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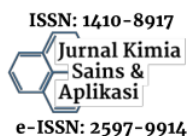
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Abstract

A large amount of Cassava peel as biomass waste is generated by agricultural activities, and it led to a new pursuit to exploit the utilization of biomass waste. This research aimed to study the potential of Cassava peel as raw material for bioethanol production. This study was performed in 2 main processes, acid hydrolysis, and fermentation. The experiment was initiated by conducting acid hydrolysis (100°C and 60 min) on Cassava peel's starch using sulfonated carbon catalyst palm oil empty fruit bunch (5% -w/v) to produce 13.53 g/L glucose. The glucose contained hydrolysates then continued to ferment at 30°C. The effect of fermentation time (h), pH, and shaking rate (rpm) of cassava peel's starch fermentation using *Saccharomyces cerevisiae* was analyzed. The best result was found at pH 4.5 and 50 rpm for a 24 h reaction with 3.75 g/L of bioethanol concentration. This study revealed that Cassava peel is a promising feedstock for biofuel production.

1. Introduction

Energy systems are mainly based on fossil resources such as coal and petroleum. However, these fossil fuels' depletion reserves and their harmful effects on the environment are significant challenges. It is necessary to utilize renewable resources for next-generation energy systems, such as cellulose [1, 2]. Currently, scientific studies have focused on cellulose derived from the waste of biomass as the feedstock for biofuel production. Cassava peel is one of the lignocellulose materials from the food processing industry's waste [3, 4]. According to Bantacut and Ramadhani [5], every one kilogram of Cassava can produce 10–15% of its waste peels, which has been reported to consist of cellulose 37.9%, hemicellulose 37%, and lignin 7.5% [6], that is suitable as raw material for bioethanol production. Many studies have been reported about bioethanol production from Cassava peel using yeast fermentation [7, 8, 9].

Bioethanol is an alternative to fossil fuels with a high octane number. As a biofuel, it reduces gas emissions to the atmosphere [10]. It could be produced by fermentation of reducing sugar derived from the

hydrolysis process [11, 12]. Acid and enzymatic hydrolysis is generally performed to extract sugar compounds from lignocellulose material [13, 14, 15]. As environmentally sustainable chemical process issues, heterogeneous acid catalysts on cellulose hydrolysis become more popular.

Furthermore, the heterogeneous acid catalyst provides easy separation from liquid products by decantation or filtration. The catalyst can be reused for the reaction without neutralization, minimizing energy consumption and waste [16, 17, 18, 19, 20, 21]. One of the drawbacks utilization of liquid mineral acids as catalysts because the concentrated mineral acids could degrade or convert the free sugars at high temperatures (170–240°C). Thus, the heterogeneous acid catalyst is more preferred than the liquid acid catalyst [22].

Indonesia is the largest palm oil producer globally, generates more than 40 million tons of palm oil empty fruit bunches (PEFB) every year [23]. The PEFB is usually treated as waste with low-value applications such as organic mulch in plantation and additional fertilizer [24, 25]. To improve the value of PEFB, previous studies were

utilized and processed as a heterogeneous acid catalyst for chemical reactions [26, 27]. From now on, this study utilized PEFB as a solid acid catalyst on hydrolysis reaction before the yeast fermentation process in bioethanol production.

In this work, bioethanol was produced from Cassava peel. Cassava peel's starch was initially extracted and dried, then continued by hydrolysis process using sulfonated PEFB solid catalyst to generate glucose feedstock for yeast fermentation. The effect of reaction time, pH, and shaking rate on yeast fermentation was investigated. Hence, Cassava peel's potential as the feedstock for bioethanol production was evaluated by this series of work.

2. Methodology

2.1. Material

All chemicals (reagent grade) and microorganisms were purchased from Sigma Aldrich (Singapore). Cassava peel was obtained from the traditional market near Banjarbaru District, South Kalimantan, Indonesia. The starch was extracted from Cassava peel. The Cassava peels were washed and shredded. The product was mixed with distilled water and filtered by using a clean cloth. The white precipitated material (starch) was separated and dried at 80°C in the oven for 8 hours until the weight reached a constant value. The dried starch of Cassava peel was milled by a domestic blender and sieved to pass through a 250 mesh. A palm oil empty fruit bunch (PEFB) was obtained from PT. Pola Kahuripan Inti Sawit, Kintapura, South Kalimantan, Indonesia. The PEFB was washed and dried in an oven at 100°C, then ground using a high-speed blender and sieved to pass through a 60 mesh. The powdered PEFB was heated in a furnace (muffle furnace, Hema scientific instrument) at 350°C for 30 min under an inert atmosphere. The carbon material was sulfonated via a hydrothermal process, according to Nata *et al.* [28]. The sulfonated PEFB was rinsed using 50% methanol and water, then again dried at 80°C overnight to produced sulfonated palm oil empty fruit bunch (PEFB-SO₃H) catalyst.

2.2. Hydrolysis of powdered starch of Cassava peel using sulfonated palm oil empty fruit bunch (PEFB-SO₃H) catalyst

The powdered starch of Cassava peel was hydrolyzed using PEFB-SO₃H in a 250 mL glass reactor. A 5%-w/v of starch and PEFB-SO₃H (2.5 g for each) were mixed in 50 mL of distilled water and heated up to 100°C for 60 min. After the reaction was completed, the hydrolysate was then centrifuged, and the supernatant was recovered. Then the presence of glucose was analyzed by using DNS (3,5-dinitrosalicylic acid) method [29]. The hydrolysate is further used for yeast fermentation to produce bioethanol.

2.3. Fermentation

The catalytic hydrolysate containing glucose was subjected to fermentation by *Saccharomyces cerevisiae* BY4743 obtained from Biomolecular Engineering Laboratory (E2-719) Taiwan Tech., Taipei, Taiwan. A

single colony of that strain was inoculated in a 250 mL conical flask containing 100 mL Yeast-Peptone-Dextrose medium, which grew at 30°C and 150 rpm until the exponential phase was reached, and then used as a starter culture for the fermentation process. Fermentation was conducted in a 250 mL conical flask with a 40 mL working volume. The nutrient solution was prepared with yeast extract (0.2%), MgSO₄·7H₂O (0.025%), and (NH₄)₂SO₄ (0.1%) and sterilized. Initially, 16 mL of the hydrolysate was mixed with 20 mL of nutrient solution. Fermentation was started by transferring 10% (4 mL) of the starter culture to the prepared media. The system's pH was varied for 4.0, 4.5, and 5.0. Fermentation was conducted at 30°C in shaker operating at various shaking rates (50, 100, 150 rpm for a specific range of time (0-72) h. The samples were aseptically withdrawn at 4 hours' time intervals, and they were purified by filtering the suspended material from the liquid product, then ready for further analysis.

2.4. Analytical Method

The chemical composition of extracted material of Cassava peel was conducted as follows. Moisture content was determined using a gravimetric method by drying at 105°C until it reaches a constant weight. Ash content was determined by heating at 600°C in a furnace for 3 h. Fat and protein content was determined by Soxhlet extraction and the Kjeldahl method, respectively. Total starch (carbohydrate) was determined using Total Starch Kit (Megazyme Ltd., Ireland). All analysis was conducted at Biomolecular Engineering Laboratory (E2-719) Taiwan Tech., Taipei, Taiwan.

The sample's surface morphology was analyzed by Scanning Electron Microscope (SEM), which was carried out using a JEOL JSM-6500 LV microscope. Fourier-transform infrared spectroscopy (FT-IR, Bio-rad, Digilab FTS-3500) was utilized to analyze the functional groups' carbon surface in the wavelength range of 4000-400 cm⁻¹ at a scan rate of 8. A Rikagu D/MAX-B X-ray diffractometer equipped with copper K-alpha (Cu K α) radiation sources was used in the X-ray diffraction (XRD) measurements at a voltage of 40 kV and current of 100 mA.

Bioethanol concentration was detected using a gas chromatography Shimadzu QP 2010 SE equipped with a flame ionization detector (FID) and Rtx-5MS and Carbowax columns. Nitrogen was used as the carrier gas. The oven temperature was set up at 55°C for the first 3 min, which then increased to 120°C with an increased rate of 4°C/min. All experiments and assays were performed in duplicates.

3. Results and Discussion

3.1. The Starch Composition of Cassava Peel

The starch solution was extracted from fresh Cassava peel, then continued for drying and screening to produce light brown fined powder (Figure 1B). The yield of starch extraction is 40% based on fresh Cassava peel. The chemical composition of Cassava peel was analyzed, and it is given in Table 1. About 80% of Cassava peel is a carbohydrate that refers to starch, while Bayitse *et al.*

[30] and Souto *et al.* [31] found Cassava's starch peel was 47% and 60%, respectively.

Carbohydrates found in nature are present as polysaccharides which are high molecular weight polymers of monosaccharides. Starch is one example of polysaccharides consisting of amylose and amylopectin [32]. According to Charles *et al.* [33], amylose and amylopectin are arranged by monomers of glucose. Those monomers are joined together by an α -glycosidic bond. The hydrolysis process can convert starch into glucose as reducing sugar that is utilized for bioethanol production [34].

Table 1. The composition of Cassava peel.

Analyzed component	Composition (%)
Moisture	8.73
Ash	1.86
Protein	2.92
Fat	0.59
Carbohydrate	80.2

Native starch granules from different plants have dimensions between 0.5–175 μm and various shapes: spherical, oval, disk, polygonal, and rods [35]. The scanning electron microscopy (SEM) image of starch from Cassava peel granulate microstructure (Figure 1C) revealed that it has a spherical shape, and the size of granules is between 4–10 μm . The size is smaller than granules from tubers such as potato and canna with sizes of about 100 μm [36].

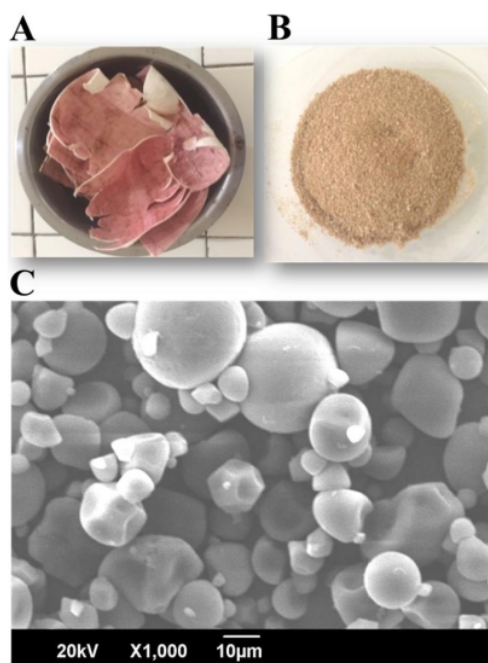


Figure 1. A. Fresh Cassava peel; B. Extracted material from Cassava peel; C. SEM image of starch from Cassava peel granulates microstructure

3.2. Catalytic Hydrolysis of Starch from Cassava Peel using PEFB-SO₃H

Acid catalytic hydrolysis of the starch was performed to breakdown carbohydrates (cellulose and hemicellulose) to produce glucose. The hydrolysis was carried out using a solid acid catalyst synthesized from a palm oil empty bunch (PEFB). PEFB was sulfonated by 6.25%-w/v of hydroxyethylsulfonic acid to generate sulfonated palm oil empty fruit bunch (PEFB-SO₃H) to activate this catalyst. The synthesized catalyst's structural morphology before and after sulfonation was shown as an SEM image (Figure 2A and 2B). The significant changes in morphology after sulfonation was observed, the numerous pores with irregular sizes are generated on the carbon shell. This structure of PEFB-SO₃H can hydrolyze the starch effectively. The Fourier transform infrared (FT-IR) spectra depicting the surface chemistry characteristics before and after sulfonation (Figure 3). After sulfonation, the peaks at 1181 and 1800 cm^{-1} correspond to the -SO₃H group's stretching vibration. This result indicates that the prepared carbon catalyst was successfully incorporated with sulfonic groups to form acid sites on the surface. The X-ray diffraction (XRD) analysis was performed to confirm the structural properties after sulfonation (Figure 4). The XRD patterns of carbon and sulfonated carbon catalyst, the broad C (002) at diffraction peak (2θ) about 15–30 might be attributed to amorphous carbon composed of aromatic carbon sheets oriented in a considerably unordered form [37].

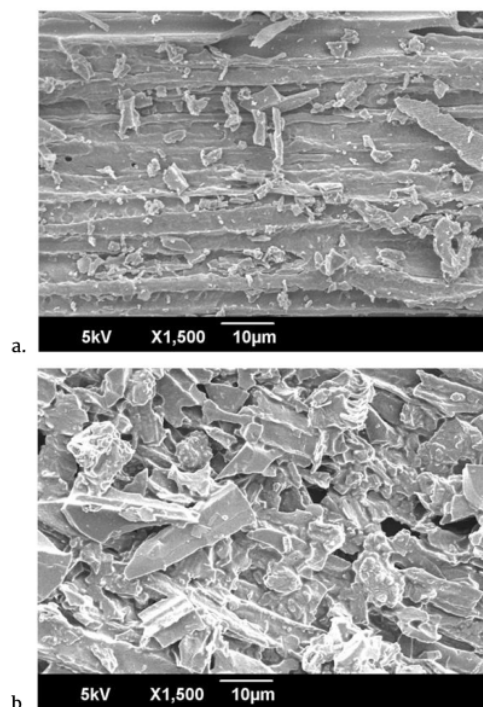


Figure 2. A. SEM for carbon acid catalyst before sulfonation, B. SEM for carbon acid catalyst after sulfonation.

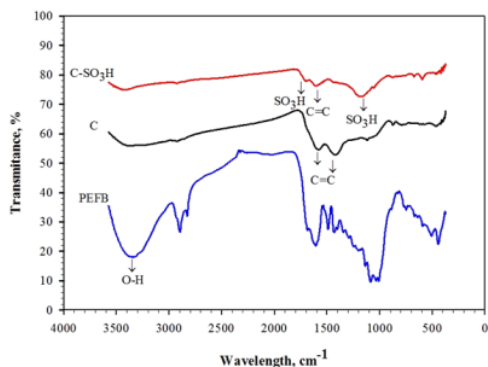


Figure 3. FTIR spectra of carbon acid catalyst before and after sulfonation

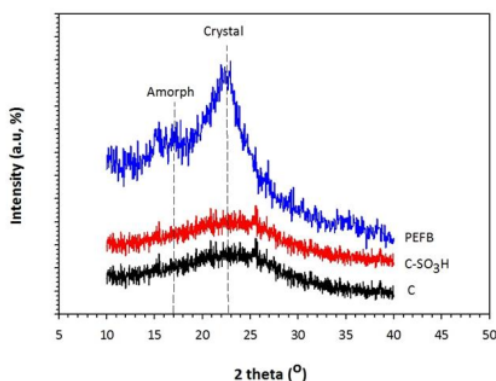


Figure 4. XRD pattern of carbon acid catalyst before and after sulfonation.

In 50 mL of distilled water, 5%-w/v of each starch (2.5 g) and PEFB-SO₃H (2.5 g) were added, and the reaction was conducted for 60 min at 100°C. As a result, 13.53 g/L of glucose was produced, and the calculated yield was 33.7%. PEFB-SO₃H catalyzed hydrolysis was earlier found to be effective in enhancing glucose production. Li *et al.* [38] and Onda *et al.* [39] prepared sulfonated carbon catalysts via various processes and gave glucose yields of 40.5% and 34.6%, respectively. Hemalatha and Brinda Lakshmi [40] performed catalytic hydrolysis from fruit waste using sulfonated magnetic carbon as a catalyst and resulted in the maximum yield of total reducing sugar, 57.97%. It was stated that sulfonated carbon acid as a catalyst in hydrolysis is more efficient than sulfuric acid hydrolysis or sulfuric acid-enzyme hydrolysis. It can produce hydrolysate with lower inhibition. Hydrolysis with sulfuric acid could produce water-soluble byproducts, including acetic acid, lignin degradation products, and furfural that inhibit the fermentation process from producing bioethanol [41]. Carbon solid acid catalyst is insoluble and stable in water or most of the solvents. It provides the effective mass transfer between the catalyst and the biomass in the water medium reduces the physical barrier in biomass hydrolysis [42, 43, 44]. According to Hu *et al.* [45], the presence of -SO₃H group as catalyst attacks the glycosidic linkages between the D-glucose units in

cellulose and then hydrolyzed it into β-1,4-glucan or glucose.

3.3. Bioethanol Production

At the beginning of the optimization process of bioethanol production, the change in bioethanol concentration based on the time of fermentation at pH 4.5 was monitored (Figure 5). In the 0 – 72 h range of fermentation time, bioethanol began to accumulate after 4 hours and continued to increase in concentration until 24 h, and then significantly decrease started from 48 h to 72 h. The highest bioethanol concentration, 3.19 g/L, was achieved at 24 h. The reduction of bioethanol production after 24 h might be due to the decrease in activity of *Saccharomyces cerevisiae*. It was shown by the decreased amount of cell mass (Figure 5). A decrease in cell mass amount might be due to the cell is in the death phase and result in cell activity reduction. It was supported by Chang *et al.* [46] whose mentioned that the production of ethanol is cell-dependent.

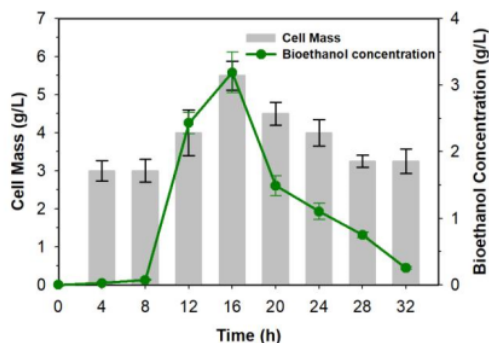


Figure 5. Effect of reaction time on the bioethanol concentration produced from Cassava peel starch at pH 4.5, 100 rpm, and 30°C.

By utilizing optimum data of fermentation time, the evaluation of pH effect on bioethanol production was performed using three different pH values; 4.0, 4.5, and 5.0 at 100 rpm. The fermentation product increased with the increase in pH up to 4.5 and decreased beyond this value. The maximum bioethanol concentration (3.19 g/L) was obtained at pH 4.5 (Figure 6). This result is similar to the study performed by Roukas [47]. He studied the effect of pH on bioethanol production from carob pod by *Saccharomyces cerevisiae* and found the maximum bioethanol concentration, yield, and fermentation efficiency were obtained at pH 4.5. An increase in pH resulted in a substantial decrease in bioethanol production and the growth of microorganisms. The inhibitory effect of high pH on the bioethanol yield could be due to the low ATP production during the metabolic changes in *Saccharomyces cerevisiae*. The enzymes in yeast cells are more active in the mildly acidic medium [48]. According to the result, pH 4.0 had low bioethanol production (2.90 g/L), presumably because the low pH encourages acid production instead of alcohol. This could be due to the formation of acetic acid, which inhibits bioethanol production. Besides, the hydrogen ion concentration in the fermentation broth can change the

plasma membrane's total charge, affecting the permeability of some essential nutrients into the cell [49]. A plasma membrane is a membrane that separates the interior of the cell from outside the environment. The plasma membrane consists of a lipid bilayer which is semipermeable. The permeability of the plasma membrane is selective. It depends on polarity. Polarity influences the ability of any material to cross membranes. The low pH of the environment increases the amount of hydrogen ion and change the polarity [50]. Most studies revealed that the optimum pH range used in fermentation for bioethanol production by *Saccharomyces cerevisiae* is between 4.0–5.5 [48, 51].

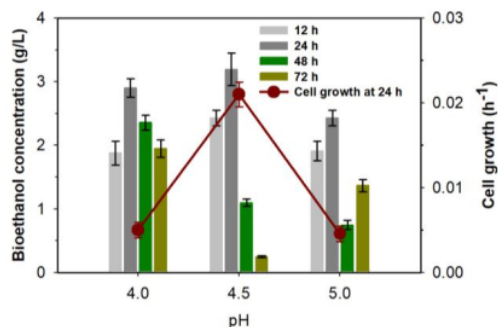


Figure 6. Effect of pH on the bioethanol concentration produced from starch of Cassava peel at reaction time range between 12–72 h, pH 4.5, 100 r.p.m, and 30°C.

To further optimize the process, the effect of the shaking rate on bioethanol production was evaluated. The reaction was conducted at three-different shaking rates, which were 50, 100, and 150 rpm. Concerning the shaking rate, bioethanol concentration slowly decreased (Figure 7) when the shaking rate increased. The maximum bioethanol concentration (3.75 g/L) was obtained at 50 rpm, pH 4.5 for 24 h fermentation time. Mohd Azhar *et al.* [52] mentioned that the shaking rate controls the permeability of nutrients from the fermentation broth to inside the cells and removes ethanol from the cell to the fermentation broth. Excess shaking rate for fermentation is not suitable for bioethanol production due to limitations to cells' metabolic activities.

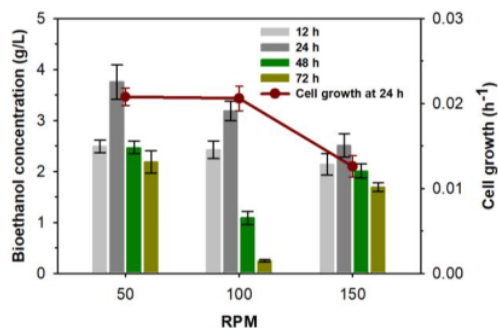


Figure 7. Effect of shaking rate on the bioethanol concentration produced from starch of Cassava peel at reaction time range between 12–72 h, pH 4.5 and 30°C

To justify the result of this study, several bioethanol productions were listed in Table 2. It indicates the waste of biomass can produce bioethanol with appropriate treatment and process. Compared to pineapple and potato peels, the bioethanol production from Cassava peel treated using sulfonated PEFB is less. The 27% yield of bioethanol was achieved in a study that, similar to research was performed by Sirajuddin *et al.* [53], which produced bioethanol from Cassava peel using HCl hydrolysis with the ultrasonic process.

Table 2. Bioethanol production from fruit and vegetable peel wastes.

Raw material	Pretreatment	Microbial Saccharification	Bioethanol (g/L)	Yield* (%)	Ref.
Pineapple peel	Alkali pretreatment and enzyme hydrolysis	<i>Saccharomyces cerevisiae</i>	5.98	-	[54]
Potato peel	SSF process using glucoamylase enzyme	<i>Saccharomyces cerevisiae</i> (SSF process)	22.54	-	[55]
Cassava peel	Ultrasonic assisted using HCl	<i>Saccharomyces cerevisiae</i>	-	20.77	[53]
Cassava peel	Acid hydrolysis using sulfonated palm oil empty fruit bunch	<i>Saccharomyces cerevisiae</i>	3.75	27.72	This work

*Yield of ethanol-based on glucose (13.53 g/L)

4. Conclusion

Bioethanol production from Cassava peel's starch was successfully performed and yielded 3.75 g/L bioethanol as the highest concentration. To generate reduction sugar such as glucose efficiently, the starch was hydrolyzed using synthesized sulfonated carbon acid catalyst, PEFB-SO₃H, which provides many advantages such as easy separation, environmentally friendly operation, reducing the possibility of product degradation, and low energy consumption. The 13.53 g/L of glucose was obtained after hydrolysis reaction for 60 min at 100°C, then continued to produce bioethanol by fermentation using *Saccharomyces cerevisiae* under various optimization parameters. It has been found that the best conditions were obtained pH 4.5 and 50 rpm for 24 h reaction time. As a result, Cassava peel's utilization provides prospective information in transforming waste of biomass into bioethanol.

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