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Article

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Enzymatic hydrolysis of cassava stems for butanol production of isolated *Clostridium* sp.

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Abstract

This research focused on the hydrolysis of cassava stems (CS) and subsequent utilization as a carbon source for the cultivation of isolated *Clostridium* sp. To yield the highest amount of reducing sugars (RS), the studies on the pretreatment with sodium hydroxide (NaOH) and the hydrolysis with cellulases, amylases, and mixed enzymes were carried out. Afterwards, the hydrolysate was utilized for the cultivation of isolated *Clostridium* sp. Experimental results revealed that CS after 1.0 M NaOH pretreatment at 121 °C for 15 min and cellulase hydrolysis (Accellerase® 1500, 2500 CMC U/g CS) obtained 10.94 ± 0.29 g/L RS concentration. Hydrolysis of CS with amylases (Termamyl® 120, 1.2 U/g CS and AMG 300L™ 3.5 U/g CS) provided 34.85 ± 0.75 g/L RS and the maximum RS amount of 47.90 ± 0.39 g/L was obtained from the hydrolysis with mixed enzymes (Termamyl® 120, 1.2 U/g CS, AMG 300L™ 3.5 U/g CS followed by Accellerase® 1500, 2500 CMC U/g CS). From the cultivation of *Clostridium* sp. G10 using CS hydrolysate, the highest dry cell weight concentration of 1.28 ± 0.07 g/L was obtained with 11.68 ± 0.31 g/L butanol. It could be concluded that CS hydrolysate was comparable with glucose for utilization as a carbon source for butanol production.
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Keywords: Acetone–butanol–ethanol fermentation; Anaerobe; *Clostridium* sp.; Cassava stem

1. Introduction

Recently, lignocellulose materials from agricultural waste have gained increasing interest since they are plentiful and full of potential to utilize as a carbon source for microorganisms [1]. Moreover, the conventional disposal including burning and landfill have caused environmental problems and global warming. Cassava (*Manihot esculenta*) has been planted largely in Africa and Asia. Thailand is one of the top-5 five countries in term of

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production and net production value of cassava since 1975 [2] and in 2017 cassava production in this country reached 30.5 million tons [3].

Due to fuel crisis and insufficient resource of fossil fuel, the demand for alternative energy has been intensifying. Renewable carbon source is one of the distinct benefits of bioenergy and boosted the opportunity for generation of ecological and economical bio-fuel. Since its resemblance properties to gasoline, butanol has potential to be an alternative fuel. These resemblances include low vapour pressure (0.43 MJ/kg), energy level (29.2 MJ/L) and non-corrosivity [4]. Butanol is a precursor for many chemicals and solvent using in various industries [4] and can be produced using *Clostridium* sp. through acetone–butanol–ethanol (ABE) fermentation [5–7]. This metabolic fermentation is largely renowned due to the availability of wide range substrates and has been utilized in industrial process and patented [8].

Several publications examined the utilization of cassava stem (CS) for ethanol and biogas production [9–12], however, none of these reports focused on acetone–butanol–ethanol production. This is the first report to directly employ CS hydrolysate for *Clostridium* sp. for acetone–butanol–ethanol fermentation. In this research, CS was hydrolysed and effectively utilized for the cultivation of *Clostridium* sp. isolated from soil sediment in pond [13]. To obtain the highest reducing sugar concentration, CS was pretreated with NaOH prior to enzymatic hydrolysis using commercial amylase and cellulase.

2. Materials and methods

2.1. Cassava stem and bacterial strain

Cassava 'Rayong 81' stem was obtained from Po-Phan sub-district, Na Chueak district, Mahasarakham province, Thailand. Subsequently, the stems were peeled and cut into 1-cm pieces, sun dried, as well as milled with a hammer miller (Retsch, SK100) Afterwards, the CS powder was screened through 300 μm (Mesh no. 50) sieve. The microorganism was isolated from soil sediment in pond [13] and identified as *Clostridium* sp. G10 (GenBank no. MG674699). It was transferred and maintained in Reinforced Clostridial Medium (RCM) (Difco™) every month at 4 °C and kept for long term storage in glycerol stock at −70 °C.

2.2. Pretreatment and enzymatic hydrolysis of cassava stem

CS powder was treated with 1.0 M NaOH at the ratio of 1:10 (w/v) and autoclaved at 121 °C, 15 psi for 15 min. The mixture was filtered through Whatman® no. 1 filter paper and the filter cake was subsequently collected and washed until pH 7.0. For cellulase hydrolysis, the commercial Accellerase® 1500 (1 mL/ 1 g of pretreated CS or 2500 CMC U/g CS) was added while pH level and the mixture volume were adjusted to 5.0 and 25 mL, respectively. Afterwards, the incubation was carried on at 50 °C for 48 h and the sample was collected every 12 h period. For the hydrolysis with α-amylase and amyloglucosidase, 15 g CS was added into 100 mL distilled water and autoclaved at 121 °C, 15 psi for 15 min. Addition of 0.05%(v/v) α-amylase (Termamyl® 120, Sigma-Aldrich) of 3.3 mL volume (1.2 U/g CS) was done and incubated at 90 °C for 2 h in water bath (Memmert, WNE 45). The addition of 0.015%(v/v) amyloglucosidase (AMG 300L™, Sigma-Aldrich) of 13.3 mL (3.5 U/g CS) was then followed before the incubation at 60 °C for 4 h. For mixed enzyme hydrolysis, the similar procedure was followed with simultaneous addition of Termamyl® 120, AMG 300L™, Accellerase® 1500 with 48 h incubation period. In all hydrolysis experiments, the determination of reducing sugar concentration based on 3,5-dinitrosalicylic acid (DNS) method was followed [14].

2.3. Preparation of medium, inoculum and ABE fermentation

T6 medium [15] was implemented for inoculum preparation and ABE production which composed of 6 g/L tryptone, 2 g/L yeast extract, 0.5 g/L KH2PO4, 0.3 g/L MgSO4•7H2O, 0.01 g/L FeSO4•7H2O, 3 g/L ammonium acetate, 0.5 g/L cysteine hydrochloride and 50 g/L glucose. Glucose was separately sterilized from the other substituents at 121 °C, 15 psi for 15 min. The CS hydrolysate was supplemented with glucose to achieve 50 g/L of reducing sugar concentration. *Clostridium* sp. G10 in glycerol stock was transferred in RCM and heat-shocked at 80 °C for 10 min Herman et al. [16]. It was anaerobically incubated at 37 °C for 48 h and later transferred to T6 medium with 50 g/L glucose and cultivated in similar condition. This inoculum was transferred into T6 medium at 10% (v/v) and the anaerobic fermentation was performed in 500-mL flasks at 37 °C for 120 h with triplicate. Data was statistically analysed by SPSS for Windows program.

2.4. Chemical analyses

All experiments were carried out in triplicate. Data was statistically analysed by SPSS for Windows program. The sampling was performed periodically and the solid part was used for determination of dry cell weight concentration. Afterwards, the culture samples were centrifuged at 5000 rpm (4200 × g) for 10 min and the supernatants were collected for quantification of reducing sugar concentration using 3,5-dinitrosalicylic acid (DNS) method [14]. The concentration of glucose, maltose, xylose, cellobiose and ABE were determined by High Performance Liquid Chromatography (HPLC). The HPLC column was Aminex® fermentation monitor (150 mm × 7.8 mm). Refractive index detector was used and operated at 37 °C and 65 °C, respectively, with 5 mM H2SO4 as a mobile phase at 0.5 mL/min. The injection size was 20 μL.

3. Results and discussion

3.1. Pretreatment and enzymatic hydrolysis

Alkali pretreatment have been a recognized process for lignin dissolving from lignocellulosic materials to obtain fermentable sugars [17]. Nevertheless, the reducing sugar concentration of merely 10.94 ± 0.29 g/L was obtained from NaOH-pretreatment followed by Accellerase® 1500 hydrolysis of CS for 48 h (Fig. 1a). Considering that CS contained 15%–34% hemicellulose and 35%–42% cellulose depending on variety and plantation area [18], the result implied that abundant glucan was absent. There was current report regarding high starch substance up to 42% of dry CS weight [10]. To hydrolyse starch, NaOH pretreatment was neglected and amylase enzymes (Termamyl® 120 and AMG 300L™) were added to examine the glucan hydrolysis process. Fig. 1b showed the reducing sugar concentration of 34.85 ± 0.75 g/L from the addition of Termamyl® 120 (2 h) and AMG 300L™ (4 h). The final procedure included the addition of Termamyl® 120 (2 h) and AMG 300L™ with Accellerase® 1500 (48 h) resulting in the reducing sugar concentration of 47.90 ± 0.39 g/L at 12 h (Fig. 1c). This value has no significant difference ($p>0.05$) from the reducing sugar concentration of 47.18 ± 0.73 g/L at 38 h. Thus, the addition of Termamyl® 120 (2 h) and AMG 300L™ with Accellerase® 1500 (12 h) was selected to prepare for ABE fermentation medium.

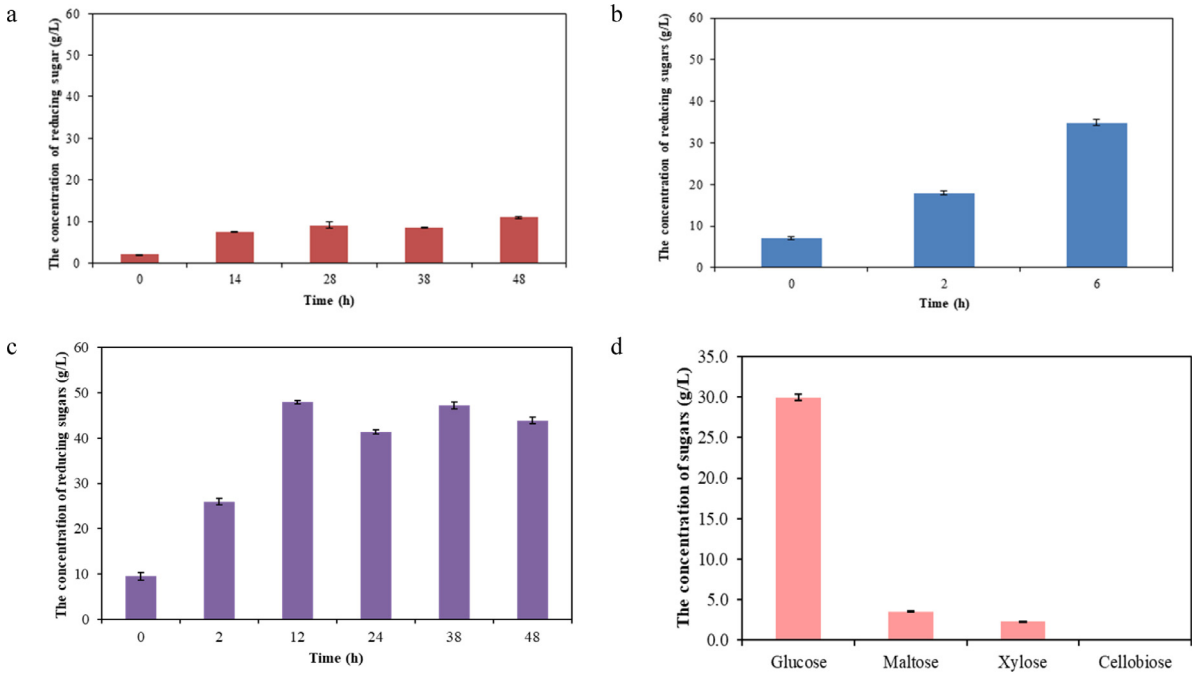

Fig. 1. Time course of the reducing sugar concentration from cassava stem measured by DNS method with (a) NaOH pretreatment and cellulase hydrolysis at 50 °C for 48 h, (b) Termamyl® 120 at 90 °C for 2 h and AMG 300L™ hydrolysis at 60 °C for 4 h, (c) Termamyl® 120 at 90 °C for 2 h and AMG 300L™ with cellulase hydrolysis at 50 °C for 48 h and (d) the concentration of glucose, maltose, xylose and cellobiose of cassava stem hydrolysate using Termamyl® 120 and AMG 300L™ with Accellerase® 1500 determined by HPLC.

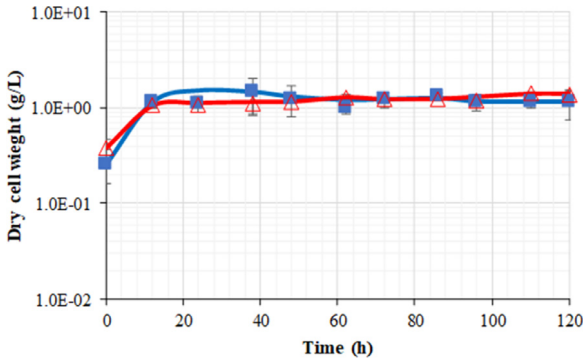

Fig. 2. Time course of dry cell weight concentration from *Clostridium* sp. G10 cultivation in T6 medium containing glucose (blue square) and cassava stem hydrolysate (red triangle) at 37 °C for 120 h under anaerobic condition.

From HPLC analysis (Fig. 1d), the CS hydrolysate contained 29.94 ± 0.37 g/L glucose, 3.54 ± 0.06 g/L maltose and 2.30 ± 0.05 g/L xylose. Small amount of xylose suggested that 'Rayong 81' stem contained small amount of hemicellulose. To compare the reducing sugar concentration, Table 1 indicated the concentration of sugars from CS with different pretreatment and enzymatic hydrolysis from various publications.

Table 1. The concentration of sugars from cassava stem with different pretreatment and enzymatic hydrolysis.

Conditions		Cassava stem concentration (%*w/v*)	The sugar concentration		Reference
Pretreatment	Enzymatic hydrolysis		Reducing sugars	Glucose (g/L)	
Thermohydrolysis (210 ° C, 45 min)	–	10	14.91 (g/L)	–	Nanssou et al. [19]
Microwave assisted NaOH (140 W, 30 min)	amylase (200U/g) amyloglucosidase (70 U/g) cellulose (20 FPU/g) β-glucosidase (1 IU/g)	5	459.0 (mg/g *CS*)	–	Sudha et al. [20]
Diluted H_2SO_4 (170 ° C, 20 min)	Liquozyme SC4X (0.01 mL /g) Spirizyme Excel XHS (0.015 mL/g)	10	–	29.9	Martin et al. [21]
Microwave assisted NaOH (300 W, 7 min)	Cellic® CTec2 (0.005 g enz. prot./ mL) Optimash BG (0.005 mL/ mL) StargenTM002 (0.001 mL/ mL)	10	59.05 (g/L)	–	Pooja et al. [11]
Repeated H_2SO_4 hydrolysis (111 ° C, 1 h & 1 h)		40	133.17 (g/L) [glucose + xylose]	94.05	Tanaka et al. [12]
–	Termamyl® 120 (1.2 U/g) AMG 300L™ (3.5 U/g) Accellerase® 1500 (2500 CMC U/g)	10	47.90 (g/L)	29.94	This report

3.2. Effect of cassava stem hydrolysate on cell growth and ABE production

The time profiles of G10 strain growth has been shown in Fig. 2. The cultivation in T6 medium with 50 g/L glucose contributed the highest dry cell weight of 1.45 g/L at 38 h. The dry cell weight in CS hydrolysate with the equivalent reducing sugar concentration of 50 g/L was 1.28 g/L after 62 h of cultivation. There was no obvious difference of dry cell weight between the cultivation of *Clostridium* sp. G10 using CS hydrolysate and glucose. On the other hand, there was slightly difference when compared the solvent production of *Clostridium* sp. G10 utilizing different carbon source (Fig. 3). From the cultivation of *Clostridium* sp. G10 using CS using glucose provided the maximum butanol concentration of 12.15 ± 0.07 g/L.

No publication report has directly utilized CS hydrolysate as a carbon source for acetone–butanol–ethanol fermentation. Torres et al. [22] reported hydrogen production from sludge in anaerobic fixed-bed reactor. Since the mixed culture was utilized, 1.56 g/L butanol was also produced from cassava starch wastewater with total carbohydrate of 2.12 g/L. The butanol concentration of 3.365 g/L was obtained from the production medium containing 60 g/L acid-hydrolysed sago (made from cassava starch) waste residues using *C. bifermentans* PNAS1 [23].

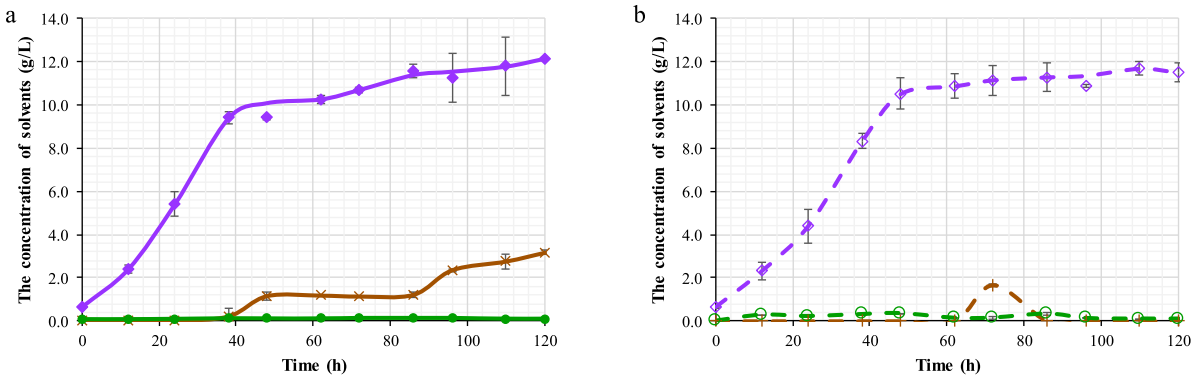

Fig. 3. Time course of the acetone (brown cross), butanol (purple diamond) and ethanol (green circle) concentration in T6 medium containing (a) glucose; (b) cassava stem hydrolysate from *Clostridium* sp. G10 at 37 °C with anaerobic condition.

4. Conclusion

In this research, cassava stem, agricultural waste, was utilized as a part of carbon source for isolated *Clostridium* sp. G10. Prior to the bacterial cultivation, the cassava stem was effectively hydrolysed by mixed enzymes (Termamyl® 120, AMG 300L™, Accellerase® 1500) and 47.90 g/L reducing sugar concentration was obtained. The concentrations of butanol achieved from T6 medium containing glucose (12.15 g/L) and cassava stem hydrolysate (11.68 g/L) were not significantly different ($p>0.05$). Renewable cassava stem has capability for the fermentation of cost-effective and sufficient biobutanol. This report has been the first to straightforwardly utilize cassava stem for butanol production.

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References

[1] Saini Jitendra Kumar, Saini Reetu, Tewari Lakshimi. Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. 3 Biotech 2015;5(4):337–53.
[2] FAO. 2019. http://www.fao.org/faostat/en/#rankings/countries_by_commodity. [Accessed 23 March 2019].
[3] Thai Office of Agricultural Economics. Industrial cassava: plantation area, harvesting area, production and production area in year 2017. 2018, http://www.oae.go.th/assets/portals/1/fileups/prcaidata/files/cassava60.pdf. [Accessed 23 March 2019].
[4] Zhu Jian-Hang, Yang Fangxiao. Biological process for butanol production. In: Cheng Jay J, editor. Biomass to renewable energy processes. New York: CRC Press; 2009, p. 246–96.
[5] Lee Sang Yup, Park Jin Hwan, Jang Seh Hee, Nielsen Lars K, Kim Jaehyun, Jung Kwang S. Fermentative butanol production by Clostridia. Biotechnol Bioeng 2008;101(2):209–28.
[6] Green Edward M. Fermentative production of butanol-the industrial perspective. Curr Opin Biotechnol 2011;22(3):337–43.
[7] Scully Sean Michael, Orlygsson Johann. Biological production of alcohols. In: Hosseini Majid, editor. Advanced bioprocessing for alternative fuels, biobased chemicals, and bioproducts. Oxford: Woodhead Publishing; 2019, p. 83–108.
[8] Lin De-Shun, Yen Hong-Wei, Kao Wei-Chen, Cheng Chieh-Lun, Chen Wen-Ming, Huang Chieh-Chen, Chang Jo-Shu. Bio-butanol production from glycerol with *Clostridium pasteurianum* CH4: The effects of butyrate addition and in situ butanol removal via membrane distillation. Biotechnol Biofuels 2015;8:168–79.
[9] Zhu Wanbin, Lestander Torbjorn A, Örberg Hakan, Wei Maogui, Hedman Bjorn, Ren Jiwei, Xie Guanghui, Xiong Shaojun. CaSsava stems: a new resource to increase food and fuel production. GCB Bioenergy 2013;7(1):72–83.
[10] Wei Maogui, Zhu Wanbin, Xie Guanghui, Lestander Torbjorn A, Xiong Shaojun. CaSsava stem wastes as potential feedstock for fuel ethanol production: A basic parameter study. Renew Energy 2015;83:970–8.
[11] Pooja NS, Sajeev MS, Jeeva ML, Padmaja G. Bioethanol production from microwave-assisted acid or alkali-pretreated agricultural residues of cassava using separate hydrolysis and fermentation (SHF). 3 Biotech 2018;8(69).
[12] Tanaka Kazumasa, Koyama Mitsuhiko, Pham Phan Thi, Rollon Analiza Palenzuela, Habaki Hiroaki, Egashira Ryuichi, Nakasaki Kiyohiko. Production of high-concentration bioethanol from cassava stem by repeated hydrolysis and intermittent yeast inoculation. Int Biodeteriorat Biodegrad 2019;138:1–7.

[13] Buakhiaw Budsaba, Sanguanchaipaiwong Vorapat. Effect of media on acetone-butanol-ethanol fermentation by isolated *Clostridium* spp. Energy Procedia 2017;138:864–9.
[14] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31(3):426–8.
[15] Kashket Eva R, Cao Zhi-Yi. Isolation of a degeneration-resistant mutant of *Clostridium acetobutylicum* NCIMB 8052. Appl Environ Microbiol 1993;59(12):4198–202.
[16] Herman Nicolaus A, Kim Seong Jong, Li Jeffrey S, Cai Wenlong, Koshino Hiroyuki, Zhang Wenjun. The industrial anaerobe *Clostridium acetobutylicum* uses polyketides to regulate cellular differentiation. Nature Commun 2017;8(1514).
[17] Kim Jun Seok, Lee YY, Kim Tae Hyun. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresour Technol 2016;199:42–8.
[18] Klinpratoom Bunpot, Ontanee Anissara, Ruangviriyachai Chalerm. Improvement of cassava stem hydrolysis by two-stage chemical pretreatment for high yield cellulosic ethanol production. Korean J Chem Eng 2015;32(3):413–23.
[19] Nanssou Paul Alain Kouteu, Nono Yvette Jiokap, Kapseu Cesar. Pretreatment of cassava stems and peelings by thermohydrolysis to enhance hydrolysis yield of cellulose in bioethanol production process. Renew Energy 2016;97:252–65.
[20] Sudha A, V. Sicakumar V, Sangeetha V, Priyenka Devi KS. Improving enzymatic saccharification of cassava stem using peroxide and microwave assisted pre-treatment techniques. Chem Ind Chem Eng Q 2017;23(3):357–66.
[21] Martin Carlos, Wei Maogui, Xiong Shaojun, Jönsson Leif J. Enhancing saccharification of cassava stems by starch hydrolysis prior to pretreatment. Ind Crops Products 2017;97:21–31.
[22] Torres Douglas GB, Lucas Shaiane Dal Maso, Andreani Cristiane L, de Carvalho Karina Q, Coelho Silvia RM, Gomes Simone D. Hydrogen production and performance of anaerobic fixed-bed reactors using three support arrangements from cassava starch wastewater. Engenharia Agricola 2017;37(1):160–72.
[23] Johnravindar Davidraj, Murugesan Kumarasamy, Wong WC, Elangovan Namasivayam. Waste-to-biofuel: production of biobutanol from sago waste residues. Environ Technol 2017;38(13–14):1725–34.