

ENZYMATIC HYDROLYSIS PROCESS FOR INCREASING GLUCOSE LEVELS FROM COCONUT HUSK WASTE

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ABSTRACT: Coconut husk waste is waste that has not been used optimally, generally only as a craft material. Seeing the composition of coconut husk, it has the potential to be used as an alternative fuel, one of which is to produce bioethanol products. The purpose of this research was to utilize coconut husk waste as raw material for bioethanol production and to assess the effect of the amount of enzymes and time of hydrolysis on the glucose levels produced. In this research, the authors focused on obtaining glucose levels from coconut husks by hydrolysis using cellulase enzymes with activity of 700 EGU/g. The variations used in this research were the volume of cellulase enzymes (2, 3, 4, 5, 6) ml and the hydrolysis time (4, 8, 12) hours. After the coconut husk undergo physical and chemical treatment using 10% NaOH, there is a decrease in lignin levels from 44% to 14% and there is an increase in cellulose levels from 24% to 38%, and the use of a cellulase enzyme volume of 2 ml with a hydrolysis time of 4 hours was more optimal with a glucose level of 0.32%.

Keywords: Coconut husk waste, glucose, cellulase enzymes, hydrolysis process

1. Introduction

Fuel is a basic necessity for the community and its use tends to increase every year, while the source of petroleum fuel used today is getting low. Therefore, we need alternative materials that can be used as a substitute for petroleum. One of them is bioethanol. Bioethanol is a liquid fermented carbohydrate (starch) using the help of microorganisms. Bioethanol can be used as an alternative fuel to address current fuel needs.[1].

To produce bioethanol, there are several stages in general consisting of several series of processes, consisting of raw material preparation, delignification, hydrolysis, fermentation, and purification. However, in this research, only focused on the hydrolysis process with the aim of knowing the best glucose levels that can be produced [2]. The raw material preparation stage for making bioethanol can be obtained by selecting raw materials from various plants, either those that directly produce simple sugars such as sugar cane, sweet wheat or those that produce carbohydrates such as corn, cassava and wheat.

The second stage is delignification, this delignification process aims to reduce the lignin

content found in raw materials which can generally be done using acid or alkaline solutions. The third stage is hydrolysis. Hydrolysis of cellulose and hemicellulose can be carried out chemically or enzymatically to produce glucose and xylose reducing sugar monomers [3].

One of the materials that can be used to make bioethanol is coconut husk waste



Figure 1 coconut husk

The availability of coconut husk waste is quite large, with the production of Indonesian coconut fruit averaging 15.5 billion grains/year or equivalent to 1.8 million tons of husk, and 3.3 million tons of husk dust. On average, one coconut fruit produces 0.4 kg of husk which contains 30% fiber. The chemical composition of

old coconut coir is lignin (45.8%), cellulose (43.4%), hemicellulose (10.25%), pectin (3.0%). If we are able to convert cellulose to glucose, it will increase the conversion of coir coconut into ethanol [4].

According to Asyeni's research entitled "Utilization of Coconut Husk into Bioethanol with the Acid-Pretreatment Delignification Process". With variations in the concentration of H_2SO_4 and CH_3COOH of (1.3, and 5%), at a temperature of 121 C and with a delignification time of 60 minutes, the results of this research were that the lowest lignin levels were obtained using H_2SO_4 at a concentration of 5%, it is 18.8125%, whereas if you use CH_3COOH the lowest lignin content is at a concentration of 5%, it is 22.8965% [5].

According to Ade Irmawan's research entitled "Optimization of Reducing Sugar Levels in the Process of Hydrolysis of Corn Stalks Waste with Variations in Concentration of Cellulase Enzymes and Duration of Hydrolysis" explains that the results of reducing sugar levels in each time variable are different. Where the results of reducing sugar levels at variations of time (5, 6, 7, 8, 9 hours) are directly proportional to variations in enzyme concentrations, (2; 2.5; 3; 3.5; 4%) which has continued to increase where the sugar yield The best reduction was at a concentration of 4%, namely 1596.7 mg/L, but at a concentration of 3% enzyme, the result of reduced sugar decreased compared to the previous enzyme concentration (2% and 2.5%) [5].

According to the research of Betaria entitled "Making Bioethanol from Coconut Husk Waste with Acid Hydrolysis and Fermentation Methods Using Yeast Tape". During the hydrolysis process using acid, the variation in the concentration of HCl used is 10%, 20%, 30%. So that, the resulting glucose levels are 17.4; 14.27; and 11.5%. The best result from the hydrolysis process is using HCl at a concentration of 10%, which is 17.4% which is used for the next fermentation stage

2. Research Methodology

2.1 Experimental

The research was conducted at the Chemical Analysis Laboratory of ITN Malang. The following are fixed variables for the Delignification Process: 100 gr coconut husk size 80 mesh, 10% NaOH concentration, 28 hours Delignification time, 28°C heating temperature, while the fixed variables for the hydrolysis process: Type of cellulase enzyme, 10 grams of

coconut husk, pH 5, the hydrolysis temperature is 37°C. Variable changes were hydrolysis time of 4, 8, 12 hours and variations in the number of enzymes: 2 mL, 3 mL, 4 mL, 5 mL, 6 MI [7].

2.2 The materials and equipment used:

The tools used are as follows: 80 mesh sieve, Basin, Beakerglass, sample bottles, Erlenmeyer, Furnace, LPG gas, watch glasses, stirring glasses, stove, three-neck flask, measuring flask, baking sheet, magnetic stirrer, grinding machine, mortar, oven, Pans, Dropper Pipettes, Volume pipettes, Knives, Thermometers, Scales The materials used are as follows Aquadest, NaOH, H_2SO_4 , cellulase enzymes, coconut husk.

2.3 Study Procedure

The research procedure carried out includes three stages, they are material preparation, delignification process and the last stage is the hydrolysis process, which are as follows:

2.4 Material Preparation

Coconut coir is crushed roughly then analyzed for cellulose, hemicellulose, and lignin content before physical treatment (Chesson method), coconut husk is then cleaned first using aquadest, then dried in an oven at 105°C for 24 hours to remove the moisture content, then The dry coconut husk is blended into a powder with a uniform size of ± 80 mesh, then stored in a desiccator dialate. And cellulose, hemicellulose, and lignin analysis were performed (Chesson method).

2.5 Process of Delignification

Coconut coir powder weighed 100 grams is put into a beaker glass, add a 10% concentration of NaOH solution, and soak the sample solution at 28°C for 28 hours, filter the delignification results and neutralize it to pH 7, dry again using an oven with a temperature of 105°C for 12 hours, followed by a lignin, cellulose and hemicellulose analysis procedure. All work is done twice (duplo)

2.6 Enzyme Hydrolysis

Taking the best sample (coconut husk) from the delignification process, weighing the sample as much as 10 grams, inserting the weighed sample into Erlenmeyer, adding enzymes with variations of 2 mL, 3 mL, 4 mL, 5 mL, and 6 mL into each Erlenmeyer, then add 100 mL of water, the hydrolysis process is carried out with a time

variation of 4 hours, 8 hours, and 12 hours, the hydrolysis process is carried out at 37°C at pH 5, after the hydrolysis process is finished, it is followed by taking samples to analyze the glucose levels.

The delignification process aims to remove lignin, reduce the crystallinity of cellulose, and increase the porosity of the material. The following is a graph comparing the levels (%) of hemicellulose, cellulose, and lignin with initial conditions, physical treatment and chemical treatment.

3. Results and Discussions

3.1 Delignification Process

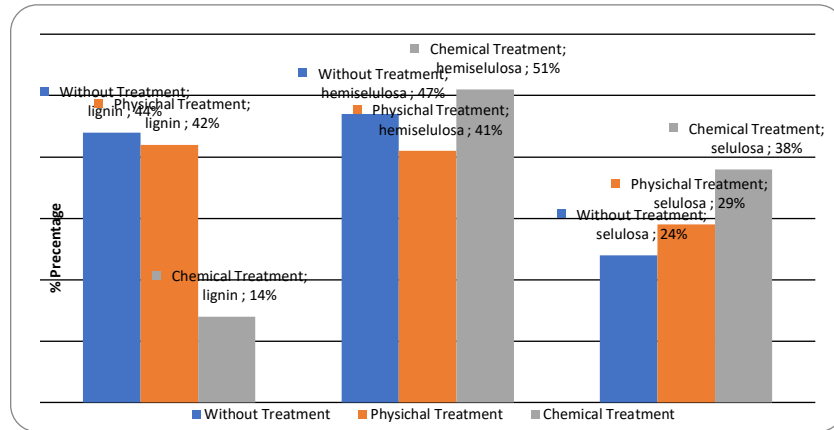


Figure 2 Graph Comparison of hemicellulose, cellulose, and lignin levels with initial conditions, physical treatment, and chemical treatment

In the graph above, it shows that the coconut husk is not treated, the hemicellulose content is 47%, 24% cellulose and 44% lignin, then when the coconut husk is physically treated with coir it is milled then sieved using an 80 mesh sieve. The data obtained from the data obtained hemicellulose 41%, 29% cellulose, and 42% lignin from the data proving that physical treatment can reduce lignin levels. After physical treatment is carried out, it is followed by chemical treatment with the delignification method using 10% NaOH. 80 mesh coconut fiber soaked for 28 hours using 10% NaOH at room temperature with a pressure of 1 atm.

This reduction in lignin levels is in accordance with the theory of Julfana Rika which states that hemicellulose extraction can use solvents such as NaOH and KOH. Among these solvents the best to use is NaOH[8]. Hemicellulose has an amorphous structure so that the use of NaOH can remove lignin and extract hemicellulose. In Safaria's research, NaOH solution can attack and damage the lignin structure in the crystalline and amorphous parts and separate part of the hemicellulose [9].

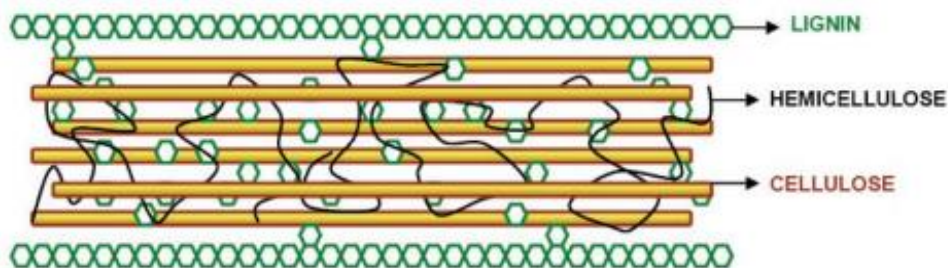


Figure 3 Structure of Lignocellulose Components [10]

From the picture above, it can be seen that the lignin layer protects the hemicellulose and cellulose contained in coconut husks, while for the hydrolysis process we need cellulose to be

converted into glucose. Therefore, the lignin layer must be removed or reduced.

Hemicellulose has a lower molecular weight than cellulose and is resistant to heat treatment.

Unlike cellulose, hemicellulosic polysaccharides are amorphous and less branched in structure, so their solubility potential is very different. Hemicellulose can be separated from cellulose by alkaline because the bonds are weak so that it is easily hydrolyzed [11].

The cellulose content increased from the initial 24%, then after physical treatment the cellulose content increased to 29%, and after chemical treatment, the cellulose content increased to 38%. This increase in cellulose levels is in accordance with Agustini's theory which states that the amount of lignin and cellulose is inversely related [12]. The more lignin levels are broken down, the cellulose levels will increase. This is because the lignin layer that protects cellulose has been reduced, so that cellulose can appear optimally. And in the following picture is a picture of the chemical delignification process in coconut husk samples.



Figure 4 Delignification Process

And the picture below is a mechanism for breaking the bond between lignin and cellulose by NaOH compounds.

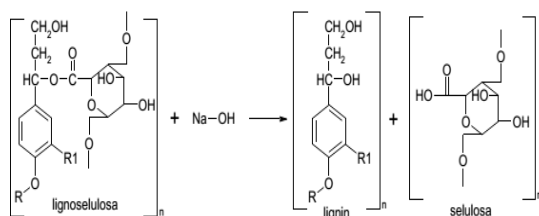


Figure 5 Breaking of the bonds between lignin and cellulose by NaOH [13]

The OH⁻ ion from NaOH will break the bonds from the basic lignin structure while the Na⁺ ion will bind with lignin to form sodium phenolics. This phenolic salt is soluble [8]. The results of delignification of coconut husk are in the form of a residue and a filtrate called black liquor. The black liquor is blackish brown. The mechanism

of breaking lignin compounds is where the base groups of the cooking solution (NaOH) degrade or attack alpha and beta lignin. The degraded lignocellulose is unstable, so it triggers condensation which causes the breakage of lignin bonds from hemicellulose and cellulose [14].

3.2 Hydrolysis Process

Hydrolysis is the process of breaking down complex sugars into simple sugars. In the hydrolysis process, cellulose is converted into cellobiose or sucrose and then into simple sugars such as glucose. Meanwhile, the result of hydrolysis of the hemicellulose component is a mixture of simple sugars such as glucose, galactose, xylose, and arabinose [15]. In complete hydrolysis cellulose will produce glucose, while hemicellulose produces several sugar monomers pentose (C₅) and hexose (C₆). Hydrolysis can be carried out chemically (acid) or enzymatically [16].



Figure 6 Hydrolysis Process

Basically, the mechanism of cutting the bond chain by cellulase enzymes is very complex because it involves the synergy of the work of 3 major components, namely endo-1,4-β-D-glucanase which functions to break cellulose bonds randomly by starting a random attack on the internal side of the amorphous region of the cellulose fiber so that the side that is open can be attacked by cellobiohydrolase. Then the action of exo-β-1,4-glucanase which cuts the ends of the individual chains of cellulose. exo-β-1,4-glucanase or called cellobiohydrolase attacks the non-reducing exterior of cellulose so that cellobiose is produced as its main structure.

Next is the work of β -glucosidase which functions to cut cellobiose into glucose molecules.

Cellulase enzymes can convert unsubstituted cellulose into cellobiose which is then further

hydrolyzed with β -glucosidase. Breaking this bond will produce oligosaccharide derivatives of cellulose, which are finally converted into glucose monomers [17].

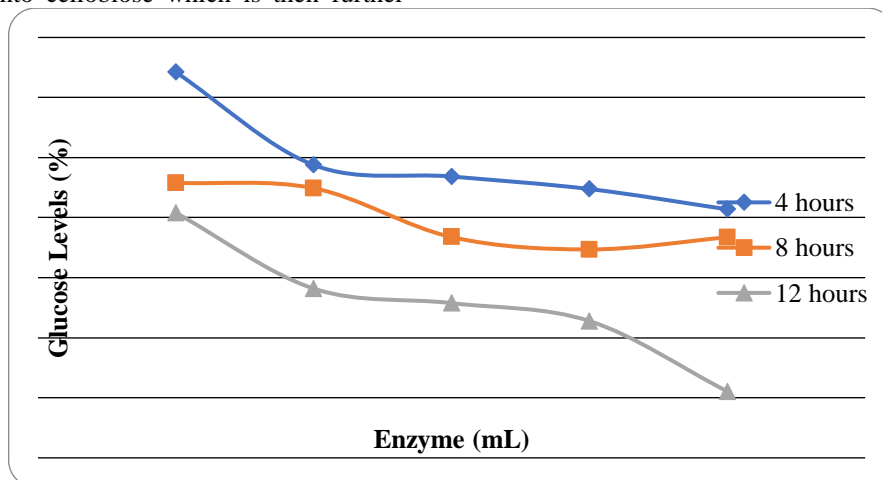


Figure 7 Graphic analysis of glucose levels against enzymes and hydrolysis time

Figure 7 shows that the results of glucose levels at each time variable are different. Where the results of glucose levels at each time variable have decreased. The highest glucose level was at 4 hours with the amount of enzyme of 2 ml, it is 0.3211%. This is not in accordance with the theory which says that the longer the hydrolysis time, the more glucose levels will be produced because the more time for the breakdown of cellulose into glucose. This is due to several other factors that cause the results of glucose levels to tend to be small and decrease among them, the first is because the temperature during the hydrolysis process is not constant, this is due to the use of hotplates with a temperature range of 37-40°C, the second factor is time, less needed to make cellulase enzymes work optimally. Hydrolysis using enzymes tends to take longer than using acids or alkalis. Some of the disadvantages of enzymatic hydrolysis include that it takes a longer time, and the action of the enzyme is inhibited by the product [15].

4. Conclusions

Based on the results of observations and calculations that we have done during the study, a conclusion can be drawn

- After coconut fiber undergoes physical and chemical treatment using 10% NaOH, there is a decrease in lignin levels from 44% to 14% and there is an increase in cellulose levels from 24% to 38%.

- The use of cellulose enzyme volume of 2 ml with a hydrolysis time of 4 hours is more optimal than the hydrolysis process using cellulase enzymes with a volume of cellulose enzymes of 3 ml, 4 ml, 5 ml, 6 ml with a glucose level of 0.32%.

5. Suggestions

In future researches, it is more concerned with factors that influence the enzyme hydrolysis process. Using a more constant temperature, and for hydrolysis using enzymes must be carried out for a longer time.

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