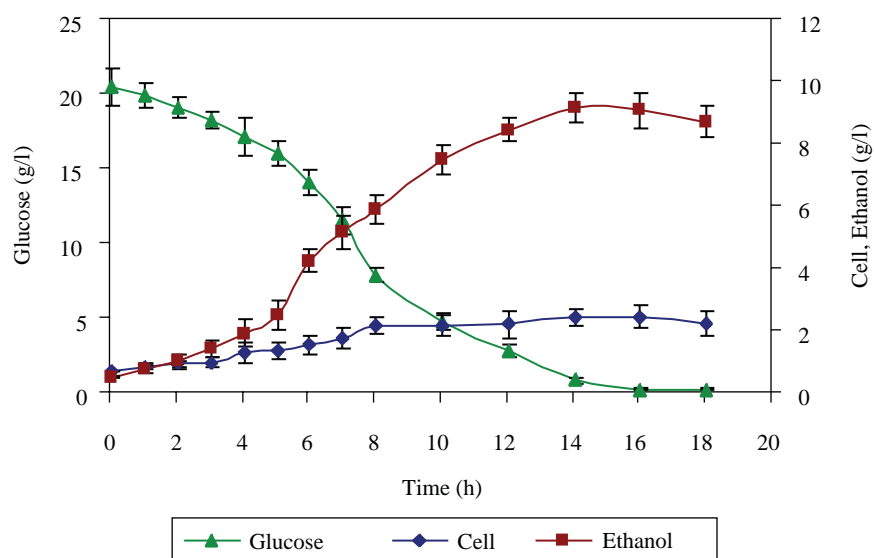
Fig. 5 Ethanol fermentation of *S. cerevisiae* grown on glucose as a substrate.
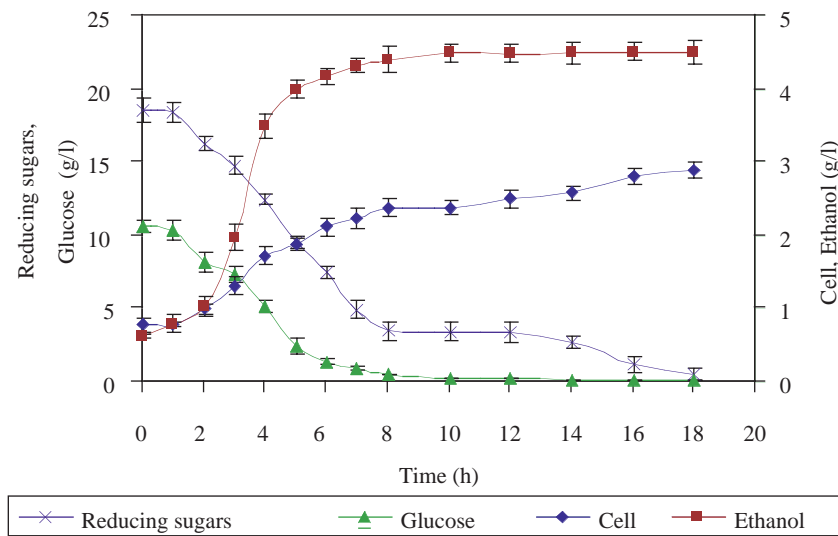
Fig. 6 Ethanol fermentation of *S. cerevisiae* grown on hydrolysate
of cassava peels as a substrate.

The fermentative parameters of the experiments for glucose and hydrolysate as substrates in shaked flasks were shown in Table 2. According to Saha and Bothast [19], in order to attain a high yield of fermentation, with a limited biomass production, by keeping the glucose-to-ethanol or (based on glucose only) conversion rate at high and constant level during all the fermentation time, it is important that the aeration level should remain high during the cell growth stage and low during the production stage.

The results obtained in this work for ethanol production showed that further studies, which take into account the bioreactor design, are necessary to optimize the conditions so that the kinetic behavior of the yeast can be improved.

Table 2 Fermentation kinetic parameters of *S. cerevisiae* grown on glucose
and cassava peels as substrates in shaking flask condition.

Parameters	Glucose	Reducing sugars
Initial substrate concentration (g/l)	20	18.42
Fermentation time (h)	18	10
Substrate consumed (%)	98.51	94.32
Maximum ethanol concentration (g/l)	9.25	4.53
Maximum cell concentration (g/l)	2.52	2.84
Volumetric ethanol productivity (Q_p, g /l h)	0.51	0.45
Ethanol yield ($Y_{P/S}$, g /g)	0.43	0.44
Cell yield ($Y_{X/S}$, g/g)	0.12	0.17
Ethanol yield based on biomass ($Y_{P/X}$ in g P/g X)	3.68	1.90
Theoretical yield of ethanol (%)	90.21	82.72

S, substrate (glucose); P, product (ethanol); X, cell mass. Maximum theoretical
ethanol coefficient: 0.51 g/g.

According to the experiment, the fermentable sugars of cassava peel hydrolysate are able to produce ethanol but in this study the fermentable sugars of the hydrolysate was low. However, ethanol production of the hydrolysate has to further study to improve ethanol yield. From this experiment it suggested that the *S. cerevisiae* was appropriate for fermentation of filtrate from cassava peel hydrolysate of diluted sulfuric acid hydrolysis treatment.

4. Conclusions

Acid hydrolysis of cassava peels was conducted with using various concentration of sulfuric acid, hydrochloric acid and acetic acid. The yields of high reducing sugars were obtained from cassava peel hydrolysis using 0.1 M sulfuric acid at 135 C. The maximum yields obtained by hydrochloric acid hydrolysis were lower than those by sulfuric acid hydrolysis. However, the concentrations of hydrochloric acid required were lower, particularly

at 0.025 M. The maximum conversion of 60 % was obtained for cassava peel hydrolysis at 135°C and 0.1 M sulfuric acid. The results showed that cassava peel hydrolysates containing fermentable sugars can be used as a substrate for ethanol production by yeast.

5. Acknowledgements

6. References

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