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Bioproduction of Ethanol in SHF and SSF from Cassava Stalks

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Abstract

Cellulose biomass is being investigated as a potential substrate for bioethanol production. Cassava stalks were successfully converted to ethanol by fermentation using *Saccharomyces cerevisiae* TISTR5048, *S. cerevisiae* KM1195, *S. cerevisiae* KM7253 and co-culture of *S. cerevisiae* TISTR5048 and *Candida tropicalis* TISTR5045. The objective of this study was to assess the fermentable sugars production from cassava stalks by dilute-acid, dilute-base and distilled water pretreatment and enzymatic hydrolysis. Another objective was to study the conversion of the fermentable sugars into ethanol by mono-culture and co-culture of yeast strain of *S. cerevisiae* and *C. tropicalis*. Cassava stalks were milled to flour and dried overnight in hot-air oven. Cassava stalks at 1.5% (w/v) in 0.1 M sulfuric acid was pretreated for 30 min at 135°C under the pressure of 15 lb/in^2. The pretreated cassava stalk suspensions were neutralized to pH 5.5 for saccharification process. The enzyme solution (cellulose, xylanase and pectinase solubilized in buffer pH 5.0) was used for hydrolysis of the pretreated cassava stalk at 50°C for 24 h. The hydrolysate was supplemented with additional nutrients. The culture was incubated at 30°C. The pretreatment of the stalk with dilute-acid hydrolysis resulted in 0.57 g/g sugar yield enzymatic hydrolysis, which was higher than dilute-alkaline-pretreated and distilled water-pretreated stalk. The sugar hydrolysate was bioconverted to ethanol with separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). The highest ethanol yields of 98.43% and 95.29% were obtained in SHF and SSF, respectively by *S. cerevisiae* KM1195. The fermentation time of SSF process was 24–32 h shorter than that of the SHF (≈56 h), but the ethanol productions (5.42-6.22 g/L for SSF; 5.9-6.23 g/L for SHF) were not significantly different.

Keywords: ethanol, cassava stalk, fermentation

1. Introduction

Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized. Bioethanol, a renewable fuel is becoming increasingly important as a consequence of major concern for depleting oil reserves, rising crude oil prices and greenhouse effect (1). Lignocellulosic feedstock is considered as an attractive raw material not only for the liquid transportation fuel but also for the production of chemicals and materials. Besides terrestrial plants,

aquatic plants are also promising renewable resource. Over the past few years, ever since the energy crunch began, there has been a tremendous interest in energy saving both on new and existing structures. Using certain materials and techniques can result in big savings. Today, the idea of utilizing biomass from agricultural and livestock wastes as a raw material for production of ethanol has attracted the interest of researchers especially in agricultural practicing countries. Thailand has an abundance of agriculture by-products available which are usually directly discharged as solid waste; causing environmental issues.

Thailand is an agricultural country. Each year the country produced not only agricultural product but also more than 50 million tons of agricultural residues (2). Cassava stalk is the fourth largest agricultural residues which accounted more than 4 million tons per year (2). Cassava stalks considered as useless agricultural residues. To fully utilize the cassava stem as a feedstock for ethanol production, pretreatment is required to render the cellulose fibers more amenable to the action of the hydrolytic enzymes. This study is aimed to investigate bioethanol production from cassava stalk cellulose acid and enzyme hydrolysate using mono-culture and co-culture of yeasts fermentation in SHF and SSF.

2. Materials and Methods

2.1 Substrate preparation

Cassava stalks were collected, washed manually using tap water to remove adhering dirt and then they were dried at 45°C in a hot-air oven for 4 days. The dried cassava stalks were cut into small pieces, milled with hammer mill and screened to select the fraction of particles with a size of 45-697 μm. The dried powder of cassava stalks were stored in the desiccators until needed.

2.2 Hydrolysate preparation

The 1.5 g dried powder of cassava stalks was pretreated with 100 ml of 0.1 M sulfuric acid and autoclaving under 15 lb/in^2. The pretreatment temperatures were varied from 120-135C and pretreatment times were varied from 10-90 min. The hydrolysate was prepared by adding with 250-ml filter-sterilize cellulase (Sumitime C; Shin Nihon Chemical Co. Ltd., Japan) solution (cellulase activity: 20 Filter paper units (FPU) (g substrate)$^{-1}$, α-amylase 100 units (g substrate)$^{-1}$, amyloglucosidase100 units (g substrate)$^{-1}$, xylanase activity: 500 units (g substrate)$^{-1}$ and pectinase activity: 250 units (g substrate)$^{-1}$ in 0.1 M sodium phosphate (pH 5.0) into the pretreated dried powder of cassava stalks and reacted at 50°C and 120 rpm for 48 h. After the enzymatic reaction, the hydrolysate was centrifuged at 21,000 x g for 10 min. The supernatants were determined for the reducing sugars and glucose. The supernatant with the highest sugars yield was supplemented with additional nutrients to give a base medium composition of: 1 g/L yeast extract; 2 g/L $(NH_4)_2SO_4$; 1 g/L MgSO4•7H_2O.

2.3 Cassava stalk cellulose hydrolysate medium

Fermentation medium composed of: 1 g/L yeast extract; 2 g/L $(NH_4)_2SO_4$, 1 g/L $MgSO_4$•7H_2O.

2.4 Batch fermentation

Batch fermentation was conducted in a 250 ml conical flask with a working volume of 100 ml. The fermentation medium was inoculated with 5% v/v inoculum (20 h culture, 1 × 10^{7}cells/ml). The fermentation temperature was kept constant at 30 ± 0.2°C in an incubation shaker. The broth was kept under agitation at 50 rpm. Samples were taken at regular time intervals during fermentations to determine the concentrations of cell mass, ethanol and residual sugars in the broth. All experiments were carried out in duplicate.

2.5 Analytical methods

Total solids (TSs) moisture and crude protein in cassava stalk were determined according to standards (3). Cellulose, hemicellulose and lignin contents were determined by the detergent extraction method (4).

2.6 Biomass determination

The biomass was determined by measuring the culture dry weight that was dried at 105 °C for 2 day in hot air oven.

2.7 Sugar determination

Total reducing sugar was estimated using dinitrosalicylic acid (DNS) reagent (5).

2.8 Ethanol determination

The fermentation was carried out at 30°C for 18 h. The fermentation broths were filtered through a 0.45 µm 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.

3. Results and Discussion

3.1 Composition of cassava stalk

The average composition of cassava stalk is summarized in Table 1.

Table 1. Average composition of cassava stalks.

Constituents	% of dry weight
Hemicellulose	11.62 ± 0.24
Cellulose	21.43 ± 0.17
Lignin	22.64 ± 0.37
Crude protein	2.72 ± 0.29
Starch	8.41 ± 0.32

Cellulose content of cassava stalk was relatively high compared to that of hemicellulose. The result indicated that cassava stalks could be a good source of cellulose for bioconversion.

3.2 Cassava stalk cellulose acid and enzyme hydrolysate preparation

The results shows that dilute sulfuric acid hydrolysis (0.1M) under autoclaving at pressure of 15 lb/in^2, in the pretreatment temperature of 135°C with the pretreatment time of 10 min and enzyme hydrolysis as described in the Materials and Methods was very effective in releasing a good amount of sugar from cassava stalks (Table 2). Higher temperature, higher yield of glucose and reducing sugars were released. Approximately 14% and 21% of glucose and reducing sugars were released at 120°C which were less than those at 135°C, respectively. So, temperature at 135°C was suitable to hydrolyse the cassava stalks for sugar production. Table 3 indicated that approximately 52.73% and 38.07% of the reducing sugars and glucose, respectively, were released in the first 10 min of autoclaving and enzyme hydrolysis, at 30 min of autoclaving and enzyme hydrolysis, 74.12% and 54.27% of the reducing sugars and glucose, respectively, were observed. After 60 min of autoclaving, sugar yield were 84.47% and 62.40% of the reducing sugars and glucose, respectively. These sugars were derived primarily from starch and cellulose component. The sugars yield (84.47%) was rather high, showing that starch and cellulose almost practically hydrolyzed.

Table 2. Effect of temperature on average sugar composition of cassava stalk cellulose acid and enzyme hydrolysate.

Temperature (°C)	**Glucose (g/L)**	**Reducing sugars (g/L)**
120	5.02 ± 0.16	6.78 ± 0.11
125	5.29 ± 0.12	7.04 ± 0.14
135	5.71 ± 0.07	7.91 ± 0.05

Table 3. Effect of pretreatment time on average sugar composition of cassava stalk cellulose acid and enzyme hydrolysate.

Time (min)	Glucose (g/L)	Reducing sugars (g/L)
10	5.71 ± 0.12	7.91± 0.14
30	8.14 ± 0.17	11.12 ± 0.09
60	9.36 ± 0.07	12.67 ± 0.16
90	9.62 ± 0.16	12.84 ± 0.11

3.3 Ethanol production in SHF

The highest values of ethanol yield per unit biomass (C_E), the maximum ethanol production (P_{max}), ethanol production rate (Q_E) and product (ethanol) yield coefficient ($Y_{p/s}$) were found to be 0.415 g (g-biomass)$^{-1}$, 6.23 g/L, 0.593 g/L/h and 0.502 g (g-total sugar)$^{-1}$, respectively, by the fermentation of *S. cerevisiae* KM1195. The lowest C_E, P_{max}, Q_E and $Y_{p/s}$ values were found to be 0.393 g (g-biomass)$^{-1}$, 5.90 g/L, 0.316 g/L/h and 0.477 g (g-total sugar)$^{-1}$, respectively, by the fermentation of mono-culture of *S. cerevisiae* KM7253. It was found that mono-culture of *S. cerevisiae* KM1195 could produce relatively higher ethanol yield than the co-culture (Table 4).

Table 4. Ethanol production by SHF with mono-culture and co-culture.

Strain	C_E	P_{max}	Q_E	$Y_{p/s}$
S. cerevisiae TISTR5048	0.398±0.007a	5.97±0.006a	0.612±0.004a	0.479±0.003a
S. cerevisiae KM1195	0.415±0.005b	6.23±0.003b	0.593±0.005b	0.502±0.002b
S. cerevisiae KM7253	0.393±0.009a	5.90±0.012a	0.316±0.004c	0.477±0.002a
SHF with co-culture inoculation of *S. cerevisiae* TISTR5048 with *C. tropicalis* TISTR5045	0.405±0.005b	6.07±0.002c	0.781±0.007d	0.489±0.002c

C_E Ethanol yield per unit biomass (g(g-biomass)$^{-1}$)

Q_E Ethanol production rate (g/L/h)

P_{max} Maximum ethanol production (g/L)

$Y_{p/s}$ Product (ethanol) yield coefficient (g(g- total sugar)$^{-1}$)

Values in the same column with the different letters are significantly different ($P < 0.05$).

Table 5. Ethanol production by SSF with mono-culture and co-culture.

Strain	C_E	P_{max}	Q_E	$Y_{p/s}$
S. cerevisiae TISTR5048	0.360±0.006a	5.42±0.004a	0.911±0.007a	0.422±0.009a
S. cerevisiae KM1195	0.414±0.003b	6.22±0.007b	0.969±0.005b	0.486±0.004b
S. cerevisiae KM7253	0.371±0.005c	5.57±0.009c	0.327±0.003c	0.435±0.005c
SSF with co-culture inoculation of *S. cerevisiae* TISTR5048 with *C. tropicalis* TISTR5045	0.394±0.007d	5.89±0.003d	0.664±0.009d	0.461±0.007d

C_E Ethanol yield per unit biomass (g(g-biomass)$^{-1}$)

Q_E Ethanol production rate (g/L/h)

P_{max} Maximum ethanol production (g/L)

$Y_{p/s}$ Product (ethanol) yield coefficient (g(g- total sugar)$^{-1}$)

Values in the same column with the different letters are significantly different ($P < 0.05$).

3.4 Ethanol production in SSF

The highest C_E, P_{max}, Q_E and $Y_{p/s}$ values were 0.414 g (g-biomass)$^{-1}$, 6.22 g/L, 0.969 g/L/h and 0.486 g (g-total sugar)$^{-1}$, respectively, by the fermentation of *S. cerevisiae* KM1195. The lowest C_E, P_{max}, Q_E and $Y_{p/s}$ were 0.360 g (g-biomass)$^{-1}$, 5.42 g/L, 0.911 g/L/h and 0.422 g (g-total sugar)$^{-1}$, respectively, by the fermentation of mono-culture of *S. cerevisiae* TISTR5048. It was found that mono-culture of *S. cerevisiae* KM1195 could produce relatively higher ethanol yield than the co-culture (Table 5).

The enzymatic response was evaluated as a function of the temperature and time of pretreatment. The experiments were carried out in order to find the optimal conditions. The temperature ranged from 120°C to 135 °C, and the acid concentration was 0.1 M in the optimal test.

Figure 1 and Figure 2 show the time-course for growth, sugar utilization and ethanol concentration in the cellulose acid hydrolysate medium at initial pH 5.0 ± 0.2 of mono-culture and co-culture of *S. cerevisiae* and *C. tropicalis*. The fermentation parameters are summarized in Table 4 and 5. The yield (C_E) and productivities (P_{max}, Q_E and $Y_{p/s}$) in SHF and in SSF of *S. cerevisiae* KM1195 were relatively higher ethanol yield than the co-culture of *S. cerevisiae* and *C. tropicalis* when grown in a medium starch and cellulose acid hydrolysate. This showed that co-culture of *S. cerevisiae* and *C. tropicalis* fermentation employed for the treatment of starch and cellulose acid hydrolysate had partially used reducing sugars as substrate but not affected to improve the fermentability. The ethanol production rate (g/L/h) of co-culture comparing to mono-culture of *S. cerevisiae* KM1195 was higher about 24 % in SHF while ethanol production rate (g/L/h) of co-culture reduced about 32% in SSF.

However, the ethanol yield of co-culture for the starch and cellulose acid hydrolysate was rather similar to that obtained with *S. cerevisiae* KM1195 (Table 4 and 5). This shows that there might be some leftover reducing sugars in the treated starch and cellulose acid hydrolysate that are not used in the fermentation performance of *S. cerevisiae* KM1195.

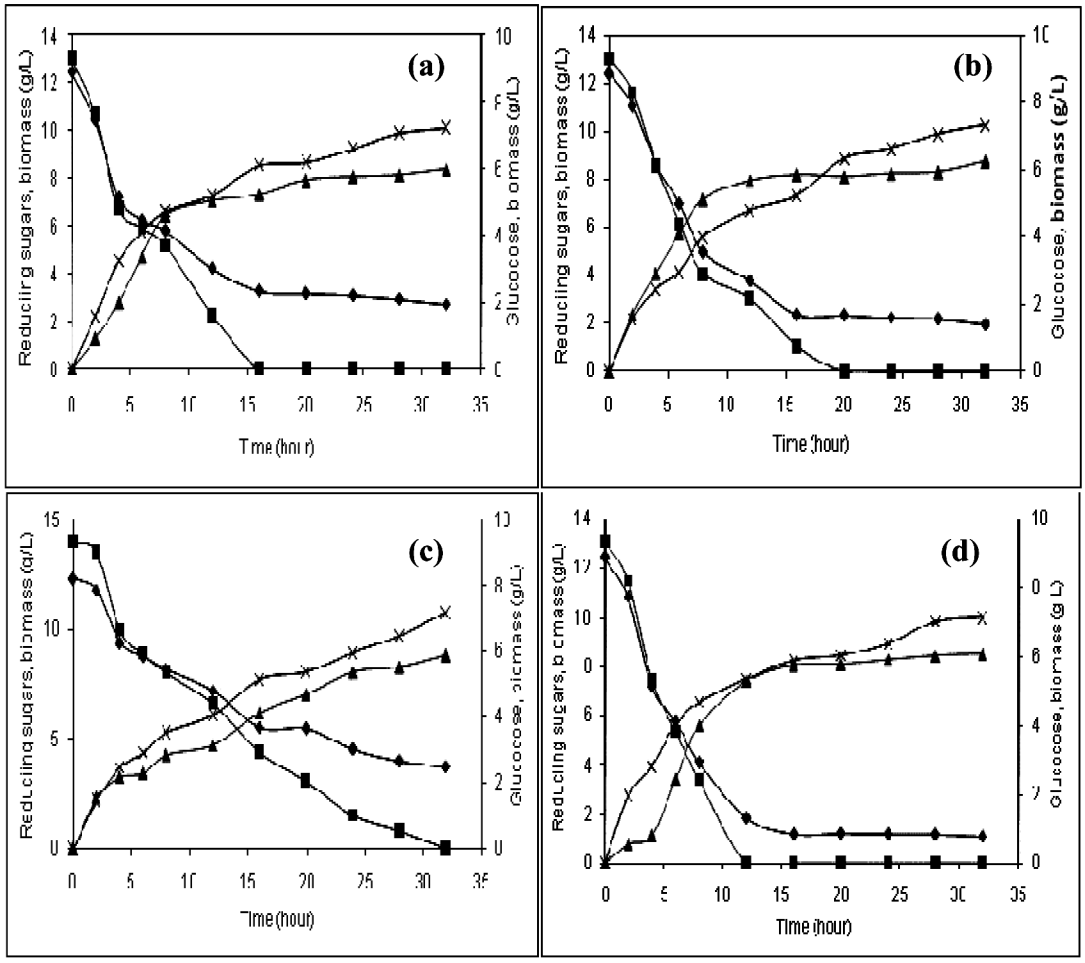

Figure 1. The time course of growth (x), reducing sugars (◆), glucose (■) and ethanol (▲) concentration in SHF by *S. cerevisiae* TISTR5048 (a), *S. cerevisiae* KM1195 (b), *S. cerevisiae* KM7253 (c) and co-culture of *S. cerevisiae* TISTR5048 and *C. tropicalis* TISTR5045 (d) at 30 ± 0.2 °C and pH 5.0 ± 0.2 using simulated synthetic hydrolysate medium.

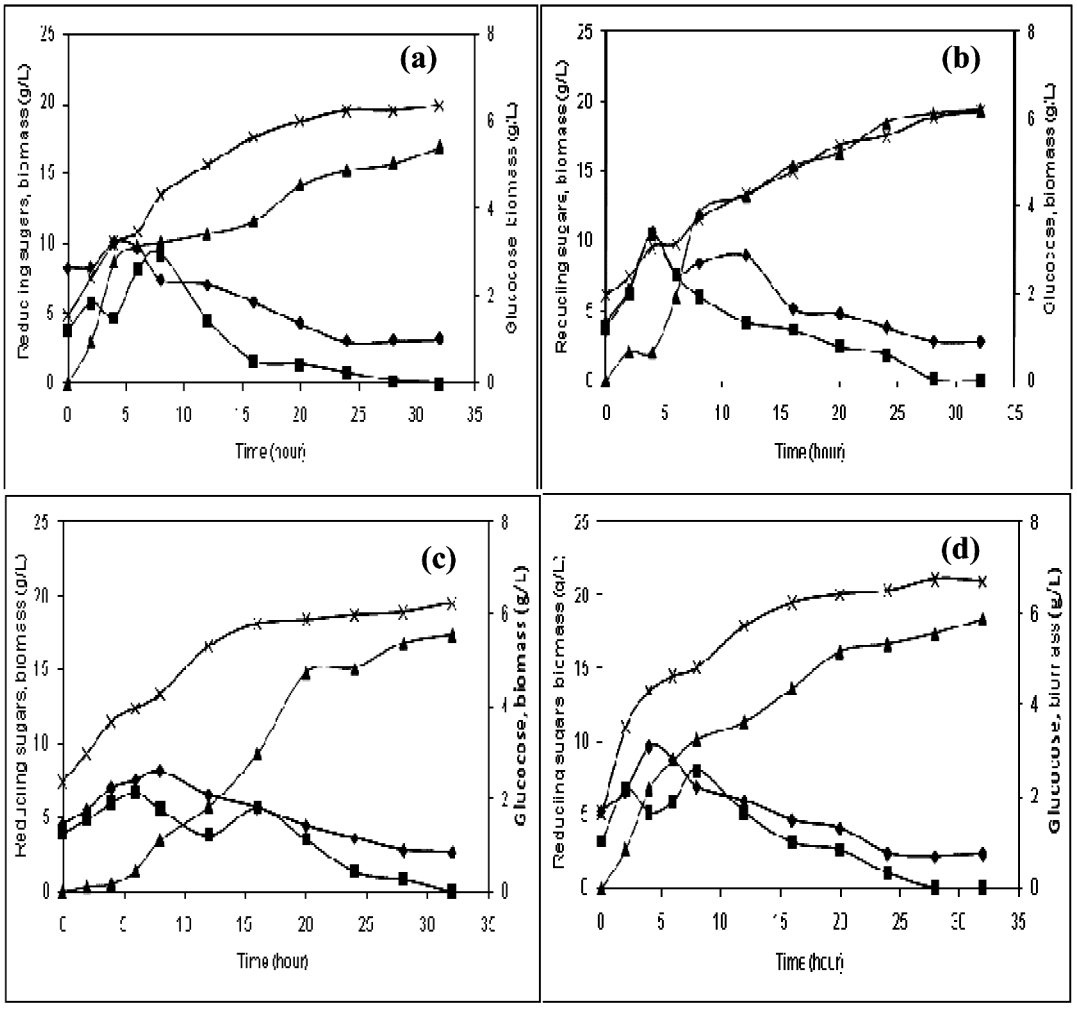

Figure 2. The time course of growth (x), reducing sugars (◆), glucose (■) and ethanol (▲) concentration in SSF by *S. cerevisiae* TISTR5048 (a), *S. cerevisiae* KM1195 (b), *S. cerevisiae* KM7253 (c) and co-culture of *S. cerevisiae* TISTR5048 and *C. tropicalis* TISTR5045 (d) at 30 ± 0.2 °C and pH 5.0 ± 0.2 using simulated synthetic hydrolysate medium.

4. Conclusions

The maximum values of ethanol yield (C_E), productivity (P_{max}, Q_E and $Y_{p/s}$) and percent sugar utilization were obtained, when co-culture of *S. cerevisiae* TISTR5048 and *C. tropicalis* TISTR5045 or mono-culture of *S. cerevisiae* KM1195 was grown in treated cellulose hydrolysate medium both in SHF and SSF at temperature 30 ± 0.2°C and pH 5.0 ± 0.2. Therefore, the fermentation of cassava stalk for ethanol production was carried out in a high yield by optimum treatment and culture of yeast strains.

5. Acknowledgements

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6. References

(1) Hu G, Heitmann, JA, Rojas OJ. Feedstock pretreatment strategies for producing ethanol from wood, bark, and forest residues. BioResources. 2008; 3 (1): 270–94.

(2) Puthikitakawiwong T, Boonsu R, Joompha O. Production of biocoal from cassava stalk. In: Carvalho MG, Afgan NH. (Eds.), New and Renewable Energy Technologies for Sustainable Development; 2004. (Instituto Superior Tecnico, Portugal) Evora, Portugal, 28 June –1 July 2004: 165–70.

(3) Association of Official Analytical Chemists (AOAC). Methods of analysis of the association of official analytical chemists. Association of Official Analytical Chemists, Washington, DC; 1975.

(4) Robertson JB, van Soest PJ. The detergent system of analysis and its application to human foods. In: James WPT, Thiander O. (Eds.), The Analysis of Dietary Fibers in Food. Marcel Dekker, New York, 1981. P.123–58.

(5) Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem. 1959; 31: 426–48, 591