Optimization Method for the Bioethanol Production from Giant Cassava (*Manihot esculenta* var. Gajah) Originated from East Kalimantan

Krishna Purnawan Candra[1,2,*], Kasma[1], Ismail[1], Marwati[1], Wiwit Murdianto[1], and Yuliani[1]

[1]*Department of Agricultural Product Technology, Faculty of Agriculture, Mulawarman University, Jl. Tanah Grogot, Kampus Gunung Kelua, Samarinda 75119, East Kalimantan, Indonesia*

[2]*Environmental Science Study Program, Post Graduate Program, Mulawarman University, Jl. Ki Hajar Dewantara, Kampus Gunung Kelua, Samarinda 75119, East Kalimantan, Indonesia*

* ***Corresponding author:***

email: candra@faperta.unmul.ac.id

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Abstract: *Here is the first report of bioethanol production from giant cassava, a variety of cassava originated from East Kalimantan. Hydrolysis on freshly grated cassava with two different acids was studied separately. The experiment was conducted as a single factor experiment in Completely Randomized Design (CRD) with five treatments (0.0–1.0 M of acid solution), each replicated three times. Reducing sugars, unhydrolyzed substance (fibers), and hydrolysate clarity were determined. The experiment was continued by studying fermentation condition using factorial experiment (2 x 4) in CRD. The first factor was yeast concentration (*Saccharomyces cerevisiae*, 5 and 10%) and the second factor was fermentation time (2–11 days). Biomass and alcohol content in fermentate were determined. The data were analyzed by ANOVA, excluding alcohol content that was analyzed by the non-parametric statistic. Optimization using regression analysis showed that hydrolysis by H_2SO_4 was more effective than HCl. Hydrolysis solution of 0.58 M H_2SO_4 gave an optimum reducing sugar in hydrolysate (5.6%), which equivalent to a yield of 28.18%. Starter concentration affected significantly on biomass and alcohol content ($p < 0.001$) of fermentate, while fermentation time affected significantly only on alcohol content ($p < 0.001$). Optimum condition of cassava hydrolysate fermentation (100 mL) was using 5% yeast for 8 days, which gave a yield of 14.17% bioethanol.*

Keywords: *cassava; bioethanol; acid hydrolysis;* S. cereviceae

■ INTRODUCTION

Bioethanol is ethanol (C_2H_5OH) derived from bioresource rich in carbohydrates like simple sugar (glucose, sucrose, and fructose), homopolymer (starch and cellulose) and heteropolymer (hemicellulose and seaweed) polysaccharide. Its characteristics of environmentally friendly, home ground, and renewable make it as promises fuel as a capacity limitation of fossil fuels. Even with the lower price of gasoline in the last decade, ethanol plays a very important role as it is the safest and lowest-cost octane available [1]. It is also has a broad range of application in perfumes, paints, lacquer, and explosive industry, that makes ethanol market growth in the coming years [2]. Bioethanol is produced by 4 main steps process, namely hydrolysis (saccharifying), fermentation, distillation, and dehydration [3]. The unhydrolyzed biomass and the biological agent used in fermentation are by product and become feedstock [1].

Hydrolysis process is needed when using non-simple sugar raw material in bioethanol production followed by milling and liquefying process for starchy materials or milling and pre-treatment for lignocellulose materials. It can be performed by acid or enzymatic hydrolysis [4]. Sulphuric and hydrochloric acid are the acids usually used in the acid hydrolysis process. However, the yield is varied on the type of acid and the raw materials [5-6].

Indonesia has huge biodiversity of plants, that some of them have potential to be utilized as bioethanol raw material like a simple sugar, e.g. arenga, coconut, sorghum, nypa sap [7-10], and molasses [11]. Raw material which is rich in starch, e.g. cassava [12], sweet potatoes [13], sago [6], sorghum, banana [14], iles-iles [15], and breadfruit [16]; rich in lignocellulose, e.g. sugarcane bagasse [17], coconut husk [18], macaranga wood [19], cassava peel [20], durian seed [21], and rice straw [22] are one of the raw materials for bioethanol generation. The raw material in the form of heteropolymer polysaccharides, e.g., seaweed [23-24] also can be used in the production of bioethanol.

Simple sugar, starch, and seaweed need relatively lower energy than lignocellulose, which makes them showing higher energy yield efficiency. Cassava needs the energy of 61 MJ for 1 kg of ethanol [3]. In 2015, harvested area of cassava in East Kalimantan was 2,384 ha with the productivity of 22.64 tons per ha, which was dominated by cassava var. Gajah [25]. This cassava is the new variety (certified by Centre for Plant Variety Protection and Agriculture Licensing of the Ministry of Agriculture of Indonesia No.182/PVHP/2013 in 2013 November, 18) that was developed in 1992 through natural cross-linking between Ceara rubber (*Manihot glaziovii* Müll Arg) and cassava (*Manihot esculenta* Crantz) [26]. Production of cassava var. Gajah is much higher, reach 30-40 kg per tree [27]. Its productivity is even higher than Mukibat cassava, a scion of Ceara rubber in *Manihot esculenta* root [28-29].

Until now, there is no scientific report about producing bioethanol from cassava var. Gajah. Here we describe the development of the acid hydrolysis method and fermentation process of cassava var. Gajah to produce bioethanol.

■ EXPERIMENTAL SECTION

Materials

Cassava var. Gajah tubers at the age of 8–9 months with an average diameter of 10 cm were obtained from a local farmer at Berambai, Sempaja sub-district, Samarinda, East Kalimantan. Starch, H_2SO_4, $Na_2S_2O_3$, luff schoorl reagent, KI, and HCl obtained from Riedel-Haen and Sigma. *Saccharomyces cerevisiae* used was commercial yeast for bread making (Haan) obtained from the local market.

Experimental Design and Data Analysis

This experiment was conducted in two steps, i.e., optimization of acid hydrolysis (H_2SO_4 and HCl) method and fermentation process. This acid hydrolysis was studied separately to prevent the negative interaction between the two types of acid when used as combining hydrolyzing agent [30]. Optimization of hydrolysis process was performed as single factor experiment for each type of acid, arranged in Randomized Completely Design (RCD) with 5 levels of treatments (0.0, 0.1, 0.4, 0.7, and 1.0 M), each repeated three times. The best acid hydrolysis condition was then used in the fermentation process step. It was conducted as a factorial (2 x 4) experiment arranged in RCD; each treatment was repeated three times. The first factor was yeast concentration (5 and 10%) and the second factor was fermentation time (2, 5, 8, and 11 days). Data were analyzed by ANOVA continued by Tukey test at α of 0.05. Non-parametric statistical analysis using Friedman and Wilcoxon test was applied for non-normal distributed data. Regression analysis was applied to determine the optimum hydrolysis condition.

Procedure

Optimization of acid hydrolysis process

Total and reducing sugar content was performed by Luff Schoorl method [31]. The clarity of hydrolysate was determined by spectrophotometer at a wavelength of 660 nm, and residual solid following hydrolysis process was determined by weighing following filtrating the hydrolysate. Cassava was selected, peeled, washed and grated. Acid hydrolysis (100 °C, 1 bar for 30 min.) was carried out by adding 20 g of grated cassava to 100 mL acid solution (0–1.0 M H_2SO_4 or HCl) in a 500 mL boiling flask connected to the condenser. Afterward, the hydrolysate was filtered and the filtrate was determined for clarity and reducing sugar, while the solid fraction was then weighed to calculate the unhydrolyzed substance (fibers).

Optimization of fermentation process

Biomass resulted from fermentation process was determined by weighing the sediment followed by centrifugation of batch culture, while alcohol content of the filtrate was determined by alcoholmeter/hydrometer [23]. The fermentation process was performed by adding dry yeast (5–10 g) to 100 mL neutralized hydrolysate filtrate obtained from the hydrolysis process using 0.58 M H_2SO_4 solution. The anaerobic fermentation process was carried out at room temperature for 2–11 days.

■ RESULTS AND DISCUSSION

Optimization of Acid Hydrolysis Process

Acid concentration affected significantly ($p < 0.001$) on the efficiency of the hydrolysis process. Pirt and Whelan [32] recommended the use of sulphuric acid instead of hydrochloric acid. Hydrolysis in H2SO4 gave a more stable suspension of the sample compared to HCl-hydrolysed sample [34]. In this experiment, sulphuric acid showed more effective in the hydrolysis process (Table 1) of cassava var. Gajah than hydrochloric acid. The acid hydrolysis at 100 °C for 30 min resulted in a negative parabolic trend of reducing sugars released with optimum concentration at 0.5781 and 0.6133 M for H_2SO_4 and HCl, respectively (Fig. 1).

In this research, we could not demonstrate the hypothesis that acid hydrolysis on fresh tubers would give more sugar than enzymatic hydrolysis because it will release sugar from fiber, which not in the case of enzymatic hydrolysis. The acidic and hot condition might be responsible for the breakdown of some sugar released. We observed that acid concentrations affected different trend on solid residue and clarity value (Table 1). An increasing of acid concentration resulted in a linear decreasing value of solid residue and the opposite a linear increase of clarity. It means that applying a higher concentration of acid affected more damage on

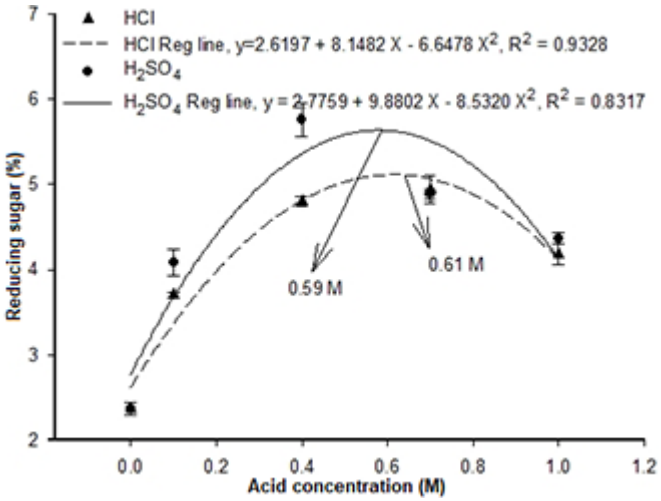

Fig 1. Optimization of acid hydrolysis (saccharifying) of freshly grated cassava var. Gajah tubers. Data and condition are referred to Table 1

Table 1. Effect of acids on hydrolysis parameters of cassava var. Gajah

Type of acid and conc. (M)		Hydrolysate characteristics			The yield of reducing sugar (%)
		Solid residue (%)[1]	Clarity (Abs at 660 nm)	Reducing sugars (%)	
HCl	0.0	(9.87 ± 0.02) ^{a}	(0.64 ± 0.01) ^{a}	(2.36 ± 0.04) ^{d}	(11.82 ± 0.21) ^{d}
	0.1	(8.89 ± 0.11) ^{b}	(0.59 ± 0.02) ^{a}	(3.70 ± 0.02) ^{c}	(18.52 ± 0.10) ^{c}
	0.4	(5.84 ± 0.02) ^{c}	(0.24 ± 0.05) ^{b}	(4.80 ± 0.03) ^{a}	(24.01 ± 0.15) ^{a}
	0.7	(3.51 ± 0.20) ^{d}	(0.17 ± 0.02) ^{b}	(4.94 ± 0.09) ^{a}	(24.69 ± 0.47) ^{a}
	1.0	(2.52 ± 0.04) ^{e}	(0.12 ± 0.00) ^{b}	(4.18 ± 0.07) ^{b}	(20.91 ± 0.34) ^{b}
H_2SO_4	0.0	(9.87 ± 0.02) ^{a}	(0.64 ± 0.01) ^{a}	(2.36 ± 0.04) ^{d}	(11.82 ± 0.21) ^{d}
	0.1	(6.81 ± 0.26) ^{b}	(0.37 ± 0.09) ^{ab}	(4.08 ± 0.09) ^{c}	(20.40 ± 0.45) ^{c}
	0.4	(6.47 ± 0.12) ^{b}	(0.22 ± 0.04) ^{b}	(5.76 ± 0.11) ^{a}	(28.79 ± 0.55) ^{a}
	0.7	(2.59 ± 0.14) ^{c}	(0.18 ± 0.04) ^{b}	(4.89 ± 0.03) ^{b}	(24.44 ± 0.16) ^{b}
	1.0	(1.94 ± 0.04) ^{c}	(0.18 ± 0.00) ^{b}	(4.36 ± 0.04) ^{bc}	(21.81 ± 0.19) ^{b}

Notes: A 20 g of freshly grated cassava was hydrolyzed in 100 mL of acid solution in 500 mL flask connected to the condenser at 100 °C, 1 bar for 30 min. Data (mean ± std.error) were obtained from 3 replications. [1]Compared to the initial weight of cassava as (w/w). Data were analyzed by ANOVA continued by Tukey test α 0.05. For each hydrolysis agent, data in the same column followed by different letter show significantly different.

Table 2. Effects of yeast (*S. cerevisiae*) concentration, fermentation time and their interactions on biomass residue and alcohol content in 100 mL batch culture

(a) Biomass Residue (g)

Time (days)	Yeast starter conc. (w/v)		Average
	5%	10%	
2	10.44 ± 0.14	19.43 ± 1.13	14.94 ± 2.07
5	10.70 ± 0.26	20.62 ± 0.26	15.66 ± 2.22
8	10.94 ± 0.26	21.48 ± 0.59	16.21 ± 2.37
11	10.95 ± 0.26	21.46 ± 0.57	16.21 ± 2.37
Average	(10.76 ± 0.12) [a]	(20.75 ± 0.39) [b]	

(b) Alcohol Content (%)

Time (days)	Yeast starter conc. (w/v)		Average
	5%	10%	
2	(0.00 ± 0.00)	(0.00 ± 0.00)	(0.00 ± 0.00) [a]
5	(1.50 ± 0.00)	(1.83 ± 0.17)	(1.67 ± 0.21) [b]
8	(2.50 ± 0.00)	(2.83 ± 0.08)	(2.67 ± 0.17) [c]
11	(2.50 ± 0.00)	(2.70 ± 0.03)	(2.60 ± 0.09) [c]
Average	(1.63 ± 0.31) [a]	(2.84 ± 0.34) [b]	

Notes: A 100 mL of neutralized hydrolysate filtrate (2.74-3.19% sugar conc.) was fermented anaerobically by *S. cerevisiae*. Data (mean ± std.error) were obtained from 3 replications. Data in each shaded row/column followed by a different letter showed significantly different (Tukey, α 0.05). **(a)** Biomass as a solid fraction was weight following centrifugation and decantation. Data were analyzed by F test continued by Tukey test (α 0.05). There is no interaction between yeast conc. and fermentation time. **(b)** Data were distributed non-normal, and the interaction effect was not analyzed because of the lack of method. Data were analyzed by Friedman and Wilcoxon test for fermentation time and yeast conc. factor, respectively.

monosaccharides than the breakdown of polysaccharides itself into monosaccharides. Hafid et al. [33] showed that fermentable sugar production from food waste would decline with the increase of acid concentration.

Susmiati et al. [12] reported that applying two steps of acid hydrolysis was not effective on cassava var. Darul Hidayah obtained from Sukabumi. However, they showed that their study showed the same effect to this study on reducing sugars content (negative parabolic trend). They reported that the optimal concentration of acid (0.5 M H_2SO_4) resulted in higher reducing sugars yield, i.e. 25% from the cassava.

These findings lead us to be more creative to find a new method to optimize the hydrolysis of the cassava flesh, which is still rich in fiber. Acid hydrolysis produced reducing sugar concentration in hydrolysate of 5.6% (28.18% of yield) shown in this study is still needed to be improved by modification of the hydrolysis procedure or by applying enzymatic hydrolysis or any combination of both hydrolysis methods. It is very important to find the appropriate hydrolysis temperature and time. Hafid et al. [33] showed that higher acid concentration would produce higher fermentable sugar, however appropriate time should be determined because the longer hydrolysis time would decrease the sugar produced. Enzymatic hydrolysis of polysaccharides showed higher reducing sugars, i.e. about 20.49 and 19.32% in hydrolysate following liquefaction and saccharification of iles-iles and sorghum starch, respectively [15].

Fermentation of Cassava Hydrolysate Filtrate

Yeast concentration affected significantly ($p < 0.001$) on biomass residue in the batch culture, while fermentation time ($p = 0.092$) and interaction of both treatments ($p = 0.940$) affected insignificantly on biomass residue (Table 2a). On the other hand, both yeast starter

($p = 0.008$) and fermentation time ($p < 0.001$) affected significantly on alcohol concentration in the batch culture. Due to the non-normal distribution data, the interaction between both treatments was not analyzed for this parameter (Table 2b).

The two yeast (*S. cerevisiae*) concentration (5 and 10%) in the batch culture showed similarly rate growth of yeast. Observation on the second day found that biomass multiplied doubled as each initial concentration and slightly increase in the next observation days (5–11 days). However, ethanol concentration in the batch culture with an initial 10% of yeast concentration was just slightly higher than the other one at each observation day. In application, bioethanol industries focus not only on the main product (bioethanol) but also CO_2 and biomass [1]. The higher yield of biomass would be an important part to be considered in the bioethanol industry.

Feeding of 10% of dry yeast for 8 days in anaerobic fermentation of cassava var. Gajah yielded the highest ethanol concentration in batch culture, i.e. 2.83%. It means that the fermentation of the hydrolysate following acid hydrolysis by 0.59 M H_2SO_4 for 30 min gave an ethanol yield of 14.15%. The conversion efficiency of sugar to ethanol in this research was actually higher than one reported, i.e. 1.2–2.25% [16-17,35], but still lower than other reports i.e. 4.2–10.0% [12,15,20,36-40], 28.18–29.33% [39,41].

To improve the efficiency of sugar to ethanol conversion in a further experiment, some efforts in terms of starter will be considered to be applied, e.g. by feeding fresh starter (10% v/v) instead of dry yeast [17,36], applying immobilized yeast [40], or using superior yeast type [36-37]. Sugar concentration in fermentation media is also a factor to be considered, which sugar concentration of 10% showed the best effect on alcohol production [40]. Addition of protein (yeast extract) and mineral ($MgSO_4$, $(NH_4)_2SO_4$) boosted the ethanol production [38,40,42-43]. However, some mineral supplements like $CaCl_2$ and KH_2PO_4 gave negative effect to *S. cerevisiae* on bioethanol production [42].

■ CONCLUSION

Acid hydrolysis by H_2SO_4 and HCl of 0–1.0 M conducted at 100 °C, one bar for 30 min. showed the same effect, i.e., a negative parabolic effect on saccharifying of the freshly grated cassava var. Gajah. Sulphuric acid is found to be more efficient than HCl in the saccharifying process. The optimum concentration of H_2SO_4 and HCl on the process were 0.58 and 0.61 M, which resulted in a yield of reducing sugar of 28.20 and 25.60%, respectively. Anaerobic fermentation of neutralized hydrolysate filtrate from cassava var. Gajah by 5% of *S. cerevisiae* in a system of 100 mL for 8 days gave the highest yield of ethanol (14.17%). The finding of optimum sulphuric acid concentration in cassava hydrolyzing for fermentable sugar can be used as basic information to develop bioethanol production from cassava var. Gajah.

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