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Hydrolysis of Corn Stalk Enzymatics using Cellulation Enzyme as an Efforts to Increase Reducing Sugar Levels

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Abstract : Bioethanol can be processed from various types of starchy plants (cassava, corn, seed sorghum, sago), sugary plants (sugar cane, sweet sorghum, beets) as well as cellulose (straw, sawmill residue, bagasse, soybean seed skin). Cellulose is the main constituent of plant cells and is an abundant organic compound on earth. Cellulose material which is not a food ingredient can be used as bioethanol raw material. One of them is corn stalk waste. Corn stalk waste is obtained from the corn harvest. In the corn harvest, there is a cellulose waste that can be utilized, including corn leaves and corn cobs. This study aims to utilize corn stalk waste to obtain optimum reducing sugar levels using variations in cellulase enzyme concentration and hydrolysis time. Before the hydrolysis process, initial processing is carried out using alkaline solutions, namely NaOH, for the delignification process. The variables used in the study include: cellulase enzyme concentration: 2%; 2.5%; 3%; 3.5%; 4% and hydrolysis time: 6, 8 and 10 hours. The effect of Physical treatment and Chemical treatment affect the levels of Lignin and cellulose. After the corn stalk waste has undergone physical and chemical treatment using 10% NaOH, there is a decrease in lignin levels from 12% to 4% and there is an increase in cellulose levels from 40% to 75%. While for the best result is obtained at the enzyme concentration of 3.5% with hydrolysis time of 10 hours which produces a reducing sugar of 2214.900 mg/L.
Key words : Corn stalk waste, Reducing sugar, Cellulase enzyme.

Introduction

Corn plant is one of the plants that can be used as a source of bioethanol. According to (Muniroh et al., 2011) corn stalk biomass is a waste which is still not widely used as a product that has added value. Corn stalk, including biomass, contains lignocellulose which is very possible to be used as bioethanol because it has quite a lot of cellulose content.

In 2017, corn production in Indonesia reached 4,467,933 Ha (Suwandi, 2016). This is quite a large amount considering the amount of corn harvest in the previous year only reached 4,387,584 Ha. However, this

large amount is not comparable to the waste produced. Because of 100% corn produced, only 30% is corn products and the rest is waste. One of the utilizations of corn stalk is as a raw material for making bioethanol.

Figure 1. Dried corn tree

This is caused by the corn stalk which has cellulose content of (30 – 50 %), hemicellulose of (15 – 35 %), lignin of (13 – 30 %), and water of (9 – 11 %), and ash of 6% (Muniroh, 2011). Previous researcher (Arif, 2016) used the same material, i.e. corn stalk waste in which the hydrolysis process used cellulase enzymes and obtained the best glucose level of 3.3458% with 8 hours of hydrolysis time and enzyme concentration of 3%. Whereas (Sutarno, 2011) with the same material at the hydrolysis time of 8 hours also obtained glucose levels of 30.884 mg/L using mixed cellulase enzymes from *Trichoderma reesei dan Aspergillus niger*. Glucose levels were obtained at 0.324% with the hydrolysis process using H_2SO_4 2% in the study (Yonas, 2013).

Cellulose

Cellulose is composed of D-glucose which is bound through bond of β (1⟶ 4). This linear structure causes cellulose to be crystalline and not soluble. In nature, cellulose is usually associated with other polysaccharides such as hemicellulose or lignin to form the main framework of plant cell walls. Cellulose which consists of thousands of glucose units can be interconnected and form structures. Crystallized structures and the presence of lignin and hemicellulose are the main obstacles to hydrolyze cellulose.

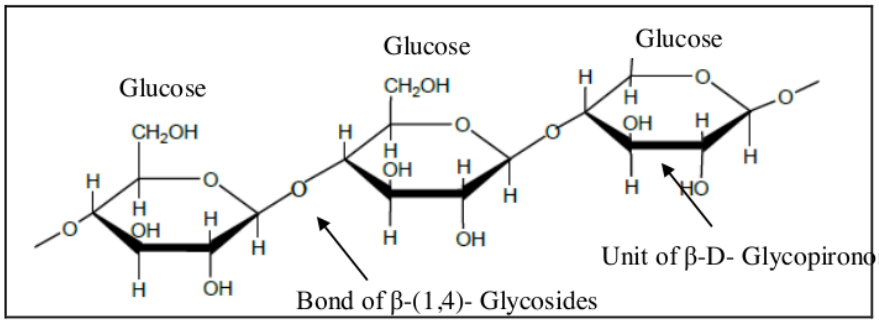

Figure 2. Structure of cellulose

Delignification Process

The delignification process aims to remove lignin, reduce crystallinity of cellulose, and increase porosity of the material. Preliminary treatment can be carried out physically and biologically. Some delignification process treatments include:

Physically, in the form of mechanical enumeration, milling, and flouring to reduce the size of the material and reduce the crystallinity of cellulose. Chemically, includes acids, bases, oxidative delignification, and organosolv processes. And biologically, by using brown weathering fungal microorganisms, white rot fungi, and softening fungi to degrade lignin and hemicellulose in lignocellulose materials. Meanwhile, the factors that influence the delignification process: Temperature, time and concentration of NaOH (Arif, 2016).

In the delignification process physically, chemically and biologically, the decrease in lignin levels is mostly in the delignification process chemically (Agustini, 2015).

In this study, the delignification process was carried out chemically using a base method, in processing lignicellulose generally using bases such as sodium, calcium and ammonium hydroxide. NaOH, KOH, $Ca(OH)_2$ and NH_4OH are alkaline solutions which have been shown to effectively degrade lignicellulose biomass. For example, NaOH can increase the rate of degradation of hardwood from 14% to 55% by reducing the level of lignin from 55% to 20%. Delignification of various lignicellulose biomass such as wheat straw, grass, hardwood, and soft wood using NaOH is also able to reduce lignin levels to be less than 26% (Hidayat, 2013).

Cellulose Hydrolysis Process

Hydrolysis is the process of polysaccharides solution in lignocellulose biomass, i.e. cellulose and hemicellulose to be the constituent sugar monomers. The enzymatic process is similar to the acid hydrolysis processes but the acid catalyst is replaced with an enzyme. The reducing sugar content in sweet potatoes produced in enzymatic hydrolysis was higher, i.e. 12.61% compared to the results of acid hydrolysis which was 6.20%.

Biological cellulose hydrolysis can be carried out using either cellulase enzymes or cellulase-producing microorganisms. There are three enzymes that play a role in cellulose reformation into glucose, namely; (a) The endogluconase enzyme, which functions to cut the long glucose chain into a shorter chain randomly, (b) Cellbiohydrolse enzyme, which functions to cut every two glucose chains (cellobiose), (c) β-glucosidase, which functions to cut cellobiose into glucose molecules (Irawati, 2016).

Enzymatic hydrolysis has several advantages compared to acid hydrolysis, including: there is no sugar degradation resulted from the hydrolysis, the process conditions that work with (low temperature), the potential to produce high yields and the cost of maintaining equipment is relatively low because there are no corrosive materials. Some of the disadvantages of enzymatic hydrolysis include taking a longer time, and the enzyme's work is inhibited by the product.

Cellulose enzyme

Cellulase enzyme is an enzymes that plays an important role in the process of bioconversion of cellulose organic wastes into glucose, single cell proteins, cattle fodder, ethanol and others. This enzyme is an enzyme that can hydrolyze β bond (1-4) in cellulose. Cellulase enzyme includes extracellular enzyme, an enzyme released from cells to the environment to hydrolyze polymers in the environment. This enzyme is produced in cellulotic microbial cells and then released from cells into the digestive system to digest cellulose.

Perfect enzyme hydrolysis requires the synergy action of three types of this enzyme, they are:

a. Endo-1,4-β-D-glucanase (*endocellulase, carboxymethycellulase* or CMCase), which reduces cellulose polymers randomly in internal bond of α-1,4-glycosides to produce oligodextrins with varying chain lengths.
b. Exo-1,4-β-D-glucanase *(cellobiohydrolase),* which parses cellulose from reducing and non-reducing agents to produce cellobiose and/or glycose.
c. B-glucosidase (cellobiase), which parses cellobiose to produce glucose.

Several factors that influence the work of enzymes, i.e.: The concentration of enzymes, pH (acidity) of cellulase enzymes in the results of the study shows two optimum conditions, i.e. condition of low acid of pH 5 and low base of pH 8, temperature, based on statistical results can be concluded that the optimum temperature is at temperature of 37^0C with the cellulose activation of 2.19 x 10^{-2}Unit/mL, Time of Contact and Final Products, enzyme reaction always involves two things, they are the substrate and the final product. In some cases, the final product can also reduce the work productivity of the enzyme (Irawati, 2016).

Experimental

The fixed variables used in this study include corn stalk powder with a size of 60 mesh, material mass of 10 gram, **Delignification process** used room temperature, time of 28 hours, type of solvent: NaOH 10%. **Hydrolysis process** used the type of cellulase enzyme, pH 5 and the temperature of 37 ^{o}C

Meanwhile, the changing variables used in this study include: Cellulase enzyme concentrations of 2%; 2.5%; 3%; 3.5%; 4% and hydrolysis time is 6 hours, 8 hours, 10 hours, 12 hours, 14 hours.

The materials used:

Materials used are: Aquades, Cellulase enzyme, H_2SO_4, Corn stalk waste resulted from corn harvest, NaOH.

The materials used in this study include 1 kg of leaf, 6 hours of drying time, 370 grams of white crystal sugar, 500 ml of aquades, 150 grams of dextrin, tween 80 with various kinds of volume: 0.5 ml, 0.75 ml, 1 ml, 1.25 ml, 1.5 ml.

Equipment used

Equipment used are: *beakerglass,* sample bottle, *Erlenmeyer, Furnace, Shaker Waterbath,* Glass, watch, Stirrer glass, Three-neck flask, Measuring flask, Milling machine, Mortar, pH meter, Drop pipette, Stamper, Scales, Thermometer.

Study Procedure

This study was conducted in these following stages:

Preparation of raw materials, namely corn stalk waste was dried under the sun, then cut into pieces with a size of ± 3 cm, after it was dry, then it was blended using blender. The result was sifted to produce a powder of 60 mesh and taken as heavy as 100 gr. Then, pre-treatment (delignification) was conducted, the sample of 100 gr was put into three beakerglasses and prepared NaOH with a concentration of 10%, then 1000 ml of NaOH was added to the beakerglasses which already contained the sample and stirred until the sample was completely immersed. Immersion was carried out for 28 hours at room temperature and then an HHSLA analysis was carried out.

The stages of Hydrolysis process with Cellulase enzymes are as follows: weighed the sample as much as 50 gr, then put into Erlenmeyer and added enzymes with variations of 2%, 2.5%, 3%, 3.5%, 4%of the sample weight into each Erlenmeyer and added 1500 mL of water. The hydrolysis process was carried out with a time variation of 6, 8, 10, 12, 14 hours, at temperature of 37 ^{o}C and pH 5. After after hydrolysis had been complete, 25 ml of sample was taken to test the glucose level.

Results and Discussions

Delignification process

The following is a graph of the comparison of levels (%) of lignin, cellulose, hemicellulose and HWS with initial condition, physical treatment and chemical treatment.

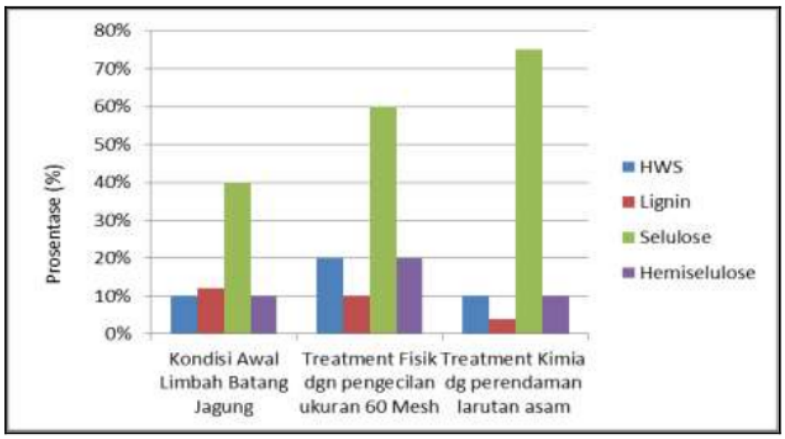

Figure 3. Graph of Comparison of levels (%) of HWS, lignin, celluloses and hemicelluloses in various treatments.

In conditions where there is no treatment of corn stalk, Hemicellulose level of 10%, cellulose level of 40%, lignin level of 12% and HWS level of 10%, then when the corn stalk got physical treatment with corn stalk was milled, then filtered using a 60 mesh sieve. Data obtained were hemicellulose level of 20%, cellulose level of 60%, lignin level of 10% and HWS level of 20% of data requiring physical treatment can reduce lignin level. After physical treatment, it was proceed with chemical treatment using alkaline delignification method. 60 mesh corn stalks were soaked for 28 hours using 10% NaOH at room temperature at a pressure of 1 atm. It obtained hemicellulose level of 10%, cellulose level of 75%, lignin level of 4% and HWS level of 10%

The mechanical or physical delignification process aims to reduce the particle size of raw materials. Reducing the size of raw materials into small parts is one of the most effective ways to increase the accessibility of lignocellulose-resistant enzyme and can increase the activity of cellulase enzymes in lignocellulose materials (Taherzadeh, 2008).

In addition to physical delignification, it can also be carried out by chemical means by giving acidic or alkaline solutions. The delignification method used in this study is alkaline solution. Solutions used such as sodium, potassium, calcium, and ammonium hydroxide. The use of bases causes changes in the structure of lignin by degrading the esters and the glycosidic side chains. The use of bases also causes the partial decryption of cellulose, partial solvation of hemicellulose and results in enlarged cellulose. This process is done by immersing the biomass in an alkaline solution at a predetermined temperature and time. The neutralization stage needs to be conducted before entering the enzymatic hydrolysis stage to remove lignin and inhibitor substances (eg salt, fenoloit acid and aldehyde) (Menon, 2012).

The figure below is a mechanism to parse bonds between lignin and cellulose by NaOH compounds.

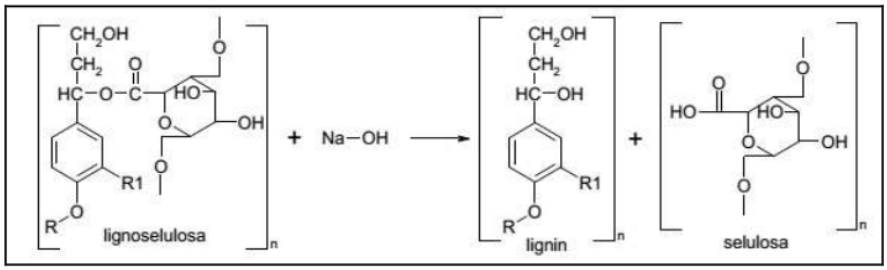

Figure 4. Termination of the bond between lignin and cellulose by NaOH (Fengel, 1995)

The mechanism of delignification by NaOH solution can be seen in Figure 5.2. Here, NaOH will enter and terminate the bonds of the basic structure of lignin and bind to lignin to form sodium phenolate. This phenolic salt is polar so it is easy to be soluble in polar solvents. In the following figure is a figure of the chemical delignification process in a corn stalk sample.

Figure 5. Chemical delignification process

The dissolved lignin is marked in black colour in a solution called black liquor. The black liquor shows that the lignin layer has been separated from cellulose. This condition will increase the productivity of microorganisms in producing cellulase and the effectiveness of hydrolysis becomes higher (Sutarno, 2011).

Hemicellulose looks different in each treatment. In the condition of whole corn stalk, hemicellulose content is 10% then after reducing the size of 60 mesh, the level of hemicellulose is increased to 20% but after the delignification process, the hemicellulose level is decreased to 10%. Probably, this happens because the chemical delignification process using alkaline NaOH compounds causes hemicellulose contained in the material degrades to be cellulose. This happens since hemicellulose is soluble in alkaline compounds.

Hemicellulose has a lower molecular weight than cellulose and is resistant to heat treatment. Unlike cellulose, hemicellulose polyscharides are amorphous and the structure is less branched, so the potential for solubility is very different. The hemicellulose can be separated from cellulose by alkali because the bond is weak so it is easily hydrolyzed (Placket, 2011).

In the study (Fachry, 2013), the percentage of cellulose obtained was 30%–50%, hemicellulose was 15%–35% and lignin was 13%–30%.

Reducing sugar Level in the Enzymatic Hydrolysis Process

Hydrolysis is the process of parsing polysaccharides in lignocellulose biomass, namely cellulose and hemicellulose into the constituent sugar monomers. In perfect hydrolysis, cellulose will produce glucose, while hemicellulose produces several pentose sugar monomers (C_5) and hexose (C_6). Hydrolysis can be carried out chemically (acid) or enzymatically (Seftian, 2012).

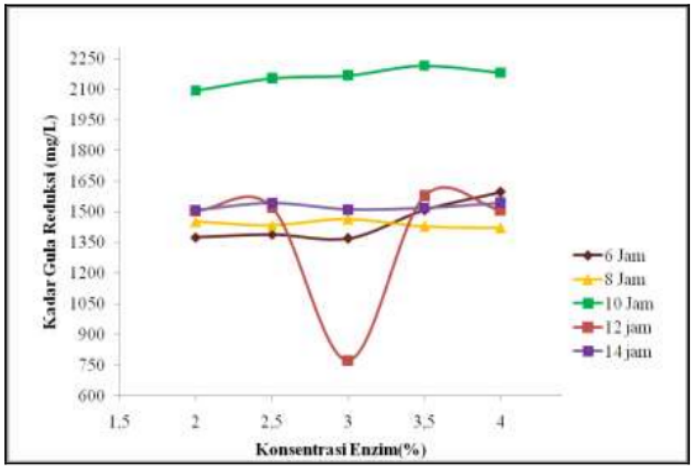

Figure 6. Analysis Graph of Reducing sugar Level to the Enzyme Concentration and Reducing sugar Level

In Figure 5.4 it can be seen in the graph that the results of reducing sugar level at 6 hours are directly proportional to the concentration of enzymes, this can be seen from the graph which continues to increase where the best reducing sugar is at concentration 4% which is 1596.7 mg/L but at the enzyme concentration 3% of the results of reducing sugar is decreased compared to the concentration of previous enzymes (2% and 2.5%). According to (Azis, 2012), one of factors that influence the hydrolysis process is pH (acidity), like proteins in general, the structure of the enzyme ion depends on the environmental pH. Enzymes can form positive ions, negative ions or charged ions (zwietter ions). Thus, changes in environmental pH will affect the effectiveness of the active side of the enzyme in forming substrate enzymes. Low pH or high pH can also cause a denaturation process which results in decreased enzyme activity. Therefore, enzymes have different optimum pH. In accordance with the statement, the condition of the reducing sugar level in the sample 3% is decreased because it is influenced by the condition of the environmental pH which was not in accordance with the optimum pH conditions of cellulase enzymes.

Whereas at 8 hours and 14 hours of the graph look more constant because it can be seen from each enzyme concentration has the reducing sugar level that is not too different where the best results are at 8 hours with a concentration of 3% which is 1464.43 mg/L , while for 14 hours the best result is at enzyme concentration 4%, which amounted to 1544.87 mg/L.

At the hydrolysis time of 10 hours, the reducing sugar level has the highest concentration. But for the time 10 with a concentration of 3.5% has the highest value for its reducing sugar level which is 2214.9 mg/L and the result is the most optimal result of several variables tested. And this result is in agreement with the study conducted by (Arif, 2016) who obtained optimum reducing sugar level with enzyme concentration 3% of 3.3458% at 8 hours. In addition, in the study of (Sutarno, 2011) using a mixture of Tricoderma ressei and aspergilus niger cellulase with a variation of the ratio of 2: 1 with a time of 8 hours resulted in the highest reducing sugar level of 30.884 mg/L.

For 12 hours, there was a significant reduction in reducing sugar levels at enzyme concentration 3%, if seen from the higher enzyme concentrations tendency of 3.5% and 4%, the decrease occurred due to a mismatch of pH with optimum conditions for the performance of cellulase enzymes.

So in general, it can be concluded from this study that the longer the hydrolysis time, the higher the yield of reducing sugar level. In addition, in this study, the best results are obtained on the variable of hydrolysis time of 10 hours and the concentration of 3.5% enzyme with reducing sugar level of 2214.9 mg/L.

Conclusions

Based on the results of observations and calculations that we have carried out during the study, following conclusions can be drawn

- After the corn stalks have undergone physical and chemical treatments using NaOH 10%, there is a decrease in lignin levels from 12% to 4% and there is an increase in cellulose levels from 40% to 75%.
- The use of concentration 3.5% in the hydrolysis process using cellulase enzyme is more optimal than the concentration of 2%, 2.5%, 3%, 4% with the results of reducing sugar level of 2214.900 mg/L.
- The longest hydrolysis time produced the highest reducing sugar level in the amount of 2214.900 mg/L, compared to the length of time 6, 8, 12 and 14 hours.

Suggestions

In further study, it is expected to pay more attention to factors that influence the enzyme hydrolysis process. pH meter is used to measure acidity more accurately.

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