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

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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 physicochemical pretreatment method, in which lignocellulosic material was soaked in 3% NaOH, then heated with microwave and boiling water. The following process is enzymatic hydrolysis with variations of cellulase enzyme 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 the optimum enzyme activity is the addition of 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, it was 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*

1. Introduction

Until now, many studies have used biomass waste as a raw material to produce reduced sugar. Reducing sugar can be made from the hydrolysis of lignocellulosic materials because the lignocellulosic structure can be converted into reducing sugars and has the potential to be

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

Pretreatment is classified into several methods: physical, physicochemical, chemical, and biological pretreatment [5]. Some of the standard pretreatment methods can be combined. Microwave heating is generally used 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 heating 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 and are 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 it 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 yield high sugar concentrations and an 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 Semboro, Jember, and rice husks taken from rice processing in Wirolegi, Jember; cellulase enzymes, *Trichoderma viride* culture was obtained from the microbiology laboratory, FMIPA Universitas Jember; sodium citrate, citric acid, sodium hydroxide (NaOH), aquadest, dinitrosalicylic acid (DNS), potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$), and sodium metabisulfite ($Na_2S_2O_5$).

2.2 Pretreatment

Twenty-five grams of bagasse and rice husks were each soaked in 250 ml of 3% NaOH solution for 30 minutes. Raw materials that have been washed can be heated in the microwave for 4 minutes or 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 50 ml of citrate buffer solution, pH 4.8, was added. The cellulase enzyme used was *Viscozyme cassava* CL with an enzyme activity of 709 EGU/g. Each enzyme activity of 0.434, 0.871, 2.61, and 3.49 FPU/g was added in a flask, then hydrolyzed in an incubator shaker with a temperature of 50 ºC and a speed of 160 rpm for 24 hours. The sample was taken every 0, 6, 12, and 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 the absorbance obtained after analysis using the DNS method: the higher the absorbance, the higher the 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*), which 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 of 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. This is because the enzymes and raw materials collide and react more, so the conversion is higher. The higher the enzyme activity added, the higher the sugar concentration obtained. Higher enzyme activity will hydrolyze more cellulose into sugar; the higher the enzyme activity, the faster the reaction speed will increase [10]. The highest sugar concentration was obtained from adding enzyme activity 3.49 FPU/g (M_4) at 24 hours, namely 4.077 g/L. Most 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 enzyme reaction rate will increase because the kinetic energy increases.

Table 2. Enzymatic hydrolysis of 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 adding 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 the enzyme, where the reaction speed will increase as the concentration of the enzyme increases [8].

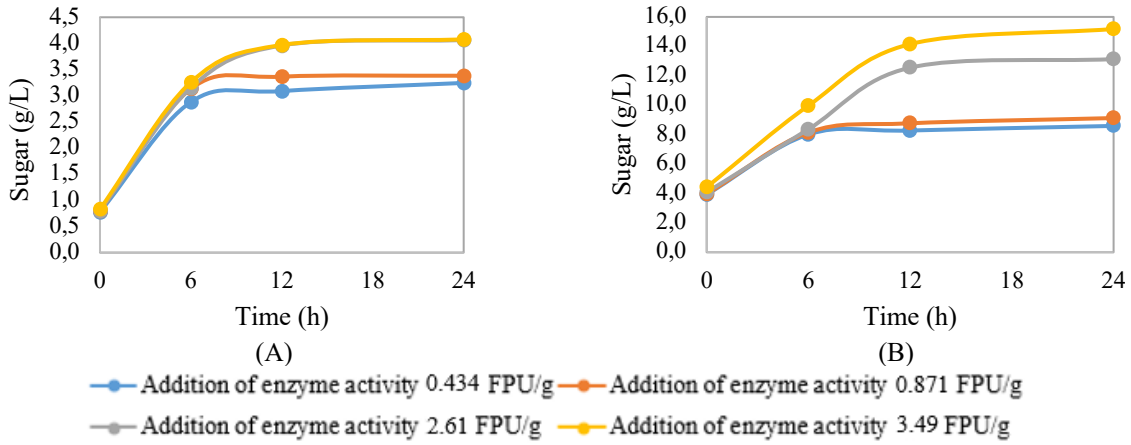

Figure 1. Graph of sugar concentration with cellulase enzyme variation treatment (A) microwave heating pretreatment (B) boiling water heating pretreatment

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 adding the slightest 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 enzyme addition 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 the enzyme concentration, the higher the sugar concentration obtained, which can increase the hydrolysis rate to a specific 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. If it exceeded the optimum time, sugar inhibitors would form so that the sugar concentration produced was lower or relatively constant [13]. It can be concluded that the optimum time for hydrolysis is 12 hours. The results of substrate hydrolysis will be continuous 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 hydrolysate 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* break 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

Table 4 shows that the average sugar concentration obtained is 0.8679 g/L with a yield of 23.95 mg sugar/g substrate. Hydrolysis results with pretreatment heated in boiling water obtained a higher concentration than hydrolysis with pretreatment using microwave heating. This can be interpreted as a reduction in lignin content, so there is a lot of decomposition of the 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 enzymes combine 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 received 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 crucial to increase the accessibility of cellulose-degrading enzymes [16]. The conversion of lignocellulosic biomass materials into sugars is done through pretreatment to open the biomass structure, release sugar groups from cellulose and hemicellulose, and increase the material's porosity [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 methods [5].

Several advantages of using a microwave as a pretreatment method are 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 boiling water heating are that there is no need to reduce the particle size of the substrate, it is effective and cost-efficient because there is no addition of other chemicals, and it is not corrosive. The purpose of 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 NaOH solution damaged the lignin structure, the crystalline and amorphous parts, and the solution also separated some of the lignin and hemicellulose. It 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 damage the lignin structure. The longer the delignification process uses heat, the more lignin is degraded.

4. Conclusion

Enzymatic hydrolysis with adding variations in enzyme activity obtained a higher sugar concentration than hydrolysis with adding *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, 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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