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Utilization of Cassava Pulp and Cassava Wastewater for Bioethanol Production

Tawida Srimuang[1]* and Supawadee Polprasert[2]

[1] Faculty of Health Sciences, Siam Technology College

[2] Department of Environmental Health Sciences, Faculty of Public Health, Mahidol University

[1] 46 Charansanitwong Rd., Watthaphra, Bangkok 10600, Thailand

[2] 420/1 Rajvithi Rd., Rajchathawee, Bangkok 10400, Thailand

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Abstract

The purpose of this study was to optimize the bioethanol production from cassava pulp and cassava wastewater. The study was divided into two phase: 1.) Pretreatment and enzymatic hydrolysis 2.) Bioethanol fermentation. The effect of substrate and ratio of loading 1, 3, 5, and 7% (w/v) with the mixed enzyme α-amylase and glucoamylase ratio of 1:1 (v/v).The results showed that at 7% substrate loading, the highest reducing sugar concentration was 89.55±1.13 g/L. Regarding, the best ratio of α-amylase and glucoamylase at 1:1 (v/v) with fermentation time of 4 h gave the highest yield of 0.58±0.02 (g product/g substrate). In addition, the highest glucose yield of 0.36±0.04 (g product/g substrate) was observed in 2 h fermentation time after hydrolysis with α-amylase and glucoamylase, using cellulase and xylanase ratio of 1:1 (v/v). The results indicated that usage of mixed enzyme proved to spend shorter hydrolysis time and enhance the fermentable sugar production from cassava waste. The enzymatic hydrolysate was used as substrate for bioethanol fermentation, using *S.cerevisiae*TISTR5339. The results showed that the ethanol yield at fermentation time at 6 h with *S.cerevisiae*5, 10, 20 and 30% (v/v) were 0.27±0.027, 0.23±0.002, 0.19±0.022 and 0.16±0.011 (g ethanol/g substrate), respectively. The result indicated that ethanol yield decreased with increasing cell concentration of *S.cerevisiae*TISTR5339 from 5% (v/v) to 30% (v/v).

Keywords : Cassava Pulp; Cassava Wastewater; Enzymatic Hydrolysis; Bioethanol Fermentation

**** Corresponding Author. Tel.: +669 5956 5132, E-mail Address: tawidas@siamtechno.ac.th***

1. Introduction

Due to the limited and non-renewable petroleum resources, alternative renewable energy source from lignocellulosic materials is of great interest. In Thailand, cassava (*Manihot esculenta Crantz*) starch industry is growing rapidly. Thailand is the most of cassava starch production in the world and continue the trend of exports increased steadily, which comprises 14-18.1%of the total export value of the country after rice and sugar. Cassava pulp (CP) is a residue from industry, that is 10-15% of fresh cassava roots [1] or with about 1.5 million ton/day. Moreover, the production of cassava starch 1 ton generated cassava wastewater (CWW) from processing up to 11-22 cubic meters. Most of solid waste residues from cassava processing used as animal feed, fertilizer, mushroom cultivation. However, during rainy season, cassava pulp spoils rapidly causing environmental problems including a strong and offensive putrefaction odour and local water contamination [2].

In addition, biosugars are one of the important intermediate products that can be produced from cassava processing waste [3]. Due to its high organic and low ash contents. Cassava pulp also offers other advantages such as easier hydrolysis process, low collection cost and lack of competition with other industrial uses [4]. As described previously, cassava wastewater has a very high organic content (BOD 9,750 mg/L, COD 20,433 mg/L) [6] because it contains residual cassava constituents. CWW showed promise when used as a substrate for solvent production by microorganisms. Besides the lower raw material cost, the processing cost is likely to be lower than the regular solid cassava to ethanol process since the CWW from the starch factory can be used instead of fresh water [5] and cassava wastewater from the starch-processing factory was used in place of fresh water in the fermentation, resulting in not only saving water and reducing discharge water, but also in an increased ethanol production, due to the starch content in the CWW. The conventional combination process between chemical hydrolysis and enzymatic hydrolysis of starchy-lignocellulosic substrate, spend long time, high cost, and acid or alkaline contamination occurred. Because it is the major component in CP, therefore, the chemical pretreatment are not necessary [6].

In this study, the efficiency of enzymatic hydrolyze using mixed enzymes including α-amylase, glucoamylase, cellulase and xylanase for enhance the sugar production is also investigated. Moreover, the yeast *Saccharomyces cerevisiae*TISTR5339 is evaluated for bioethanol production.

2. Research Methodology

This study investigated the bioethanol

production from cassava pulp and cassava wastewater from a cassava starch factory. The study was divided into two phases: 1) Pretreatment and Enzymatic Hydrolysis 2) Bioethanol Fermentation. Hydrolysis of α-amylase, glucoamylase, cellulase and xylanase were used for enzymatic hydrolysis. *Saccharomyces cerevisiae*TISTR5339 was used for bioethanol production. Fermented sugar and type of sugar were analyzed to investigate the efficiency of enzymatic hydrolysis. Ethanol concentration was evaluated to determine the best condition for bioethanol fermentation by *Saccharomyces cerevisiae*TISTR5339.

2.1 Materials and Methods

Cassava pulp was obtained from Choncharoen cassava starch industry in Chonburi province, Thailand. Dry cassava pulp was prepared by sun drying for 48 h and heating at 90°C for 24 h, and then crushed with a 1.00 mm, the figure of cassava pulp is shown in **Fig. 1A** and its components are shown in Table 1

Table 1 The components of cassava pulp

Components	Content
Starch (% w/w)	50.12
Cellulose (% w/w)	21.76±0.91
Hemicellulose (% w/w)	14.09±1.81
Lignin (% w/w)	1.44±0.20
Ash (% w/w)	1.87±0.05
pH	5.77±0.08

Cassava wastewater was collected from Choncharoen cassava starch industry in Chonburi province, Thailand. The CWW was stored in a refrigerator at 4°C until any further use. The CWW was analyzed for its characteristics, consisting of initial concentration of Chemical oxygen demand, Total suspended solids, Total dissolved solids, Total volatile solids, Suspended solids and pH., The figure of cassava wastewater is shown in **Fig. 1B.** and its components of cassava wastewater are shown in **Table 2.**

Table 2 The components of cassava wastewater characteristics

Parameters	Average
pH	3.9±0.02
SS (mg/L)	2,700±173
TS (mg/L)	10,724±62
TVS (mg/L)	8,054±68
TDS (mg/L)	8,124±23
COD (mg/L)	12,240

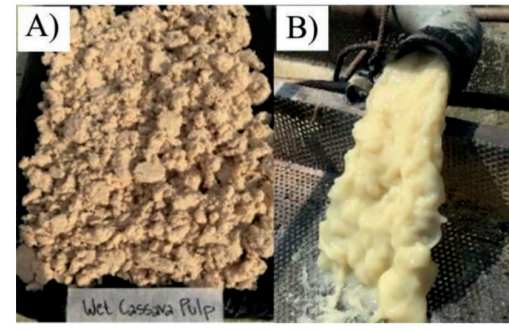

Fig. 1 A) Cassava Pulp, B) Cassava Wastewater

Commercial enzymes used in this study including α-amylase from *Bacillus licheniformis*, glucoamylase from *Asper gillus niger*, cellulase and xylanase from *Trichoderma reesei* were purchased from

Reach biotechnology Co., Ltd, Bangkok, Thailand. Unit activity of each enzymes is shown in **Table 3.**

Table 3 Enzymatic used in hydrolysis

Enzymes	Unit Activity
α-amylase	40,000 IU/mL
Glucoamylase	20,0000 IU/mL
Cellulase	1,300 IU/mL
Xylanase	10,0000 IU/mL

*Saccharomyces cerevisiae*TISTR-5339 was purchased from the Thailand Institute of Scientific and Technological Research (TISTR). In this process of bioethanol production indicated that initial cell concentration was obtained at 5.4×10^6 CFU/mL.

2.2 Experimental Methodology

Substrate loading of 1, 3, 5 and 7 % (w/v) was prepared by using CP 1, 3, 5 and 7 (g) in CWW 100 mL, respectively and autoclaved (121°C 15 mPa) for 15 min. The effect of substrate loading was observed from α-amylase: glucoamylase ratio of 1:1 (v/v) with different substrate loadings of 1, 3, 5 and 7 % (w/v).

Enzymatic hydrolysis was carried out in 2 steps. In the first step, the best ratio of α-amylase and glucoamylase for starch hydrolysis was investigated. After that, the appropriate ratio of cellulase and xylanase for other residual fiber hydrolysis was determined.

Fermentation was carried out in 250 mL Erlenmeyer flask using the best condition hydrolysate which was initially inoculated with the culture suspension of *S. cerevisiae*TISTR5339 (5, 10, 20, 30% v/v). The fermentation was operated at room temperature (28-30°C) with rotary shaking at 150 rpm for 72 h. Samples were removed at various time intervals and analyzed for sugar concentration by High Performance Liquid Chromatography (HPLC) and ethanol production by Gas Chromatography (GC).

The samples were collected at various time intervals. The samples were centrifuged at 9,000 rpm for 30 min and the supernatant was used for analysis. The supernatant was first filter through the GF/C filter membrane and then appropri ate aliquots were taken from the filtrate into 0.2 μm polypropylene membrane syringe filter for sugar (Reducing sugar and glucose) and ethanol concentration analysis. The experiment was repeated three times.

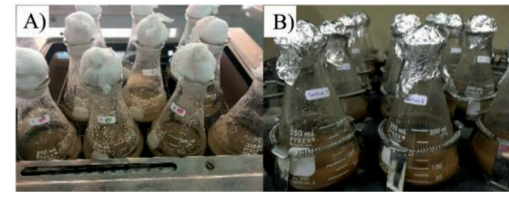

Fig. 2 A) Enzymatic Hydrolysis
B) Bioethanol Fermentation

3. Results and Discussion

3.1 Effect of substrate loading on fermentable sugar production

The results of the effect on initial ratio of cassava pulp and cassava

wastewater on the reducing sugar concentration. Results indicated that the highest reducing sugar concentration of 89.55 g/L was obtained at the highest substrate loading of 7% (w/v) chosen as the best result from your study for enzymatic hydrolysis. As shown in **Fig. 3.** when increasing cassava pulp concentration over 7% (w/v), the sample was not filtrated due to the difficulty of heat and mass transfer at high solid loading [7]. However, Zhu et al. [8] reported that a pulp concentration of 12% was as the best substrate concentration for a one-step enzymatic hydrolysis with glucose yield of 0.41 g/g. Whereas, the substrate concentration was over 12% (w/v), the glucose yields were slightly decreased. Furthermore, Li et al. [7] indicated that the ethanol production significantly decreased when the pulp concentration was over 8% (w/v) due to resistance to heat and mass transfer and result in a decrease in the fermentation efficiency, therefore was important to find an optimal substrate concentration.

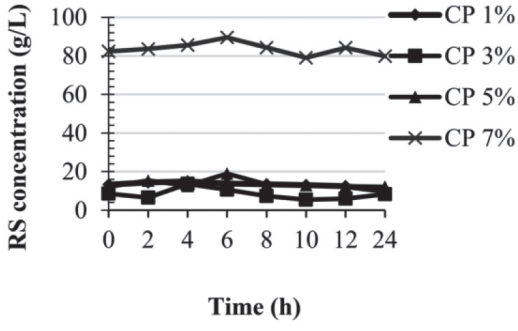

Fig. 3 Reducing sugar (RS) concentration (g/L) with enzymatic hydrolysis of α-amylase and glucoamylase; 1:1 (v/v)

3.2 Effect of enzymatic hydrolysis on fermentable sugar production

Regarding the four sets of initial α-amylase and glucoamylase a ratio of 1:1, 2:1, 3:1 and 4:1, with 7% (w/v) substrate loading investigated, results showed that at the lowest α-amylase content a ratio of 1:1 (v/v), the highest glucose yield (0.58 ± 0.02 g/g) was observed at hydrolysis time of 4 h as show in **Fig. 4.**

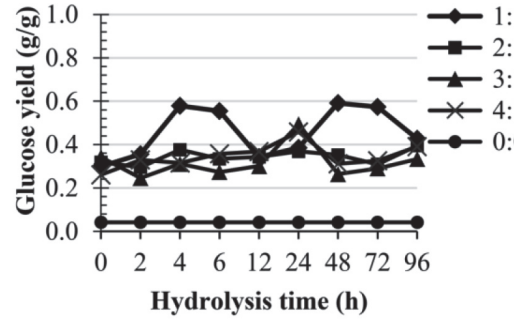

Fig. 4 Glucose yield (g glucose /g substrate) obtained at different ratio of α-amylase:glucoamylase

When increasing α-amylase and glucoamylase ratios, the glucose yields were not remarkably increased. Chotineeranat et al. [9] found that the combination of 285.6 MWU of -amylase and 0.21 DU of glucoamylase per gram of cassava pulp at 0.5:0.5 ratio gave the highest reducing sugar concentration, which was higher than that using only one enzyme type.

As shown in **Fig. 5**. the highest yield of glucose concentration was obtained from the cellulase and xylanase ratio of 1:1 at 2 h followed by mixed enzyme

α-amylase and glucoamylase of 1:1 at pH 5.0, and temperature of 50°C for 4 h. The results showed that the shorter hydrolysis time spent to achieve the desired yield, using these enzymes.

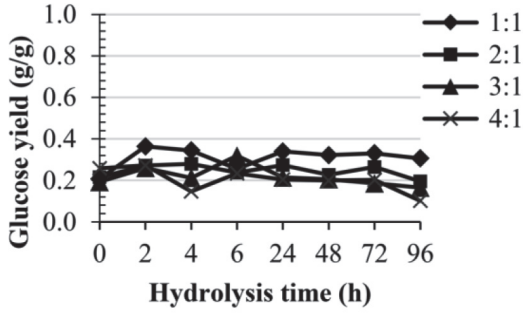

Fig. 5 Glucose yields (g glucose / g substrate) obtained at different ratio of cellulase: xylanase

3.3 The best condition for bioethanol fermentation using *Sacchromyces cerevisiae* TISTR5339

The ethanol production derived from enzymatic hydrolysate containing glucose concentration 65.11 g/L were fermenter using *S. cerevisiae* TISTR5339 of cell concentration 5, 10, 20 and 30% (v/v) with substrate loading of 7% (w/v). The highest ethanol concentration of 18.88±1.89 g/L, ethanol yield of 0.27±0.027 g/g, and ethanol productivity of 3.15±0.315 g/L/h were obtained at fermentation time of 6 h with 5% (v/v) cell concentration of *S. cerevisiae* TISTR 5339 as shown in **Fig. 6.** and **Fig 7.** Reporting results obtained of standard deviation from measurement within the same sample of three replicate. The ethanol yields and fermentation efficiency decreased with increasing cell concentration of *S. cerevisiae* TISTR5339 from 5% to 30% (v/v). Sueast et al. [10] reported that when fermenting with 89.2 g/L reducing sugars by *S. cerevisiae* was obtained at 24 h, it was found that 36.2 g/L of ethanol production and 0.25 g/g of ethanol yield. When compared with our study, the results showed that shorter fermentation time required for the similar yield due to using different mixed enzyme on enzymatic hydrolysis process. However, Yoonan et al. [11] found that the higher ethanol yield of 0.2 g/g was obtained at 18 h fermentation time with pretreatment of 0.025 sulfuric acid.

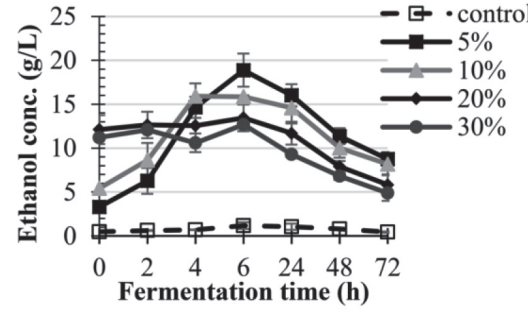

Fig. 6 Ethanol concentration (g/L) from 7% (w/v) substrate loading at different cell concentration of *S. cerevisiae* TISTR

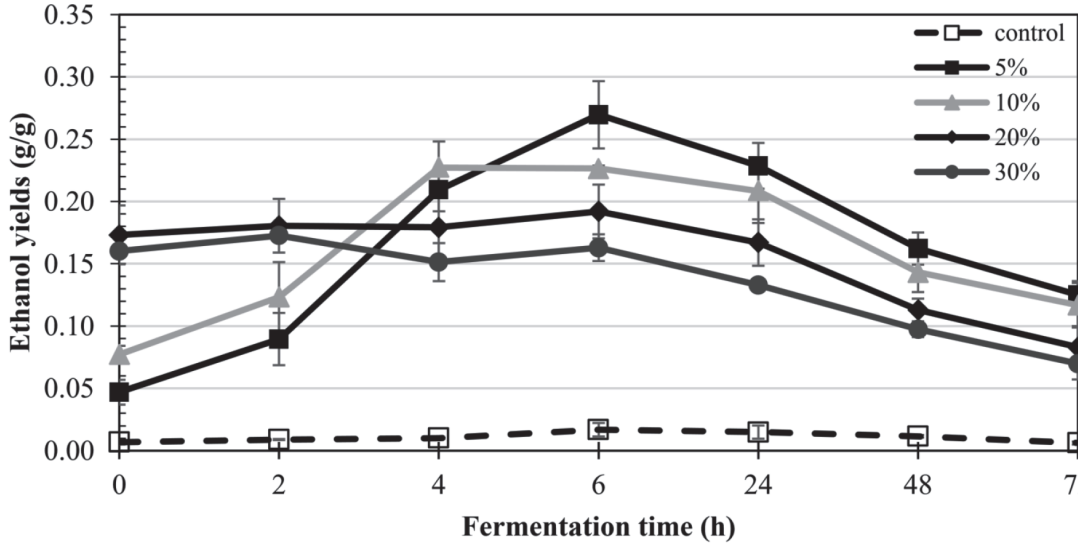

Fig. 7 Ethanol yields (g/L) from 7% (w/v) substrate loading at different cell concentration of *S. cerevisiae*TISTR

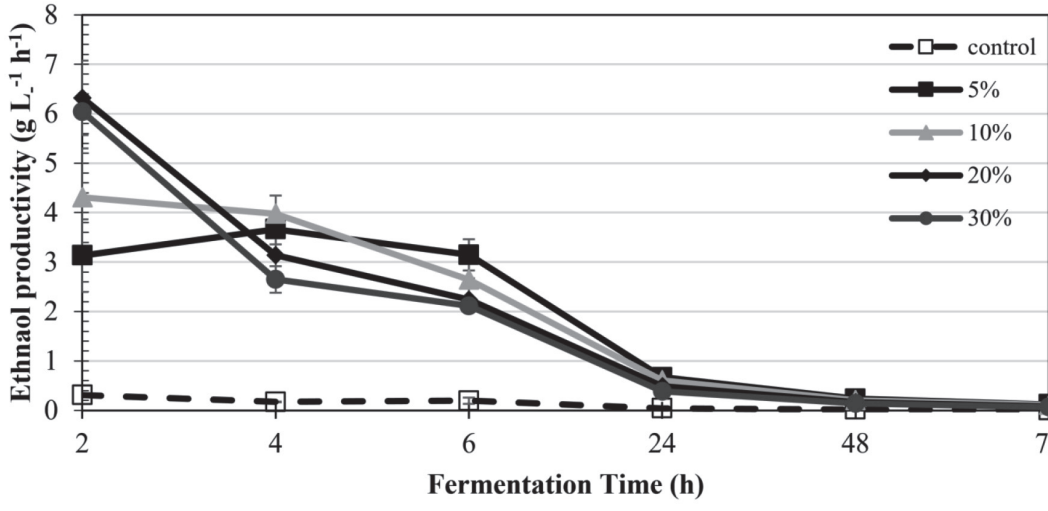

Fig. 8 Ethanol productivity (g/L/h) from 7% (w/v) substrate loading at different cell concentration of *S. cerevisiae*TISTR

Zhu et al. [8] found that a high pulp concentration can lead to a high ethanol concentration. However, when the pulp concentration increased to over 16%, the operation was difficult and a significant decrease in fermentation efficiency was observed.Moreover, the highest fermentation efficiency was achieved when using 4% of CP concentration.

From theoretical balanced equation,

it was found that the fermentation 1g of $C_6H_{12}O_{6,}$ resulting in the production of 0.43 g of ethanol by equations for yeast fermentation. Therefore, in this study, the fermentation using *S.cerevisiae*TISTR, 1 g of glucose produced 0.71 g of ethanol. Paturau [12] found that a famous series of experiments that the maximum practical yield is 48.40 because some of the dextrose is consumed in side reaction necessary for ethanol synthesis. Products of the side reaction are many and include glycerol, succinic acid, and acetic acid.

4. Conclusion

Overall, results of this work indicate that the cassava pulp and cassava wastewater have the potential to generate fermentable bioethanol under the conditions investigated. Fermentable sugar concentration was affected by substrate loading. The maximum reducing sugar concentration was attained at substrate loading of 7% (w/v). The variation in the ratio of α-amylase: glucoamylase studied had a noticeable effect on glucose yields. The optimal ratio of α-amylase and glucoamylase of 1:1 at pH 5.0, temperature of 50°C with fermentation time of 4 h gave the highest yield. Furthermore, more glucose yield was obtained when using cellulase and xylanase ratio of 1:1 for 2 h followed by mixed enzyme amylase and glucoamylase ratio of 1:1. Lastly. The fermentable bioethanol concentration was affected by the best condition. The maximum bioethanol concentration was attained at *S. cerevisiae*TISTR5339 of 5% (v/v) at room temperature (28-30°C) with fermentation time of 6 h gave the highest yield.

5. Acknowledgement

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