The Influence of Temperature, Time and Initial Heating on Enzymatic Hydrolysis of a Used Paper to Produce Glucose

Fuadi, A.M*., Harismah, K*.
*Department of Chemical Engineering
University of Muhammadiyah,Surakarta, Jawa Tengah, Indonesia
am_fuadi@ums.ac.id

Abstract —**Glucose is one of materials that can be used to produce ethanol. Ethanol is an alternative fuel to potentially anticipate the reduction of petroleum oil. Discussion about the decline of Indonesia's oil reserves due to the rising demand of fuel oil has been frequently conducted. Related to this, a number of researches have been carried out to find alternative materials that can be used to produce fuel. In agricultural country, there are many waste of agriculture product. Agricultural wastes containing cellulose are materials that can be used for producing glucose; however, these materials still contain many impurities, such as lignin. The presence of lignin will inhibit the enzyme to hydrolyze cellulose, so the lignin must be removed first. This study investigates the possibility of utilizing paper waste to produce glucose. Paper waste is selected because the paper contains very high cellulose since some impurities such as a lignin have been removed during pulping process. The research was preceded by drying of paper waste to evaluate the water contain. After that, weighing 5 grams of dried waste paper and added by 200 mL of water was pulverized by using a blender. The water in this mixture was separated and the pulp was hydrolyzed using cellulose enzyme to obtain glucose. Enzymatic hydrolysis started by adding 0.7 g of cellulose enzyme, water and H_2SO_4 to maintain the pH to be 6 in the pulp. Hydrolysis was carried out at various temperature, namely at 45^{o}C, 40^{o}C, 35^{o}C and at room temperature. The results showed that at the beginning of the process, hydrolysis was carried out at high temperatures produced more glucose. However, when the process was continued, a process at higher temperatures decreases the rate of glucose production. Based on the research result, it can be concluded that hydrolysis of waste paper will produce the highest glucose, if hydrolysis is run at ambient temperature. Treatment by preheating for 1 hour at 40^{o}C can increase the yield of glucose.**

Key word: hydrolysis, paper used, enzyme

I. INTRODUCTION

Energy is a basic need for everyone. All of our activities consume energy. The one of energy resource that is widely used is fossil based oil. Consumption of oil is increase relates to the increasing of population and human activities. This trend will cause the problem, since is not followed by additional of oil reserves as an energy source. It is estimated that the availability of fossil fuels in Indonesia will be not sufficient by 2025. This issue has been often discussed in several events so it should be responded immediately. Indonesian's government gives attention to this issue seriously, so the decision of president according to energy security was generated, at 25 of January 2006 about preparing and utilizing of bio fuel as alternative fuel. Energy is not only government's responsibility but it is ours. According to this, we must anticipate how to solve this problem. Technology and innovation to explore the resource of energy is very necessary to support national energy security. Oil fossil is not renewable energy, so some time this energy will not be available any more. Studying to find the resource of the new renewable energy must be conducted. Cellulose is a carbohydrate; it is a polymer that is arranged from glucose monomer, so cellulose can be hydrolyzed to produce glucose. Containing cellulose in common wood is 40-50% [1]. Cellulose is the main part of the composition of woody plant, these materials found in all plant species until algae and fungi. Lignocelluloses in a wood and in common agricultural residue have variety composition. For instance hard wood consists cellulose of 40-55 %, soft wood is 45-50%, fresh bagasse is 33.4% and corn cob is 45 % [2]. All these resources can be processed to obtain glucose. Glucose is one of materials that can be used to produce ethanol. Cellulose formula is shown in Figure 1.

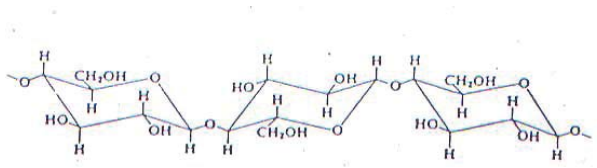

Figure 1. Structure of cellulose molecule

Indonesia is very abundant natural resources, including the availability of land and forests, so the opportunity to utilize cellulose as a raw material source of renewable energy is very strong. Furthermore, as an agricultural country, Indonesia has too many product of agriculture, so; there are many waste of agriculture product. Cellulose resources in Indonesia is too much, these are raw material of glucose which can be processed continuously to produce ethanol. Unfortunately, the utilizing of wood and agriculture waste as resource of cellulose need more complicated process because there are some impurities such as lignin. Lignin covers cellulose of

hydrolysis attack, so it must be avoided first. Lignin contain in lignocelluloses material is big enough. The containing of lignin in some lignocelluloses is presented in Table 1.

TABLE I
THE COMPOSITION OF LIGNOCELLULOSES OF COMMON AGRICULTURE RESIDUE AND WASTE

Lignocellulosic Material	Cellulose, %	Hemicelluloses, %	Lignin %
Hardwood	40-45	24-40	18-25
Softwood	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Paper	85-99	0	0-15
Newspaper	40-55	25-40	15-30
Grasses	25-40	25-50	10-30
Orchard Grass	32	40	47

Based on the Table 1, all of part of plant can be utilized to produce several organic chemicals. Sugar that is resulted from the cellulose and hemicelluloses can be fermented to be ethanol. Thus technology development for producing ethanol is focused in utilizing of residual lignocelluloses material because using sugar to produce ethanol directly impacts negatively of the economic side, because this process is much more expensive compared to fossil fuel. In Brazil, ethanol is produced from the fermentation of cane juice whereas in the USA corn is used [3].

Utilizing ethanol as a fuel has been practised in US as gasohol or oxygenated since 1980s. The containing of ethanol in this fuel is up to 10% by volume [4]. Using ethanol as a fuel has some advantages, because ethanol has more oxygen then fossil oil, so ethanol is essayer to be burnt as complete combustion, in result air pollution caused by combustion is minimize. Ethanol is not only renewable energy but also environmentally friendly energy. As renewable energy, ethanol can be produced from various raw materials as long as the material containing glucose as well as starch and cellulose. Grains, corn, potatoes and cassava are the material that contains a lot of starch, so can be made into ethanol. Americans are using corn as a feedstock for ethanol, while in Europe a lot of use of potatoes. The main substance in these materials is starch. Starch is polymer that is composed of α-D-glucose units, the same as cellulose starch can be hydrolyzed to produce glucose. In fact, the use of raw materials derived from foodstuffs can cause problems in the food supply, so it should be anticipated in order not to create the new problems.

Research to utilize lignocelluloses, agriculture and residual or waste of agriculture as a material to obtain glucose or ethanol has been conducted. Research using cassava as raw material to make bio ethanol has been done by Sukandar at all. This research was carried out to get the optimum condition in hydrolysis enzymatic of cassava using amylase enzyme from aspergilus niger ITB CC L74. Some variables that were studied are temperature, pH, and the percentage of enzyme volume. Optimum process was obtained at 4.5 of pH, and the temperature was 60^{o}C. The effectiveness of enzyme was increase relate to the increasing of enzyme percentage. However, the effectiveness of enzyme was limited by the amount of glucose, because glucose can inhibit the activation of enzyme [5]. Similar study to get glucose from waste rice water has been done. Hydrolysis was done by using amylase at 60^{o}C.

Research to improve the understanding and development of the manufacture of ethanol by using lignocelluloses material have been studied. The research utilized banana stalk waste as a materials. Material selection is based on the abundance of waste and not fully utilized. Furthermore cellulose contain in banana stalk is very high, that is about 65%. Even has high cellulose, lignin in banana stalk is still existing. The research was preceded by acid hydrolysis at various time and temperature to achieve the optimum process. Hydrolysis run at different temperatures are 80^{o}C .100^{o}C and 60^{o}C as well as a variety of time, which is 2 hours, 4 hours and 6 hours. The expectations, the results could support the national energy security, which can substitute the fuel oil up to 5% [6]. Lignin in banana stalk will cover cellulose from hydrolysis process, so this process was treated to remove lignin. Like pulping process, to remove lignin in lignocelluloses material must be done at high temperature. Absolutely this activity need more energy, so production cost will be more expensive.

Hydrolysis of rice straw to produce glucose was studied by Anwar N, at all. The rice straw is the one of agriculture wate, which contain 37.7% of cellulose, 22% of hemicelluloses and 16.6% of lignin [7]. Similar to the other hydrolysis process of lignocelluloses, the process is also initiated by delignification process. The existing of lignin in the material will cause contact between cellulase (enzyme) and cellulose is not complete, so hydrolysis is not effective, inconsequently the result of hydrolysis process is not maximum. Delignification process was conducted by using NaOH 2% at 85^{o}C for 6 hours. Enzymatic hydrolysis was done by using Fluka Biochemica enzyme. The more number of enzyme will produce more glucose.

Hydrolysis of lignocelluloses needs initial treatment to avoid the lignin. The one of material which has very high cellulose is paper. Table 1shows that paper is a material where its celluloses percentage is very high. Some lignin in raw material of paper has been removed in pulping process, especially in paper that made from chemical pulp, almost all lignin has been removed, so residual lignin in chemical pulp is almost zero. Like energy, paper is a material which is everyone needs it. Consumption of paper increase continuously, its impact the amount of waste paper also increases. Estimated, the waste of paper is about 10.11% of total waste. Just in Jakarta, the number of paper waste almost achieves 3,000 m^3 per day. Given that the papers contain very high cellulose, so paper used is very potential to be utilized as a material to produce glucose, that way this research try to make glucose from paper waste. This research studied the influence of temperature and initial heating to glucose result in hydrolysis of paper waste.

II. OBJECTIVE

Energy problems and garbage, either household waste or waste agricultural products, remains a serious problem that needs serious attention. Energy reserves are dwindling and the growing amount of waste that needs to be anticipated early. This research aims to contribute in overcoming the problem of energy and garbage. the selection of waste paper as raw material, as used paper is one of the components of the waste that has a high cellulose content, so if the garbage of paper can be used as raw material for making glucose, then there is a contribution to reduce the amount of waste, since the amount of paper used as garbage constituent very much. Thus, utilization of waste paper as a raw material for renewable energy can provide a significant contribution in support of energy security. This research studies the influence. This research studied the effect of temperature and preheating the process hydrolysis using the enzyme.

III. THEORY

A. Paper used

The main constituent in a paper is cellulose. Cellulose is a natural polymer of glucose. Cellulose chains are packed by hydrogen bonds which is called elementary fibrils. These elementary fibrils are then packed to form micro fibrils. These micro fibrils are then joining each other to form of bundles or macro fibrils [8]. In order to get maximum result from lignocellulosic hydrolysis, the bundle of lignocelluloses must be opened by process of pre-treatment, The level of cellulose content in a paper is variety; depend on the kind of pulping process which is used. Besides that, the level of cellulose content is also depending on the quality of the desired paper. To obtain high-quality paper, pulp manufacture is done in order to release lignin maximally, so containing of lignin minimal. Minimal lignin content will give the stability of paper, paper dose not easy to be yellowing. Paper that does not require high stability typically uses raw materials that still contain a lot of lignin, such as newspaper. Table 1 shows that the levels of cellulose in the paper. Cellulose content in newspaper ranges 40-55%, lignin 18-30%. Paper composition in a newspaper is almost the same as composition in raw material of paper. Pulping process to produce newspaper is done by mechanical pulping, in this process no lignin is removed, in result yield in this process is very high. Paper that resulted by chemical pulping has very high cellulose content, this is because when the pulping process, almost all the existing lignin is degraded until dissolved.. Moreover, the process continued with the process of bleaching. Existing residual lignin from the pulping process is released in the bleaching process, so it becomes the higher cellulose content. In paper-making process there is some stuffing materials which are added to improve the quality of the paper as desired. There are some paper products, each product has different content. Bleached chemical pulps are used for different types of products, but especially for paper products. Paper used has various containing of cellulose. The research utilized paper used that resulted from chemical pulping. Cellulose is the backbone of the fiber wall and the main contributor to paper strength. The quality of cellulose is affected by the length of cellulose chain. Cellulose can be classified in to three kinds, alpha cellulose, beta cellulose and gamma cellulose. This classification is based on the level of degree of polymerization. Alpha cellulose is the highest degree of polymerization, followed by beta and gamma. The higher degree of polymerization means the more its constituent glucose. The degree of polymerization of cellulose can be determined by measurement of the viscosity of solution of the cellulose. Fiber composed of cellulose, the carbohydrate structure is closely related to the crystalline of the fiber. These crystals are sometimes so tight that neither water nor enzyme can penetrate them. In order that the enzyme can penetrate well, then the crystal must be destroyed. It can be done by softening the cellulose. Paper is not only consist of cellulose, hemicelluloses is also exist. A certain amount of hemicelluloses is required for sufficient softening of the fiber wall during refining and sheet forming. However, too much hemicelluloses leads to brittleness after drying of the sheet. Thus, cellulose content has an optimum value [1]. As a material composed by cellulose and hemicelluloses, paper can be hydrolysed to obtain glucose.

$$\underset{\text{Cellulose}}{(C_6H_{10}O_5)_n} + \underset{\text{water}}{nH_2O} \longrightarrow \underset{\text{glucose}}{n\,C_6H_{12}O_6} \qquad (1)$$

B. Glucose

Initially, carbohydrates have the general formula$C_x\,(H_2O)_y$ analogous to hydrate and carbon. More widely, carbohydrates are polyhydroxy compounds are abundant in nature. These compounds can be in the form of small molecules, such as sugars, as well as a great unity in the form of polysaccharides. Sugar is the result of photosynthesis in chlorophyll which is a synthesis between CO_2 and water to form glucose and release oxygen according to the reaction:

$$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2 \qquad (2)$$

The sugar formed from this reaction is stored in plants as a source of energy, there are also stored in the form of polysaccharides, such as starch as a reserve of food, or in the form of cellulose and hemicelluloses as a source of strength to the cell wall. Carbohydrates can be classified into three, namely monosaccharide, oligosaccharides and polysaccharides. Glucose is included in the group of monosaccharide. Monosaccharide can be in the form of a glycoside, or it could be a constituent oligosaccharide units or it could be a polysaccharide constituent units. D-glucose is a monosaccharide that is most widely in nature. Glucose is monosaccharide, it cannot be hydrolysed to form the simpler of carbohydrates. Monosaccharide is important in the body, especially glucose. Glucose is formed from hydrolysis of complex carbohydrate such as starch, dextrin, hemicelluloses and cellulose [9]. Almost all of organism beings to process carbohydrates into glucose as the source of energy. Glucose is

raw material to make ethanol. Glucose is the raw material for making ethanol. Fermentation of glucose to ethanol in accordance with the equation:

$$C_6H_{12}O_6 \longrightarrow C_2H_5OH + CO_2 \quad (3)$$

C. Hydrolysis

Hydrolysis is the breakdown of a compound into simpler compounds with the aid of water. Hydrolysis process can break polymer compounds into compounds monomers. The hydrolysis of polysaccharides includes degradation process of lignocelluloses biomass, namely cellulose and hemicelluloses into sugar monomers constituent. The process of hydrolysis can be accelerated by adding either acid or enzyme. Hydrolysis also can be performed without adding acid or enzyme, but the process needs very long time.

At acid hydrolysis, acids that can be used in acid hydrolysis, among others, HCl, H_2SO_4, perchloric acid. Sulphuric acid is the most widely used on acid hydrolysis. Acid hydrolysis can be divided into two kinds of ways, i.e. dilute acid hydrolysis and concentrated acid hydrolysis. The concentrated acid hydrolysis is more widely used than the dilute acid hydrolysis, because it can convert more lignocelluloses. The mechanism of acid hydrolysis includes several stages. The hydrolysis process is begin by the interaction between protons in the acid with glycoside bond in lignocelluloses. Oxygen connecting two glucose molecules form a conjugate acid. Next, there were cuts CO bond and conjugate acid solution into the ring carbonium ion. The addition of H_2O will release glucose molecules and protons. The formed protons will interact again with the glycoside bond, and so on so that the whole glycoside bond reacts. The weakness of chemical hydrolysis process is: requires considerable energy, requires neutralization process and require corrosion resistant equipment. In consequently this process needs very high capital. Acid hydrolysis is also must be performed at high temperature. In general, the cellulose hydrolysis reaction that produces glucose written in Reaction (1). Dilute acid hydrolysis is known as two-stage acid hydrolysis, this method has been developed. The pieces of material put into the tank and heated by using the steam for 1 hour. The next step is the acid hydrolysis. Hydrolysis carried out at higher temperatures, but with a lower concentration of acid. The downside of dilute acid hydrolysis is the presence of sugar degradation and the formation of undesired side products. Degradation of sugar and side-products not only reduce yields of sugar, but a side-product can also inhibit the formation of ethanol in the subsequent fermentation step. Some inhibitor compounds that can be formed during dilute acid hydrolysis process is furfural, 5-hydroxymethylfurfural (HMF), levulinik acid (levulinic acid), acetic acid (acetic acid), formic acid (formic acid), uronat acid (uronic acid), acid 4-hydroxybenzoic acid vanilik (vanilic acid), vanillin, phenol, cinnamaldehyde, formaldehyde (formaldehyde), and several other compounds [8]. Hydrolysis can be accelerated by enzyme; this process is called enzymatic hydrolysis. Enzymatic hydrolysis. Enzymes are biological catalysts that can accelerate biochemical reactions. Enzymes have outstanding performance, such as 1 part amylase enzyme capable to hydrolyse 20000 section of starch [9]. Enzyme is a protein catalyst, which speeds up the rate of biochemical reactions. As a catalyst, enzyme will decrease activation energy. Enzyme performance is affected by several conditions including temperature, pH, etc. Enzymes are not resistant to high temperatures, at high temperatures the enzyme activity will decrease. Enzyme hydrolysis is usually carried out at low temperature, this is one of the advantages of enzyme hydrolysis than acid hydrolysis because it is more energy efficient. However, the enzymatic hydrolysis also has a weakness, which takes a long time. Enzymatic hydrolysis takes a few days, while acid hydrolysis requires only a short time [10]. General comparison between dilute acid hydrolysis and enzymatic hydrolysis shown in Table 2 [11]

TABLE 2. ADVANTAGE AND DISADVANTAGE OF DILUE ACID AND ENZYMATIC HYDROLYSIS

Comparison of	Acid hydrolysis	Enzymatic hydrolysis
Mild hydrolysis condition	No	Yes
High yield	No	Yes
Product inhibition during hydrolysis	No	Yes
Formation of inhibitory by-product	Yes	No
Low cost catalyst	Yes	No
Short time of hydrolysis	Yes	No

The main disadvantages of enzymatic hydrolysis are reaction time and the prise of enzyme. Enzyme that usually used for enzymatic hydrolysis of lignocelluloses is cellulase. Cellulase acts collectively to hydrolyse cellulose from agriculture waste. Cellulase enzyme can be produced by using some fungi for example *aspergillus niger* and utilizing of waste agriculture. Sample were collected from farmlands after harvest. Aspergillus niger intended for captive breeding using PDA media, during the process of breeding, all the equipment used should be kept to avoid contamination with other fungi [12] the similar research to obtain enzyme α-amilase was done. This research used Aspergillus niger L74 and Aspergillus Oryzae. Enzyme obtained was used to hydrolyse starch [13].

IV. METHOD

A. Material and Equipment

The materials used in this study were the material to be processed, the materials used to hydrolyze and the substance to measure glucose result. The raw material hydrolyzed was paper used. The selection of raw materials, because of high cellulose content and low lignin. Chemicals substance needed for hydrolysis include enzyme, H_2SO_4, Nelson solution. The equipments were vacuum pump, blender, heater, Erlenmeyer

glass, thermometer, pH meter, spectrophotometer, magnetic stirrer, and balance.

B. Performing Research

Research started by measuring the moisture of paper used. Then take paper used equivalent to 5 gram of dry paper. This paper then added by water so total water in the mixture is 200 mL. This mixture, water and paper, was softened using blender in order crystallinity of cellulose decrease. Water and pulp paper was separated. The pulp obtained was weighed, then hydrolysis process is done. The treatment of the hydrolysis process can be divided into three kinds, namely the process of hydrolysis at various temperatures and up to 24 hours, extending hydrolysis time up to 66 hours, hydrolysis with preheating. Hydrolysis processes performed by the addition 0.7 gram of enzyme at 6 of pH, hydrolysis by initial heating.

V. RESULT AND DISCUSSION

A. Hydrolysis at various temperature and time up to 24 hours. The result is presented on Table 3A.

TABEL 3A. THE INFLUENCE OF TEMPERATURE AND TIME ON THE HYDROLISIS RESULT

Temperature K	Glucose resulted (mg/100mL), at various time (hour)			
	0	4	20	24
Surrounding	0	19,81	41,09	108,06
308	0	35,32	36,78	105,79
313	0	33,01	35,43	61,13
318	0	32,11	33,53	44,53

Based on the Table 3A, can be made the figure relationship temperature and time to the hydrolysis result, that can be seen on Figure 2.

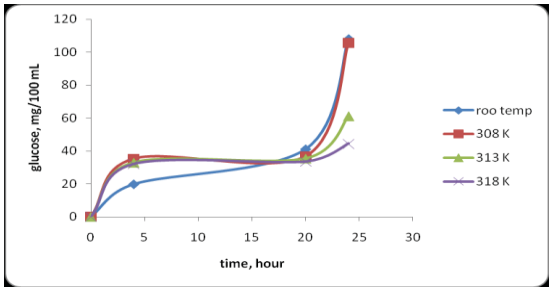

Figure 2. Relationship between time, temperature and resulting glucose

Table 3A and figure 2 show that hydrolysis up to 4 hours, hydrolysis at308 K produce the highest glucose, the lowest glucose was resulted when hydrolysis conducted at room temperature. Hydrolysis at 313 K and 318 K were lower than at 308 K, this trend is caused by activation of enzyme decreases because of increasing the temperature. Basically, increasing temperature will increase reaction rate, but reaction rate is also affected by energy activation, higher energy activation means lower reaction rate. To know how the effect of temperature on the decrease in enzyme activity, hydrolysis reaction was extended up to 66 hours. Hydrolysis results are shown in Table 3b and Figure 3.

TABEL 3B. THE INFLUENCE OF TEMPERATURE AND TIME ON THE HYDROLISIS RESULT

Temperature K	Glucose resulted (mg/100mL), at various time (hour)				
	28	44	48	52	66
Surrounding	121,32	187,75	155,93	210,23	230,64
308	131,65	150,17	152,17	155,31	175,24
313	63,95	67,81	121,97	131,37	157,75
318	50,26	52,82	86,89	90,52	97,22

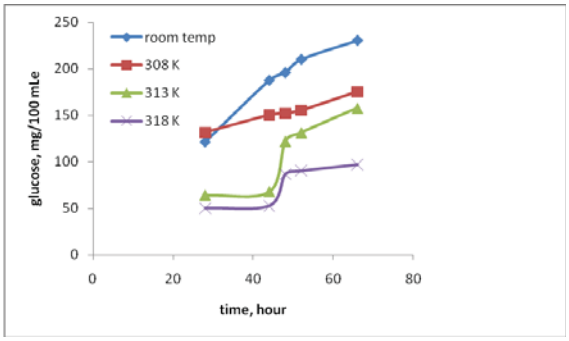

Figure 3. The influence of temperature on glucose result

Table 3B and Figure 3 support the statement that the influence of temperature on the reducing of enzyme activity is more dominant rather than effect temperature to accelerate the reaction rate. At the beginning process, the difference result of hydrolysis between hydrolysis at low temperature and at high temperature was not significant, but when the process was continued up to 66 hours, the difference result was significantly. This trend may be caused by too long heating. To make sure is it affected by too long heating or not, the research was continued by initial heating at 40°C for 1 hour, then the heater was switched off. The affect of initial heating to glucose result presented in figure 4.

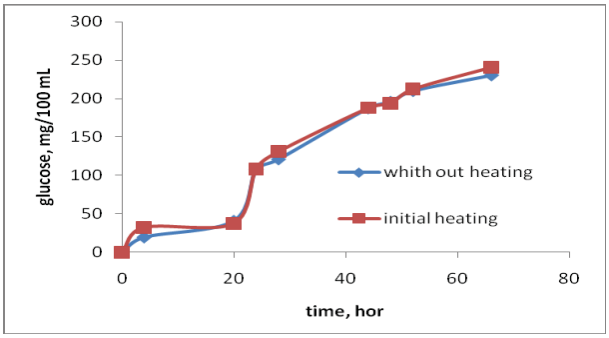

Figure 4. The influence of initial heating on enzymatic hydrolysis

Figure 4 shows; initial heating up to 1 hour has not caused a decline in the activity of the enzyme, even obtaining of glucose increased slightly.

VI. CONCLUSIONS

Based on the research result obtained, it can be concluded that:

a. Paper used can be utilized as raw material to produce glucose.
b. Long heating will decrease the activity of the enzyme, although the temperature is not too high.
c. Hydrolysis at surrounding temperature resulted in the highest glucose.
d. Initial heating up to 1 hour at 40^{o}C did not cause in reduced enzyme activity.
e. Enzymatic hydrolysis requires a long time; more research needs to be done to shorten the reaction time.

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