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# Potensi Limbah Kulit Singkong Mentega (Manihot esculenta) Sebagai Bahan Baku Bioetanol dengan Perlakuan Enzimatis

## *The potency of Mentega Cassava (Manihot esculenta) Variety Peels Waste as Raw Material for Bioethanol by Enzymatic Hydrolisis*

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**A B S T R A K**

Limbah kulit singkong mentega dapat dijadikan sebagai sumber energi berupa bioetanol. Etanol ($C_2H_5OH$) merupakan hasil konversi dari fermentasi gula menggunakan bantuan mikroorganisme. Penelitian ini bertujuan untuk mengetahui kadar bioetanol yang dihasilkan dari kulit singkong mentega yang diperoleh dari kecamatan Ibun, Kabupaten Bandung melalui perlakuan enzimatis. Prosedur penelitian dimulai dari pra-perlakuan limbah kulit singkong sebagai substrat, hidrolisis enzimatis dan fermentasi. Hidrolisis enzimatis menggunakan isolat *Trichoderma viride* dengan 3 konsentrasi inokulum 10%, 15%, dan 20%. Sedangkan proses fermentasi menggunakan isolat *Saccharomyces cerevisiae*. Gula pereduksi tertinggi sebesar 21,28 mg/L dihasilkan oleh perlakuan waktu hidrolisis selama 60 jam dan konsentrasi inokulum 10%. Kadar bioetanol tertinggi sebesar 4,9 mg/L dengan waktu fermentasi selama 96 jam.

**Kata Kunci:**
Bioetanol, fermentasi, hidrolisis, kulit singkong mentega, limbah

**A B S T R A C T**

*Butter cassava skin waste can be used as an energy source in the form of bioethanol. Ethanol (C2H5OH) is the result of conversion from sugar fermentation using the help of microorganisms. This study aims to determine the levels of bioethanol produced from butter cassava peels obtained from Ibun district, Bandung regency through enzymatic treatment. The research procedure started from pre-treatment of cassava peel waste as a substrate, enzymatic hydrolysis and fermentation. Enzymatic hydrolysis used Trichoderma viride isolates with 3 inoculum concentrations of 10%, 15% and 20%. Meanwhile, the fermentation process uses Saccharomyces cerevisiae isolates. The highest reducing sugar of 21.28 mg/L was produced by hydrolysis treatment for 60 hours and an inoculum concentration of 10%. The highest bioethanol content was 4.9 mg/L with a fermentation time of 96 hours.*

**A R T I C L E  I N F O**

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## 1. INTRODUCTION

Increasing of the world's population leads to increased energy needs such as electricity and fossil fuel. However, fossil energy is non-renewable, and the stock is limited therefore research on renewable energy has gained interest in the last 20 years (IESR, 2019) Moreover, renewable energy is preferable due to the global warming phenomena since it has a lower carbon print. Although Indonesia is a member of OPEC that exports fossil fuel but Indonesia also imported purified gasoline to fulfill the country's needs (Badaruddin, 2015). Therefore, effort needed to reduce the use of fossil fuel and start to use biofuel.

Renewable energy is an energy source that comes from natural resources and will not run out because it is formed from sustainable natural processes. Bioethanol ($C_2H_5OH$) is one of biofuel produced by starch or lignocellulose hydrolysis to produce glucose and followed by glucose fermentation into EtOH. Further processes namely distillation and purification needed to obtain 99% EtOH called *fuel grade ethanol* (FGE).

Indonesia has abundant renewable energy sources to replace fossil energy. According to the General National Energy Plan (RUEN), the contribution of renewable energy sources in 2022 consists of *Biofuel* of 13.9 million kL, Biomass of 8.4 million tons, and biogas of 498.8 million $m^3$. To date, the contribution of biofuel has been recorded at 8.4 million kL (60.4%), and biogas has only reached 28.07 million $m^3$ (5.6%) (Ermawati, 2015). Agricultural waste has chemical content that has the potential to be used as an alternative raw material for making bioethanol to avoid competition between energy and food utilization.

Lignocellulosic materials come from plants or agricultural waste with the main content of lignin, hemicellulose and cellulose and are the raw materials for second-generation bioethanol (G2) (Syadiah & Syamsu, 2021). production of tapioca produced cassava peel as waste. The cassava peel is the source of lignocellulose that is still underutilized. Producing biofuel from lignocellulose requires three steps namely hydrolysis and fermentation. Enzymatic hydrolysis using raw enzymes from fungi *Aspergillus niger* and *Trichoderma viride* or *Pseudomonas sp*., *Cellulomonas sp*., and *Bacillus sp* are preferable due to the low cost and environmentally friendly.

There was previous research bioethanol production research using cassava peel as raw material. Different microbial inoculants such as Rhizopus nigricans, *Spirogyra africana*, *Saccharomyces cerevisiae* for the simultaneous saccharification and fermentation of cassava peels (Chibuzor *et al.,* 2016). Production of bio-ethanol from cassava peels using *Aspergillus niger* and *Saccharomyces cerevisiae* (Adetunji *et al.,* 2015) followed by research objectives.

## 2. METHODS

### 2.1 Materials

Cassava peel waste obtained from Ibun sub-district, Bandung district.

### 2.2 Microorganisms

Isolates of *Trichoderma viride* BioYTV 127 and *Saccharomyces cerevisiae* BioJSC 31.1 were obtained from the microbiology laboratory of the Universitas Pendidikan Indonesia. The material used for the isolate refresh medium was PDA (Potato *Dextrose Agar). Trichoderma viride* was subcultured to produce cellulase and incubated for 7 days. Then

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*Trichoderma viride* was grown on a liquid nutrient medium to produce cellulase enzymes. *Saccharomyces cerevisiae* isolate was subcultured in PDA medium slant and incubated for 2 days. The isolate was then cultured in 50 ml YMGP (yeast malt peptoneglucose) in 200-ml Erlenmeyer. The culture was incubated in shaking incubator at 125 rpm and 30 °C for 24 h. There were 9 subcultures for each microorganisms

Other ingredients are distilled water, phenol reagents, glucose and a small amount of material for fermentation. The equipment used in this study included Iwaki test tubes, batch bioreactor, Harveur mini milling machine, Duran *baffle flask*, Xuebei brand Erlenmeyer flask, 12 Liter autoclave, laminar air flow, pH-meter, incubator, 20 mesh filter and UV-Vi's spectrophotometer, and Vista 6000 variant gas chromatography.

### 2.3 Research Procedure

The working procedure developed in this study is a modification of the stages of bioethanol production from cassava peel which has been carried out by Syadiah & Syamsu (2021). Preparation of Mentega Cassava Peel Waste as Media Soaking fresh mentega cassava skin as much as 4 kg is done for 65-72 hours, then cut into smaller parts. The mentega cassava peel was then dried for 120 hours and 2.1 kg of dry cassava peel was obtained. The cassava skin was mashed and then sieved using a 20-mesh sieve. After that, the milled cassava peels were baked in an oven at a temperature of ± 105$^{o}$C for 100 minutes.

Delignification or *pretreatment process* is carried out by taking 250 grams of cassava peel powder resulting from sieving and oven drying, put into an Erlenmeyer, then add 750 mL of distilled water and 250 mL of 8% NaOH, heated and stirred using a stirrer for 30 minutes at 160$^{o}$C. Next, do the filtering using a filter cloth. The filtered residue was washed with distilled water until a neutral pH was obtained and then baked at a temperature of 105$^{o}$C for 120 minutes. Do size reduction with a mini Harveur milling machine, then sieved using a 20-mesh sieve.

Culture

The rejuvenation of *Trichoderma viride* was carried out by inoculating stock cultures on Potato Dextrose Agar (PDA) inclined zigzag with the help of Ose wire and Bunsen fire (aseptically) in laminar air flow and then incubated for 7 days. Then *Trichoderma viride* was grown on a liquid nutrient medium to produce cellulase enzymes. Liquid media was prepared by mixing 1 L of citrate buffer solution, grams of trace elements and 1% cassava peel mentega as an inducer. The nutrient solution was then stirred using a magnetic stirrer until homogeneous. One loop of *Trichoderma viride* was transferred to liquid medium and incubated on a rotary shaker at 120 rpm at 30$^{o}$C for 7 days.

Enzymatic Hydrolysis

The sample powder from the delignification results was then hydrolyzed by weighing 20 grams of the sieve results in the delignification stage for 4 treatments. Each sample was put into an Erlenmeyer glass and added with various concentrations of *Trichoderma viride inoculum* as much as 10, 15 and 20% at room temperature for 60 hours. The solution was filtered using filter paper. The obtained filtrate was measured for glucose levels using a UVVis spectrometer.

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### 2.4 Fermentation

The fermentation process is carried out by taking as much as 150 mL of the filtrate from hydrolysis added with 5.5 M NaOH solution until the pH becomes 5. Then 15 grams of ammonium sulfate and 15 grams of $NH_3$ $SO_4$ are added and pasteurized at 85$^oC$ for 15 minutes then cooled in a sterile place. After that, 10%, 15% and 20% yeast (*Sacharomyces cerevisiae)* were added for 96 hours a day at a temperature of 27-30$^oC$. Determination of ethanol content using an alcohol meter. Analysis and Measurement Parameters
Calculation of sugar content using UV-Vi's spectrometer and ethanol content using alcoholmeter.

## 3. RESULTS AND DISCUSSION

Delignification of mentega cassava peel needs to be carried out, which aims to convert the characteristics of the raw material specifically degrading lignin and hemicellulose and alleviating the cellulosic crystallinity. The increased cellulose concentration and decreased lignin after delignification could indicate optimization of hydrolysis process (**Table 1**).

**Table 1.** Composition of Mentega Cassava Peel Waste

| Component | Composition (%) | |
|---|---|---|
| | Before | After |
| Lignin | 22.31±0.025 | 13.83±0.28 |
| Hemicellulose | 47.18±0.24 | 28.52±0.19 |
| Cellulose | 23.29±0.04 | 48.93±0.42 |
| Ash Level | 8.7±0.15 | 11±0.08 |

The high content of cellulose as much as 48.93±0.42% in mentega cassava peel can be used as a potential raw material for the manufacture of bioethanol but at the same time it is also a challenge to the efficiency of biomass conversion of mentega cassava peel. Kurniaty *et al.,* (2017) stated that the effectiveness and efficiency of biomass conversion can be increased, by: (1) selection of suitable biomass, (2) effective pretreatment, (3) production of enzymes for saccharification process, (4) fermentation of hexose and pentose and (5) downstream process. Therefore, the components of the selected biomass fiber need to be known in advance to determine their potential, including the appropriate process steps and the yield to be obtained.

The delignification process needs to be carried out on lignocellulosic materials such as mentega cassava peel. The lignin content decreased from 22.31±0.025% to 13.83±0.28%. It aims to change the properties and components of raw materials, especially to degrade lignin and hemicellulose and reduce the crystallinity of cellulose, as well as facilitate cellulase enzymes to hydrolyze cellulose to produce monomeric sugars. The mechanism of breaking the bond between lignin and cellulose by NaOH, namely $OH^-$ ions from NaOH will break bonds from the basic structure of lignin, while $Na^{+\ ions}$ will bind to lignin to form sodium phenolate (Larasati *et al.,* , 2019).

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In this study the level of delignification decreased the efficiency of lignin concentration by almost 70%, as reported by Cao et al. (2012). A lignin loss of 60% by hydrothermal alkaline pretreatment using wheat straw was obtained by Merali *et al.,* (2015). This indicates that the pretreatment ability depends on the type of lignocellulosic material, especially its chemical composition. In addition, the use of the same chemical does not guarantee the same results if there is a diversity factor from the pre-treatment conditions used (Umagiliyage et al. 2015).

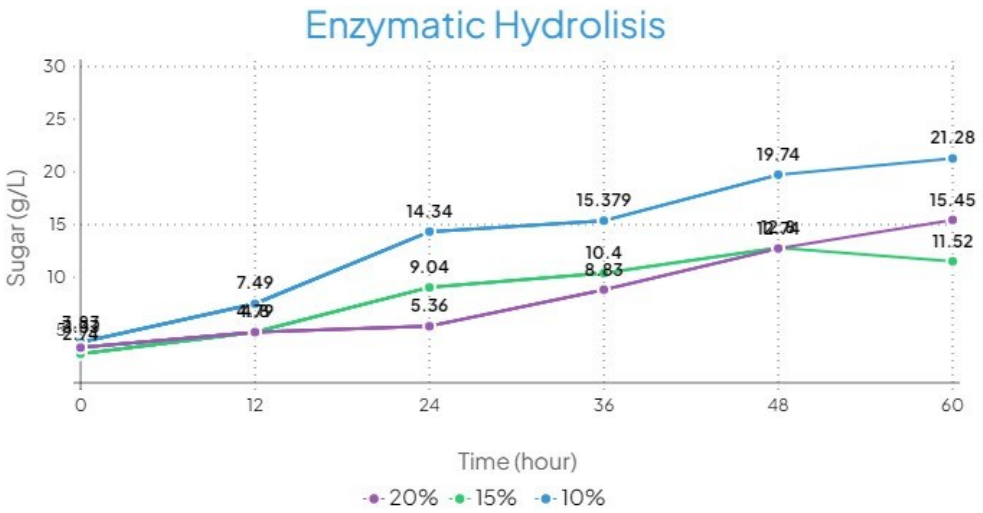


**Figure 1.** Enzymatic Hydrolysis of mentega casava peel using *Trichoderma viride*

**Figure 1,** showed the process of hydrolysis of cellulose into glucose can use commercial cellulase enzymes or microbes that produce cellulase enzymes such as *Trichoderma viride* . The presence of cellulose in mentega cassava peel can be used as a substrate or source of cellulose. *Trichoderma viride* is able to produce cellulase enzymes that play a role in the hydrolysis of cellulose into simple sugars. Sugar production from mentega cassava peels is carried out in a batch system (bulk) using pure *Trichoderma viride culture* . According to Nathan *et al.,* (2014) cellulase enzymes that play a role in the hydrolysis of cellulose into glucose units can be produced by the type of fungus *Trichoderma viride* .

The whole cultivation process is carried out by aeration and agitation. Syadiah *et al.,* (2018) used agitator with a speed of 150 rpm to produce bioethanol with sweet sorghum bagasse media. The agitation caused the microbial cell suspension to remain uniform and in a homogeneous growth medium from the beginning to the end of the cultivation process. Agitation aims to facilitate the diffusion of oxygen into the medium so that there is more contact between the substrate and the inoculum. The cellulase enzyme that breaks down cellulose into sugar is produced by *Trichoderma viride*, namely (a) Endoglucanase enzyme, which functions to cut long glucose chains into shorter chains randomly, (b) Cellobiohydrolase enzyme, functions to cut every two glucose chains (cellobiose), starting from the chain. number one (last chain) glucose, and (c) -glucosidase enzyme, functions to cut cellobiose into glucose molecules.

In this study, the concentration of the cellulose peel of cassava mentega substrate decreased along with the growth of *Trichoderma viride* and the increase in sugar production. *Trichoderma viride* is able to hydrolyze the cellulose skin of cassava mentega. This is

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evidenced by the production of monomer sugar as the resulting product. In the picture, the highest sugar value of *Trichoderma viride cultivation* was at the 60th hour with a concentration of 10% at 21.28 mg L-1. The advantages of using microbes as enzyme producers include microbes that are easy to breed, have high growth rates, are easy to control their growth, production costs are quite cheap, and can be used in a short time as expected. In this study, two different cultures were used, namely *Trichoderma viride* and *Saccharomyces cerevisiae* (**Figure 2**).

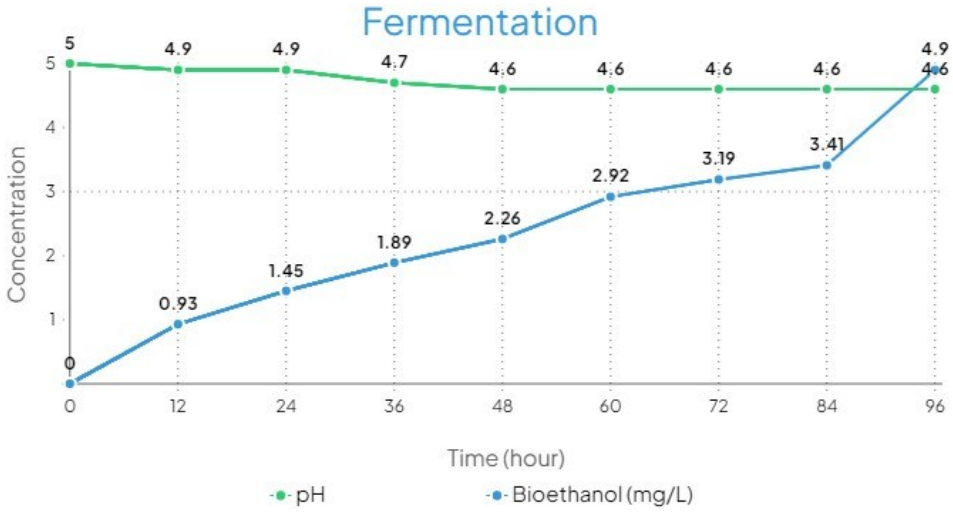


**Figure 2.** Fermentation of hydrolysate using Saccharomyces cerevisiae

pH analysis was carried out every 12 hours using a pH-meter. The value of the degree of acidity is very influential on microbial activity. Microbes have an optimum pH, namely the pH that can produce the highest activity in catalyzing a reaction. The pH value will affect the active site of the enzyme in forming the enzyme-substrate complex. The decrease in pH until the end of *Saccharomyces cerevisiae cultivation* indicated the formation of acid compounds and CO2 as a result of the respiration process. The resulting sugar decreases as the pH value. The decrease in the acidity value at the end of cultivation caused the number of microbes to decrease drastically but the accumulation of cellulase enzymes was still able to hydrolyze cellulose into sugar.

The sample with the highest glucose content from the hydrolysis results, namely 21.28 mg/L was fermented to obtain bioethanol. Prior to fermentation, the filtrate resulting from hydrolysis was increased in pH until the pH reached 5 in accordance with the opinion (Syamsu *et al.,* 2020) that the microbial growth range of *Saccharomyces cerevisiae* was pH 3.5-6.5 and at pH 5 was the optimal pH condition. Saccharomyces *cerevisiae* will grow optimally in a temperature range of 30-35$^{o}$C and peak alcohol production is achieved at a temperature of 33$^{o}$C. Temperatures that are too low will cause fermentation to take place slowly, and at temperatures that are too high cause the *Saccharomyces cerevisiae microbes* to die so that the fermentation process cannot take place (Syadiah *et al.,* 2018).

The duration of fermentation is influenced by several factors, both directly and indirectly affecting the fermentation process, including the substrate, temperature, pH, oxygen, and microbes used in the fermentation process (Monir, 2020). This study used the yeast *Saccharomyces cerevisiae* because the microbe *Saccharomyces cerevisiae* has several advantages over other microbes, which can produce up to 2% alcohol in 96 hours (Perrone

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*et al.,* 2013). *Saccharomyces cerevisiae* microbes produce invertase enzymes and zimase enzymes in the presence of these two enzymes, *Saccharomyces cerevisiae microbes* can convert sugar into ethanol. Sugars from the disaccharide group will be hydrolyzed by the invertase enzyme into monosaccharides, then the zimase enzyme will convert the monosaccharides into alcohol and carbon dioxide (Erna *et al.,* 2016).

There was a significant increase in ethanol content from the 36$^{th}$ hour to the 96$^{th}$ hour, which was 4.9 mg/L. This shows that the longer the fermentation, the higher the ethanol content produced due to the faster microbial growth. At the 108$^{th}$ hour there was a decrease in the ethanol content because in further fermentation the ethanol was converted to other compounds such as carboxylic acids and further converted to esters

## 4. CONCLUSIONS

Based on the results of the study, it can be concluded that the production of bioethanol from cassava peel by enzymatic hydrolysis with the highest yield at a hydrolysis time of 45 minutes with a reducing sugar content of 21.28 mg/L and an ethanol content of 4.9 mg/L.

## 5. AUTHOR'S NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The author confirms that this article is free from plagiarism.

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