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Research Article

# Bioconversion of Industrial Cassava Solid Waste (*Onggok*) to Bioethanol Using a Saccharification and Fermentation process

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**Abstract.** Cassava solid waste (*Onggok*) is a by-product of the starch industry containing a lot of fiber, especially cellulose and hemicellulose. It has the potential to be converted to bioethanol. This work aimed to evaluate the effect of feedstocks ratio for the optimal bioethanol production via enzymatic and acidic hydrolysis process in a batch fermentation process. The effect of alpha-amylase and glucoamylase activities was studied. The sulfuric acid concentrations in the hydrolysis process in converting cassava into reducing sugar were also investigated. The reducing sugar was then fermented to produce ethanol. Enzymatic and chemical hydrolysis was carried out with the ratio of *onggok*(g)/water(L), 50/1, 75/1, and 100/1 (w/v). In the enzymatic hydrolysis, 22.5, 45, and 67.5 KNU (Kilo Novo alpha-amylase Unit) for liquefaction; and 65, 130, and 195 GAU (Glucoamylase Unit) for saccharification, respectively of enzymes were applied. The liquefaction was carried out at 90-100°C for 2 hours. The saccharification was executed at 65 °C for 4 hours. Meanwhile, the acidic hydrolysis operating condition was at 90-100 °C for 3 hours. The fermentation was performed at pH 4.5 for 3 days. Fourier Transform Infra-Red (FTIR) analysis was conducted to evaluate the hydrolysis process. The highest ethanol was yielded in the fermentation at 8.89% with the ratio of *onggok* to water 100:1, 67.5 KNU of alpha-amylase, and 195 GAU of glucoamylase. Ethanol was further purified utilizing fractional distillation. The final ethanol concentration was at 93-94%.

**Keywords:** α-amylase; glucoamylase; liquefaction; saccharification; reducing sugar; starch.

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## 1. Introduction

The availability of fossil fuels in nature is continuing to decline due to high exploitation. The production of alternative energy from biological sources was increasingly developed to overcome the problems (Altway *et al.*, 2018). Biomass can be alternative energy to replace the fossil energy source (Altway *et al.*, 2019) (Soeprijanto *et al.*, 2020). Later, the bioethanol production from lignocellulosic biomass have been developed by several authors (Karagöz & Özkan, 2014; Kumar *et al.*, 2019; Laopaiboon & Laopaiboon, 2012). The lignocellulosic biomass such as cassava stem, corn, agricultural product, and cassava peels can be used as feedstocks for bioethanol production. However, these kinds of biomass have a conflict with the food production issue. Therefore, biomass waste is preferable used as feedstock for bioethanol production in the recent decade (Noel *et al.*, 2016).

Lignocellulosic biomass residues are one of the most abundant natural resources that can be converted to biofuels and bioproducts. (Adekunle *et al.*, 2016; Hamzah *et al.*, 2019). Recently, the utilization of the cassava solid waste (in Indonesia mostly known as *Onggok*) as bioethanol had been developed (Amalia *et al.*, 2021). *Onggok* is a residue in starch manufacturing that still has a high starch and cellulose content which can be converted

into sugar for bioethanol (Kristensen *et al.*, 2007; Obianwa *et al.*, 2016; Olanbiwoninu & Odunfa, 2012). *Onggok* is an abundant resource, conflict-free with food production, and easy to process. Some of the advantages indicate that it may be considered as the most viable option for producing bioethanol while providing environmental protection by recycling the waste into energy (Soeprijanto *et al.*, 2019).

The previous studies had reported the production of bioethanol from tapioca flour waste through the hydrolysis and fermentation process. Some parameters had been investigated regarded to yield of sugar and bioethanol such as fermentation time, amount of yeast, yeast activity, pH, and temperature. The effect of the feedstocks (cassava to water) ratio was not considered in those investigations. However, this parameter significantly influenced the yield of the final bioethanol produced. Later, alpha-amylase and glucoamylase compositions were not clearly described in those studies. These two parameters will affect the final yield owing to providing the best condition in the fermentation process. Therefore, the feedstocks ratio and the composition of the enzymes were investigated in this study (Abu Tayeh *et al.*, 2014; Amalia *et al.*, 2021; Wahyuono *et al.*, 2015)

In this study, the raw material used was *onggok* (cassava waste) obtained from home industries in Kediri,

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East Java, Indonesia. This work aimed to evaluate the effect of feedstocks ratio which is onggok to water ratio (50:1, 75:1, and 100:1 w/v) for the optimal bioethanol production via enzymatic and acidic hydrolysis process in a batch fermentation process. The effect of alpha-amylase and glucoamylase activities was studied. Besides, the sulfuric acid concentrations in the hydrolysis process in converting cassava into reducing sugar were also investigated. The enzymatic and acidic hydrolysis were employed since both produced a high yield of reducing sugar. However, the enzymatic tend to have a slower hydrolysis process than the acidic, while acidic hydrolysis is not environmentally friendly. Therefore, for clear comparison, onggok was converted to reducing sugar using both methods.

## 2. Material and Method

### 2.1. Materials

Onggok as a biomass resource was obtained from home industries in Kediri. Alpha-amylase, amylo-alpha1,6-glucosidase was supported by PT Sorini Corporation in Pasuruan. *Saccharomyces cerevisiae* used in the fermentation process was obtained from PT. Sangra Ratu Boga. Urea and  $(\text{NH}_4)_2\text{HPO}_4$  as a nutrient in the fermentation process and Sulfuric acid was used to adjust

the pH in the fermentation process were obtained from Sigma-Aldrich. While Calcium chloride ( $\text{CaCl}_2$ ) was used for stabilizing the alpha-amylase enzyme in the liquefaction process obtained from Merck.

### 2.2. Pre-treatment

In the pre-treatment process, onggok was dried at  $105^\circ\text{C}$  in the oven. Then, it was mechanically pre-treated by reducing the size to 3 mm in diameter, Onggok was stored at room temperature before being used. Fig 1 shows onggok before and after the pre-treatment.

### 2.3. Enzymatic Hydrolysis

Enzymatic hydrolysis consists of two steps. They were liquefaction and saccharification process. After pre-treatment, In liquefaction, *onggok*, and water in the ratio of 50:1, 75:1, and 100:1 (w/v, g/L) were mixed with 22.5, 45, and 67.5 KNU of alpha-amylase, respectively. the mixture was then mixed with  $\text{CaCl}_2$  solution 2% wt at  $100^\circ\text{C}$  for 2 hours. In the saccharification process, the mixture was added with 1, 2, and 3 GAU glucoamylase at  $60\text{-}65^\circ\text{C}$  for 4 hours. The schematic apparatus of the enzymatic hydrolysis experiment can be seen in Fig. 2.



Fig 1. (a) *Onggok* before the pre-treatment process and (b) *Onggok* after the pre-treatment process.

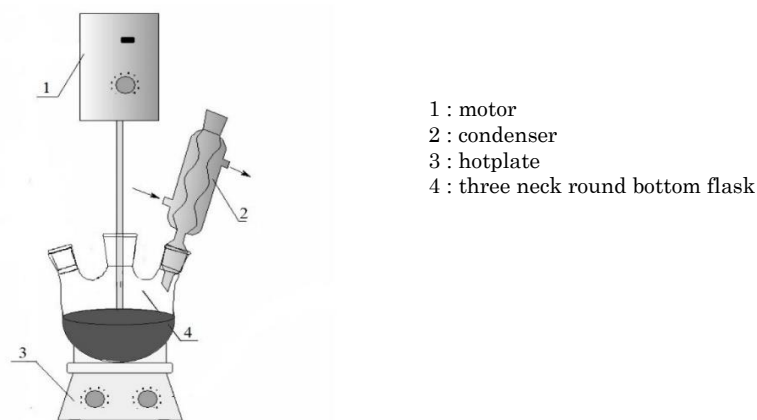
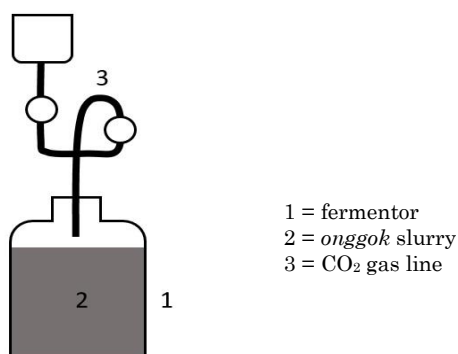


Fig 2. Schematic apparatus of enzymatic hydrolysis



**Fig 3.** The schematic apparatus of batch fermenter using baker's yeast *S. cerevisiae*

#### 2.4. Acidic Hydrolysis

The production of reducing sugars can be obtained through acid hydrolysis. The sulfuric acid was used as the acid solution with a concentration of 0.5, 1, 1.5 N. *Ongkok* was mixed with water in a ratio of 100:1 (w/v). Hydrolysis using sulfuric acid was carried out at 100 °C with various concentrations (0.5, 1, and 1.5 N).

#### 2.5. Fermentation

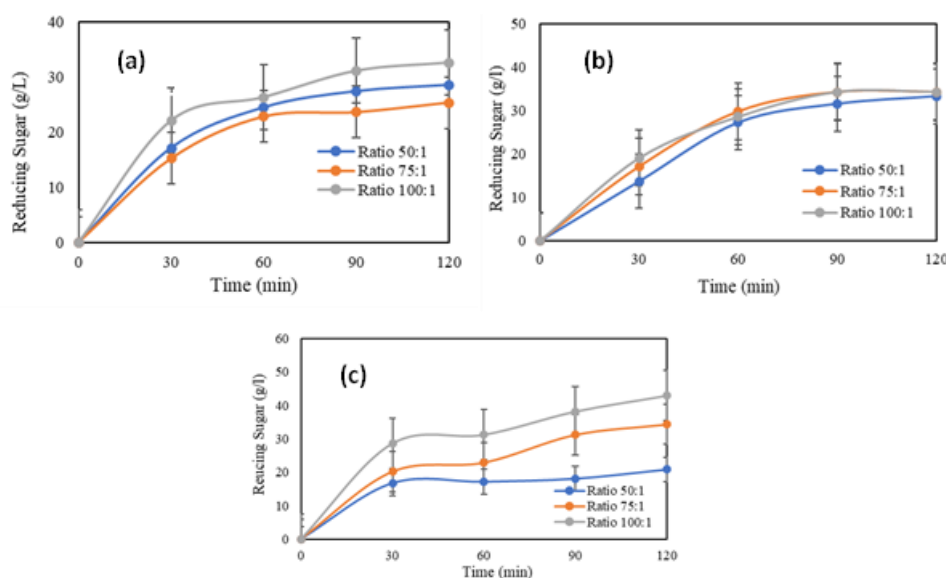
The *ongkok* slurry resulted from the enzymatic hydrolysis was introduced into the fermentor. It was mixed with baker's yeast and nutrients containing KH<sub>2</sub>PO<sub>4</sub> 0.5% wt, Urea 0.5% wt, Baker's yeast 0.2% wt. The fermentation was conducted at room temperature for 72 hours. Then,

bioethanol was produced in the fermentation process. The gas chromatograph (Shimadzu GC 2010) equipped with a thermal conductivity detector was applied to determine bioethanol content. The schematic apparatus of the fermentor can be seen in Fig. 3.

### 3. Result and Discussion

#### 3.1. Enzymatic Hydrolysis

Reducing sugar from *ongkok* starch was produced by two steps sequential enzymatic hydrolysis process, namely, liquefaction and saccharification. Figure 4 shows reducing sugar production by the liquefaction process with the *ongkok* to water ratio of 50:1, 75:1, and 100:1 (g/L and with the enzymatic activity of 22.5, 45, and 67.5 KNU, respectively. In the liquefaction process, alpha-amylase had been used. The production of reducing sugar was highly influenced by substrate concentration and enzyme activity. The highest reducing sugar of 32.7 g/L was obtained at the ratio of 100:1. Based on the results in Fig. 4, the three figures had the same patterns. The reducing sugar concentration increased along with time and substrate concentration. It indicated that Alpha-amylase consistently worked at high temperatures and the mechanical pretreatment make the liquefaction process became easier since the surface contact between substrate and enzymes was larger (Kaparaju *et al.*, 2009). The influence of alpha-amylase activity was also critical. It depicts in Fig. 4 that the amount of sugar produced was enlarged along with the activity of the enzymes. After the liquefaction process, hydrolysis in saccharification was carried out by supplementing glucoamylase into the mixture at 60-65 °C for 4 hours.



**Fig 4.** Time course of reducing sugar produced at (a) 22.5 KNU; (b) 45 KNU; and (c) 67.5 KNU in liquefaction.

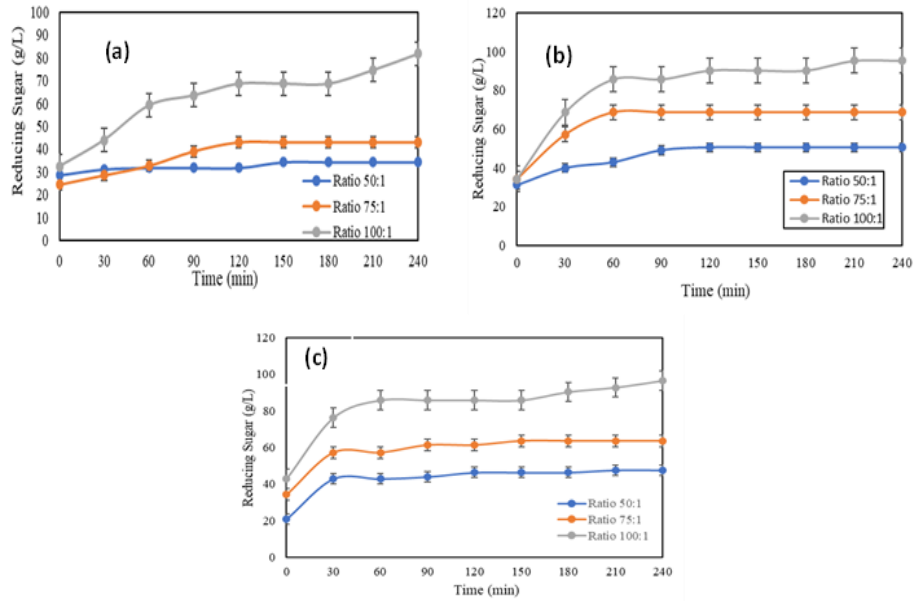


Fig 5. Time course of reducing sugar enhancement in saccharification by (a) 65 GAU; (b) 130 GAU; and (c) 195 GAU enzymes.

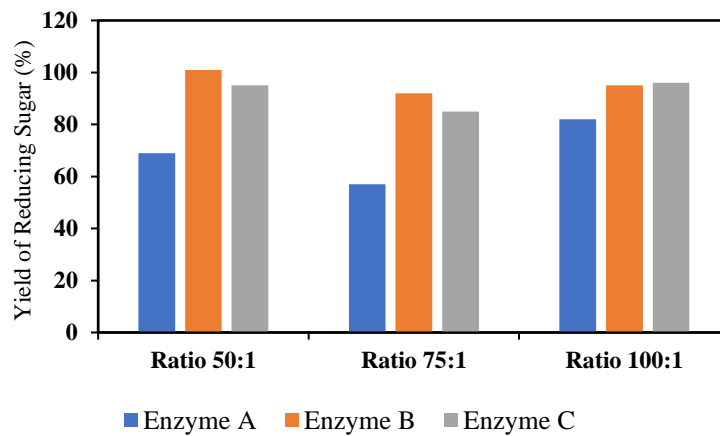


Fig 6. The effect of enzymatic activity and ratio of feedstock on the yield of reducing sugars in the enzymatic hydrolysis

Figure 5 shows that the reducing sugar enhancement in the saccharification process with the onggok to water ratio of 50:1, 75:1, and 100:1 and the enzymatic activity of 65, 130, and 195 GAU. These result of saccharification has an identical pattern with the liquefaction process. The higher amount of the enzyme activity will produce more reducing sugar. The significant difference was the increasing tendency of sugar between liquefaction and saccharification. In the 60 minutes of the liquefaction process, the amount of reducing sugar still significantly increased. While in the saccharification process, the amount of the reducing sugar is considered at a constant value. It indicated that in the liquefaction process, there was still a significant number of substrates that had not been converted to reducing sugar. While in the saccharification process, the substrates were almost completely converted (Lyu *et al.*, 2021). The enzymatic hydrolysis was selected in this study due to its high selectivity, produce higher yield, and able to operate in various conditions (Hamzah *et al.*, 2017, 2019)

### 3.2. The yield of Reducing Sugar from Enzymatic Hydrolysis

The enzymatic hydrolysis was used in this study due to its high selectivity, produce higher yield, and able to operate under various conditions (Hamzah *et al.*, 2017, 2019). According to the results obtained in Figure 5, high selectivity was described by the amount of sugar that almost completely yielded by showing constant value. Furthermore, the yield of reducing sugar from enzymatic hydrolysis was also influenced by the ratio of feedstock (50:1; 75:1, and 100:1 w/v) and activity of an enzyme (Enzyme A= 22.5 KNU + 65 GAU; enzyme B= 45 KNU + 130 GAU and enzyme C= 67.5 KNU + 195 GAU), as seen in Figure 6. The results depicted that the use of feedstock ratio of 50:1 produced reducing sugar yield of 69, 101, and 95% with the use of enzyme A, B, and C, respectively; the use of feedstock ratio of 75:1 produced a reducing sugar yield of 57, 92, and 85%; and the use of feedstock ratio of 100:1 produced reducing sugar yield of 81, 95, and 96%, respectively. These results indicated that the highest yield of reducing sugar was able to be obtained using enzyme B;

this was 101, 92, and 95% correlated to reducing sugar obtained (47.66, 63.54, and 96.38 g/L). The high yield by enzyme B ratio might be due to the maximum enzyme's adsorption towards the substrates. The adsorption of enzymes to the *onggok* is a key condition in enzymatic hydrolysis. In this study, the utilization of alpha-amylase and glucoamylase was carried out sequentially. enzyme A yields less sugar because of the lack of enzyme activity, while enzyme C might be caused by the low adsorption rate of glucoamylase to the substrate. This low adsorption rate might be due to the existence of alpha-amylase that is still attached to the substance (Tomasik & Horton, 2012).

### 3.3 Acidic Hydrolysis

In addition, reducing sugar was also produced via the acidic hydrolysis process in this study. The sulfuric acid concentrations in converting cassava into reducing sugar were investigated. *Onggok* was mixed with water in a ratio of 100 :1 (w/v). Hydrolysis using sulfuric acid was carried out at 100 °C with various concentrations (0.5, 1, and 1.5 N). Higher temperature was preferable for the breakdown of the cellulose chain to produce reducing sugar. Figure 7 shows that the reducing sugar increased from 68.63 to 91.91 g/L, along with the increase of sulfuric acid concentrations. Higher sulfuric concentration will provide more acid to completely break down the cellulose into the reducing sugar (Li *et al.*, 2008)(Jia *et al.*, 2015).

### 3.4 Fermentation

According to the reducing sugar yield in Figures 4, 5, and 7, it indicated that the enzymatic process was preferable to be applied, as the conversion of the substrate (cellulose) into the reducing sugar was higher in the enzymatic process compared to the acidic process.

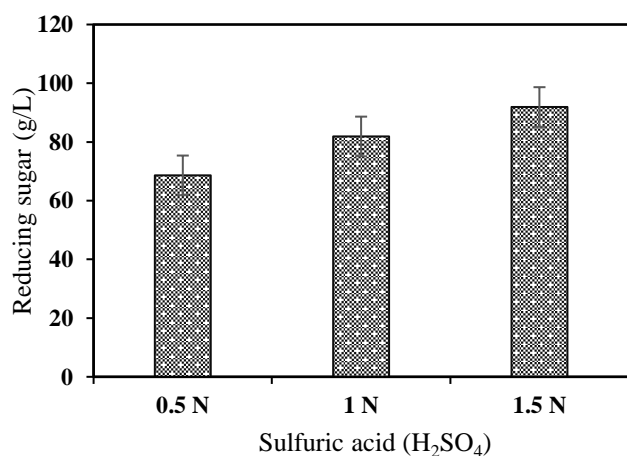


Fig 7. Effect of sulfuric acid concentration on reducing sugar production.

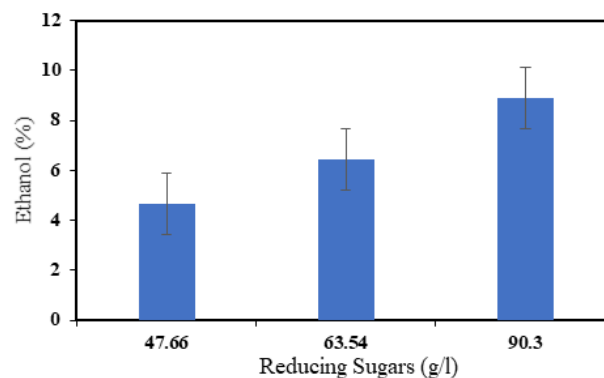
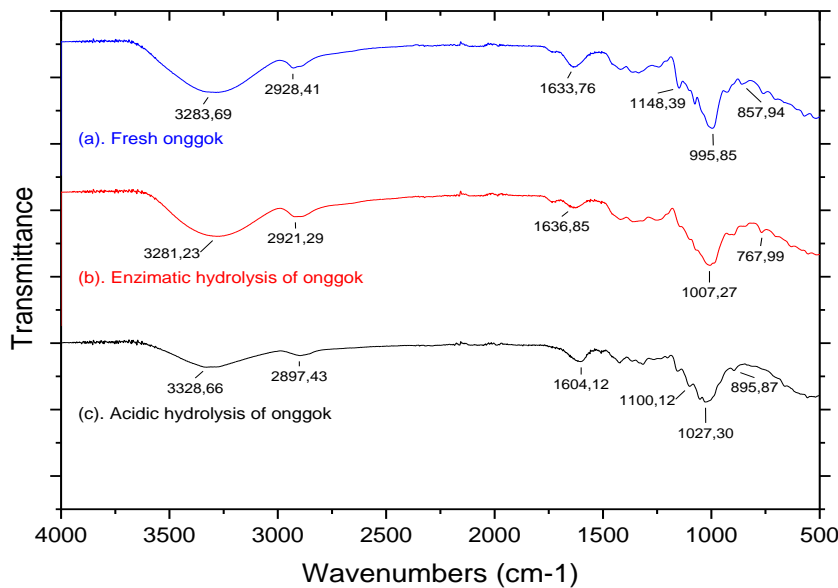


Fig 8. Bioethanol yielded from *onggok* with various amounts of reducing sugars

Besides, in the acidic process, there was still acid in the product, and it was not selective for converting cellulose into reducing sugar, therefore the amount of reducing sugar produced was lower than that of in the enzymatic process. Thus, the enzymatic hydrolysis method was selected for further process in the production of bioethanol using batch fermentation. After *onggok* was converted into reducing sugar in the enzymatic hydrolysis process, the step was continued to the fermentation process. Bioethanol was produced through a fermentation process using baker's yeast *S cerevisiae*. The production of bioethanol using the fermentation process was strongly influenced by the raw material of reducing sugar yielded by enzymatic hydrolysis. *Onggok* had been successfully converted into a decent amount of sugar by enzymatic hydrolysis. The reducing sugar then was fermented in the fermenter reactor for 72 hours (Madadi *et al.*, 2021). Fig 8 shows the yield of bioethanol generated from reducing sugar. It was revealed that 47.66, 63.54, and 90.3 g/L of reducing sugar effectively yielded 4.65, 6.44, and 8.89% of bioethanol, respectively. The low ethanol concentration is mainly due to the *S cerevisiae* having low tolerance against ethanol (Amalia *et al.*, 2021). The ethanol yielded from the fermentation process was then purified by fractional distillation to obtain a higher ethanol concentration. It attained 93-94% of ethanol concentration.

### 3.5. Fourier transform infrared spectroscopy (FTIR)

The characterization of the substrate (*onggok*) used in this study was analyzed using FTIR. The chemical composition of *onggok* was analyzed before and after hydrolysis. The samples were made by blending and drying in an oven at 105 °C, then loaded and scanned by a Nicolet iS10 FTIR Spectrometer (Thermo Fisher Scientific, USA).



**Fig 9** Fourier-transform infrared spectra of *onggok* (a) Fresh *onggok*; (b) Enzymatic hydrolysis of *onggok*; and (c) Acidic hydrolysis of *onggok*.

The spectra were measured in the wavenumber range of 400–4000  $\text{cm}^{-1}$  at the resolution of 4  $\text{cm}^{-1}$ . The FTIR characterization was also conducted to understand the success of hydrolysis and fermentation. (Kusdianto et al., 2020).

Figure 9(a) shows the FTIR spectra of fresh *onggok*. The spectra at 3283.69  $\text{cm}^{-1}$  show the O-H stretching and 2928.41  $\text{cm}^{-1}$  for the C-H stretching. Other spectra at 1633.76  $\text{cm}^{-1}$  the carbonyl aldehyde in lignin, 1148.39  $\text{cm}^{-1}$  for C-O-C stretching, and at 995.85  $\text{cm}^{-1}$  the C-O, C-C, and C-OH stretching vibrations in the hemicelluloses, cellulose, and lignin, 875.94  $\text{cm}^{-1}$  the alkane C-H bonds (Qomariyah et al., 2020). Figure 9(b) is the FTIR spectra of the *onggok* after enzymatic hydrolysis. Spectra at 3281.23  $\text{cm}^{-1}$  show the O-H stretching, 2921.29  $\text{cm}^{-1}$  shows the C-H stretching, 1636.83  $\text{cm}^{-1}$  shows the carbonyl aldehyde in lignin. Additional spectra at 1007.27  $\text{cm}^{-1}$  show the C-O, C-C, and C-OH stretching vibrations in the hemicelluloses, cellulose, and lignin, 767.99  $\text{cm}^{-1}$  for the alkane C-H bonds. After enzymatic hydrolysis, the C-O-C stretching disappears. It indicated that the carbohydrate successfully converted into reducing sugar. Figure 9(c) shows the FTIR spectra of the *onggok* after Acidic hydrolysis. The spectra at 33128.66  $\text{cm}^{-1}$  show the O-H stretching, 2897.43  $\text{cm}^{-1}$  shows the C-H stretching, 1604.12  $\text{cm}^{-1}$  shows the carbonyl aldehyde in lignin. Additional spectra at 1100.12  $\text{cm}^{-1}$  show C-O-C stretching, 1027.30  $\text{cm}^{-1}$  shows the C-O, C-C, and C-OH stretching vibrations in the hemicelluloses, cellulose, and lignin, while spectra at 895.87  $\text{cm}^{-1}$  show the alkane C-H bonds.

### 3.6. Opportunities and Challenges

All the experimental results show that the highest yield of bioethanol at 8.89% could be obtained at the *onggok* to water ratio 100:1 with 67.5 KNU of  $\alpha$ -amylase, and 195 GAU in enzymatic hydrolysis. Besides the *onggok* ratio and activity of the enzyme, the other parameters affecting the yield of ethanol such as fermentation time

course, microorganism activity, operating condition, etc need to be further studied. However, the present study had already successfully converted the *onggok* waste to bioethanol. Therefore, the *onggok* can be a good candidate as a feedstock in bioethanol production.

## 4. Conclusion

*Onggok* (cassava waste) was successfully converted into bioethanol through consecutive enzymatic and acidic hydrolysis followed by fermentation. The highest reducing sugar around 93.31 g/L was obtained at the ratio of 100:1 with 130 GAU in the enzymatic process. At the acidic process, the highest reducing sugar around 91.1 g/L was yielded using an  $\text{H}_2\text{SO}_4$  concentration of 1.5 N. The highest ethanol concentration yielded was 8.89%. It was achieved by a 100:1 (w/v) substrate ratio, 67.5 KNU of  $\alpha$ -amylase, and 195 GAU of glucoamylase. The bioethanol yielded was then further purified using fractional distillation to produce 93-94% of ethanol concentration.

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