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Hydrolysis of Mixed Sugarcane Bagasse and Rice Husk Using Cellulase Enzyme for Reducing Sugar

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Abstract. Reducing sugar can be produced from lignocellulosic raw materials. The content of polysaccharides such as cellulose, hemicellulose, and starch will be broken down into simpler carbohydrates. This study used a mixture of sugarcane bagasse and rice husks as lignocellulosic raw materials. The lignin content in the raw material must be removed through delignification or pretreatment so that enzymes can access cellulose and hemicellulose. This study used a physics-chemical pretreatment method, in which lignocellulosic material soak in 3% NaOH then heated with microwave and boiling water. The next process is enzymatic hydrolysis with variations of cellulase enzymes activity 0.434, 0.871, 2.61, and 3.49 FPU/g mixture of bagasse and rice husks. The cellulase enzyme used in this study was also derived from the fungus *Trichoderma viride*. Analysis of the sugar concentration resulting from hydrolysis used the DNS method with the 3.5-dinitrosalicylic acid reagent. The concentration of sugar from hydrolysis using a variety of enzymes with microwave heating pretreatment and boiling water pretreatment obtained the highest results which were the same at the addition of enzyme activity 3.49 FPU/g substrate at 24 hours, namely 4.077 g/L and 15.18 g/L. The optimum time for enzymatic hydrolysis is 12 hours and optimum enzyme activity is the addition of enzyme activity 2.61 FPU/g. The average concentration of sugar hydrolyzed by the addition of *Trichoderma viride* solution in pretreatment using microwave heating was 0.7611 g/L with a yield of 21.01 mg sugar/g substrate and with pretreatment in boiling water obtained 0.8679 g/L with a yield of 23.95 mg sugar/g substrate.

Keywords: *sugarcane bagasse, rice husk, enzymatic hydrolysis, lignocellulose, reducing sugar, and trichoderma viride*

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1. Introduction

Until now, many studies have used biomass waste as raw material to produce reduced sugar. Reducing sugar can be produced from the hydrolysis of lignocellulosic materials because the lignocellulosic structure can be converted into reducing sugars and has the potential to be further processed for the manufacture of butanol, acetone, ethanol, and other products with higher economic value [1]. Examples of reducing sugars are all monosaccharides (*glucose*, *fructose*, *galactose*), disaccharides (*lactose*, *maltose*) except sucrose and starch (*polysaccharides*) [2]. The content of cellulose and hemicellulose in lignocellulosic materials has the potential as a source of reducing sugar production. Polysaccharides will be crashed into simple sugar monomers such as reducing sugars [1]. Enzymatic hydrolysis can be chosen as a more environmentally friendly method than hydrolysis using acid to produce high concentrations of reducing sugars [1].

Biomass raw materials are available in abundance and are not used as food so that their use as alternative fuels or other economically valuable materials does not interfere with the availability of food. Biomass can be produced from plants, agricultural waste, and industrial waste [3]. Agricultural waste in Indonesia reaches 19.5 megatons per year for the main commodities, namely rice husks, cassava peels, sugar cane bagasse, coffee grounds, and cocoa husks (BPS Indonesia, 2018). In this study, mixed biomass from rice husks and bagasse agricultural waste is used because there is still no research that uses mixed raw materials of agricultural waste. In the manufacture of sugar reduction, raw materials through several steps are pretreatment, hydrolysis, and fermentation, so to produce reducing sugar through 2 steps namely pretreatment and hydrolysis [4].

Pretreatment is classified into several methods, are physical, physics-chemical, chemical, and biological pretreatment [5]. Some of the common pretreatment methods can be combined. Microwave heating is generally used in combination with other pretreatment methods, especially chemical treatments [6]. Pretreatment with boiling water heating is suggested as one of the leading pretreatment methods [5]. In this study, before pretreatment using a microwave and heated with boiling water, raw materials were soaked in NaOH 3%. The pretreatment methods with microwave heating and boiling water heating are more often recommended for use as well as a method that is suitable for laboratory scale, therefore the two pretreatment methods are compared with the effect of sugar concentration resulting from hydrolysis in this study.

Hydrolysis can be done chemically, biologically, and enzymatically. Enzymatic hydrolysis has several advantages compared to acid hydrolysis which provides high sugar results and relatively low maintenance costs because there are no corrosive materials [7]. Acid hydrolysis has the disadvantage that is not environmentally friendly. The hydrolysis method assisted by microorganisms can be compared with enzymatic hydrolysis in this study. From this study, it is expected that the hydrolysis results will obtain high sugar concentrations and optimum time for enzymatic hydrolysis.

2. Materials and Methods

2.1. Materials

The materials used in this study were bagasse taken from a sugar factory in Jember and rice husks taken from rice processing Wirolegi Jember, cellulase enzymes, *Trichoderma viride* culture was obtained from microbiology laboratory, FMIPA Universitas Jember, sodium citrate, citric acid, sodium hydroxide (NaOH), aquadest, dinitrosalicylic acid (DNS), potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$), sodium metabisulfite ($Na_2S_2O_5$).

2.2. Pretreatment

Twenty-five grams mixture of bagasse and rice husks were each soaked in 250 ml of 3% NaOH solution for 30 minutes. Raw materials that have been soaked can be heated in the microwave for 4 minutes and heated with boiling water for 15 minutes.

2.3. Hydrolysis

Enzymatic hydrolysis with the addition of cellulase enzyme activity, 5 grams of delignified sample was added to a flask then added 50 ml of citrate buffer solution pH 4.8. The cellulase enzyme used was *Viscozyme cassava* CL with an enzyme activity of 709 EGU/g. Each added enzyme activity of 0.434, 0.871, 2.61, and 3.49 FPU/g in a flask then hydrolyzed in an incubator shaker with temperature 50 ºC and speed 160 rpm for 24 hours. The sample was taken every 0, 6, 12, 24 hours.

3. Result and Discussion

3.1. Enzymatic Hydrolysis with The Treatment of Variations in Cellulase Enzyme Activity

Cellulase enzymes are biocatalysts that help support hydrolysis reactions. Sugar from the hydrolysis of polysaccharide components can be calculated from absorbance obtained after analysis using the DNS method. The higher absorbance, the higher sugar concentration obtained [8]. There is a component in the cellulase enzyme that can break the bonds in cellulose, namely endoglucanase (*endo*-β-1.4-D-*glucan*-4 *glucanohydrolase*) breaks down β-1.4-

glucanohydrolase bonds in the cellulose chain at random, exoglucanase (β-1.4-D-*glucancellobiohydrolase*) which breaks down cellobiose units from the end of the chain and β-glucosidase which breaks down cellobiose into glucose [9].

Table 1. Enzymatic hydrolysis sugar concentration by microwave heating pretreatment

Time (h)	Concentration (g/L)			
	M_1	M_2	M_3	M_4
0	0.7789	0.8011	0.8056	0.8323
6	2.880	3.133	3.151	3.258
12	3.089	3.365	3.961	3.970
24	3.245	3.383	4.063	4.077

Description: M_1: Addition of enzyme activity 0.434 FPU/g
M_2: Addition of enzyme activity 0.871 FPU/g
M_3: Addition of enzyme activity 2.61 FPU/g
M_4: Addition of enzyme activity 3.49 FPU/g

Based on Table 1, the sugar concentration increases with time increases. This is because the enzymes and raw materials collide with each other and react more so that the conversion is higher. The higher enzyme activity added, the higher sugar concentration obtained. This is because higher enzyme activity will hydrolyze more cellulose into sugar, also the higher enzyme activity, the reaction speed will increase [10]. The highest sugar concentration was obtained from the addition of enzyme activity 3.49 FPU/g (M_4) at 24 hours, namely 4.077 g/L. Most of the cellulase enzymes have optimum activity in the temperature range of 20 – 50 °C and the optimum pH range for cellulase activity is 4.5 – 7.0 [11]. If the temperature conditions increase to the optimum temperature, the rate of enzyme reaction will increase because the kinetic energy increases.

Table 2. Enzymatic hydrolysis sugar concentration by hot liquid water pretreatment

Time (h)	Concentration (g/L)			
	G_1	G_2	G_3	G_4
0	3.925	3.957	4.077	4.424
6	8.011	8.145	8.367	9.969
12	8.278	8.768	12.55	14.15
24	8.590	9.124	13.13	15.18

Description: G_1: Addition of enzyme activity 0.434 FPU/g
G_2: Addition of enzyme activity 0.871 FPU/g
G_3: Addition of enzyme activity 2.61 FPU/g
G_4: Addition of enzyme activity 3.49 FPU/g

Based on Table 2, the highest sugar concentration of 15.18 g/L resulted from the addition of the highest enzyme activity of 3.49 FPU/g, namely G_4 with a hydrolysis time of 24 hours. The

speed of the reaction also depends on the concentration of enzyme, where the reaction speed will increase as the concentration of the enzyme increases [8].

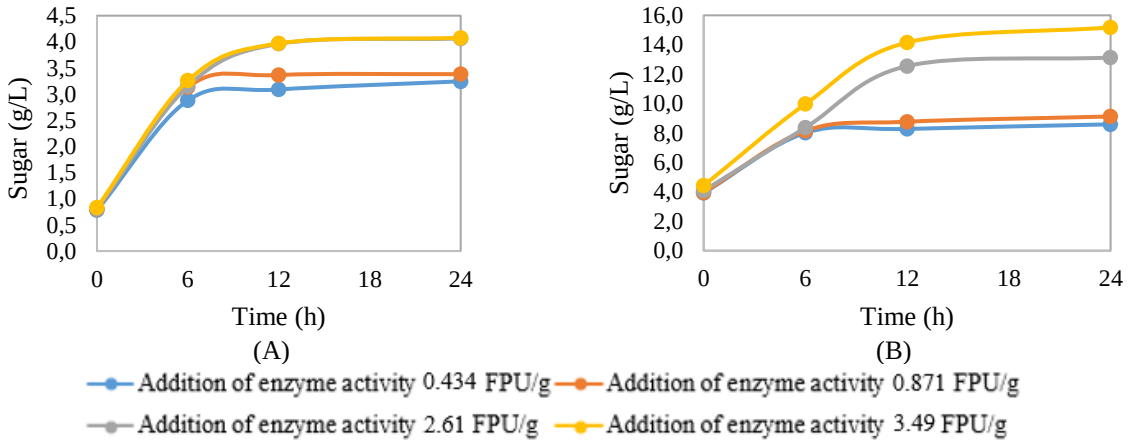

Figure 1. Graph of sugar concentration with cellulase enzyme variation treatment (A) microwave heating pretreatment (B) boiling water heating pretreatment

From Figure 1, it can be seen that the sugar concentration increased with each enzyme variation treatment. After hydrolysis for 24 hours the lowest sugar concentration was obtained from the addition of the smallest variation of the enzyme, namely 0.434 FPU/g with 3.245 g/L for pretreatment using microwave heating and 8.590 g/L for pretreatment using boiling water heating. The highest concentration from the addition of enzyme was 3.49 FPU/g with 4.077 g/L for pretreatment using microwave heating and 15.18 g/L for pretreatment using boiling water heating. From this statement, the addition of enzyme activity 2.61 FPU/g is quite an optimum enzyme activity, because the results of the sugar concentration are not much different from the treatment with the addition of enzyme activity 3.49 FPU/g.

Based on Figure 1, it can be concluded that the greater enzyme concentration, the more sugar concentration obtained and can increase the rate of hydrolysis to a certain concentration limit [12]. The increase in sugar concentration from the 12 - 24 hours of each enzyme variation treatment was not very significant or relatively constant because if it exceeded the optimum time, sugar inhibitors would form so that the sugar concentration produced was smaller or relatively constant [13]. It can be concluded that the optimum time for hydrolysis is 12 hours. The results of substrate hydrolysis will be constant with increasing enzyme concentration because the addition of enzymes is no longer effective [10].

3.2. Enzymatic hydrolysis with the addition of Trichoderma viride

The hydrolyzate sampling time was carried out on the 7th day of hydrolysis. The optimum operating temperature conditions for the growth of *Trichoderma viride* are at 20 ºC

– 36 ºC [14]. The operating temperature condition used in this study is 28 ºC, which is still included in the optimal temperature range so that the enzyme can work optimally in hydrolyzing cellulose to produce sugar concentrations. Cellulase enzymes produced from *Trichoderma viride* affect breaking the complex bonds of cellulose into simpler bonds, namely sugar.

Table 3. Sugar concentration and yield of hydrolysis addition of solution *Trichoderma viride* with microwave heating pretreatment

Repetition	Sugar concentration (g/L)	*Yield* (mg sugar/ g substrate)
1	0.9480	26.16
2	0.4273	11.79
3	0.9079	25.06

Based on Table 3, it can be seen that the average sugar concentration is 0.7611 g/L with an average yield of 21.01 mg sugar/g substrate for raw materials that are treated with microwave heating. One of the microbes that can produce cellulase enzymes is *Trichoderma sp*. *T. viride* can produce cellulase enzymes consisting of endoglucanase, exoglucanase and β-glucosidase [14].

Table 4. Sugar concentration and yield of hydrolysis addition of solution *Trichoderma viride* with boiling water heating pretreatment

Repetition	Sugar concentration (g/L)	*Yield* (mg sugar/ g substrate)
1	0.8412	23.22
2	0.8545	23.58
3	0.9079	25.06

From Table 4 it can be seen that the average sugar concentration obtained is 0.8679 g/L with a yield of 23.95 mg sugar/g substrate. The results of hydrolysis with pretreatment heated in boiling water obtained a higher concentration than hydrolysis with pretreatment using microwave heating. This can be interpreted that lignin content is reduced a lot so that there is a lot of decomposition of polysaccharide component by the cellulase enzyme from *Trichoderma viride* with pretreatment heated in boiling water.

There are 10 types of cellulosic enzymes produced by *Trichoderma viride* that work together to break down cellulose material [7]. Amorphous cellulose can be hydrolyzed by endoglucanase which is randomly soluble and crystalline cellulose can be degraded by cellobiohydrolase to produce cellobiose. These two types of enzymes work together to degrade

cellulose into cellobiose and other short cellooligosaccharides. β-glucosidase enzyme to hydrolyze cellobiose and other cellooligosaccharides produced by cellulase into glucose [7].

3.3. Effect of pretreatment for enzymatic hydrolysis

Hydrolysis by pretreatment heated in boiling water obtained a higher sugar concentration every hour based on Table 2 also obtained a higher sugar concentration based on Table 4 compared with the results of sugar concentration in Tables 1 and 3, namely hydrolysis where the raw material was pretreated with microwave heating. The pretreatment process with longer heating will damage most of the lignin structure, so enzymes can more easily access cellulose and hemicellulose so that the hydrolysis process runs more easily and a higher sugar concentration is obtained [15].

In the enzymatic hydrolysis process using lignocellulosic materials, pretreatment is an important step taken to increase the accessibility of cellulose-degrading enzymes [16]. The conversion of lignocellulosic biomass materials into sugars is carried out through a pretreatment process to open the biomass structure and release sugar groups from cellulose and hemicellulose and increase the porosity of the material [17]. Microwave heating is generally used in combination with other pretreatment methods. Microwave heating with a combination of pretreatment using an alkali has been widely studied, mainly because the results obtained by alkaline solvents are better and recommended and have been shown to produce high sugar yields and higher lignin removal compared to acidic solvents [6]. Boiling water heating pretreatment is suggested as one of the leading pretreatment methods [5].

There are several advantages of using a microwave as a pretreatment method, namely faster heating rate, shorter reaction time, and high energy efficiency. The main drawback of microwave heating is the non-uniform heat profile [6]. When lignocellulosic biomass is heated by microwave, selective heating of polar molecules is observed due to the effect of dipolar polarization. This selective heating also decreases the crystallinity of cellulose. In the presence of polar solvents, hot spots can cause rupture or explosion of some lignocellulosic structures [6]. The advantages of using boiling water heating are there is no need to reduce the particle size of the substrate, effective cost because there is no addition of other chemicals, not corrosive. The purpose of using boiling water heating is to trigger changes in the structure of lignocellulose to make cellulose more accessible to enzymes, hemicellulose on heating is maintained in the form of oligomers and the formation of monomers is minimized [5].

Based on Table 2 with the highest sugar concentration of 15.18 g/L and table 4 with an average sugar concentration of 0.8679 g/L, the highest sugar concentration was obtained from pretreatment of raw materials using boiling water heating for 15 minutes. This is because when soaking with 3% NaOH for 30 minutes, the lignin structure, the crystalline, and amorphous parts were damaged by NaOH solution, the solution also separated some of the lignin and hemicellulose and caused swelling of the cellulose [18]. Several studies have been conducted on the superiority of NaOH as a pretreatment solution for lignocellulosic materials. The strongest alkali catalyst that is effective in increasing the rate of enzymatic hydrolysis is NaOH solution compared to other alkali solvents [19]. The cellulose content after pretreatment increased. In addition to the NaOH solution, heating can also damage the lignin structure. The longer the delignification process uses heat, the more lignin is degraded.

4. Conclusion

Enzymatic hydrolysis with the method of adding variations in enzyme activity obtained a higher sugar concentration than hydrolysis with the addition of *Trichoderma viride*. the optimum time for enzymatic hydrolysis was 12 hours with the optimum variety of enzyme activity 2.61 FPU/g. Pretreatment by heating in boiling water which combined with 3% NaOH immersion obtained a higher sugar concentration because a lot of lignin was degraded, thereby increasing the accessibility of cellulose-degrading enzymes.

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