Delignification and Hydrolysis Lignocellulosic of Bagasse in Choline Chloride System

R Manurung 1, A Syahputra, M A Alhamdi, W Satria, E M Barus, R Hasibuan and M Z Siswarni
Chemical Engineering Department, Faculty of Engineering, University of Sumatera Utara Jl. Almamater Kampus USU, Medan 20155, Indonesia.
1 renitachem@yahoo.com

Abstract. Bagasse was the waste which has a fairly high content of lignocelluloses and has not been utilize optimally. With a cellulose content of up to 40%, bagasse then potentially be used as raw material for bioethanol. In this research, delignification process was carried out using sodium hydroxide (NaOH) in the ionic liquid system and without ionic liquids. The purpose of this research was to find out the highest content of cellulose which contained in the bagasse and the best hydrolysis conditions was obtained at the hydrolysis process in the choline chloride (ChCl) system. The hydrolysis stage in this research was carried out at temperature 105 °C, catalyst (H2SO4) 10% (w/w) cellulose, ChCl 10%, 15%, and 20% (w/w) cellulose and it was stirred at constant speed 120 rpm with reaction time of 30, 60 and 90 minutes. Delignification research results used ChCl obtained highest content of cellulose was 39.8%, hemicellulose 18.59%, and lignin 3.62% in cooking treatment 90 minutes and 20% ChCl. While delignification without ChCl obtained highest content of cellulose is 24.98%, hemicellulose 8.25%, and lignin 18.99% in cooking treatment 90 minutes. The maximum glucose yield of 39.4% was obtained at reaction time 90 minutes and 15% of ChCl.

1. Introduction
Sugarcane is a plant grown to be raw materials of sugar and vetsin. This plant can only grow in tropical climates. Sugarcane is of an herbage kind. The age of the plant between planting and harvesting is up to approximately 1 year. In Indonesia, the cultivation of sugar cane is done mostly in Java and Sumatra. The area of sugarcane plantations in Indonesia reaches 398,260 Ha. The process of sugarcane cultivation would produce 5% sugar, 90% bagasse and the remainder in the form of drops (molasses), and water [1]. Bagasse is one of the bioethanol raw materials that contain lignocellulose. The utilization of bagasse is still in the development process. Generally, bagasse is used for fuel, paper raw material, brake pad raw material, mushroom industry and others. Bagasse is possible to be utilized as an alternative energy source such as bioethanol or biogas. Bagasse contains 52.7% cellulose [2]. In the process of manufacturing bioethanol, lignocellulose delignification is the first phase to be done to break the bond between cellulose, hemicellulose, and lignin. Delignification process is a process of removing lignin from the raw material in order that the result of this process contains cellulose with adequate purity [3]. Cellulose delignification with Ionic Liquid media is more effective than the process without Ionic Liquid media. Ionic liquids are salts that are liquid at room temperature. This happens because the ionic liquid reduces the degree of crystallinity and increases the sample porosity so it becomes easier to delignify the bagasse [4]. Delignification of cellulose in Ionic Liquid media is more effective than without Ionic Liquid. Ionic liquids are salts that are at room temperature in the liquid form. This is because the ionic liquids have decreased the degree of crystallinity and increased the porosity of the samples making it easier to delegate fruit empty bunch.
Hydrolysis plays an equally important role in bioethanol manufacture. Bagasse has the potential to be used as a source of glucose through a process of hydrolysis by using acids or enzymes. The resulting sugar solution can then be converted into various products such as alcohol, which owns a higher economic value. Lignocellulose hydrolysis with dilute acid is applied as the most common way to obtain sugar. Ionic liquids can generally be used at room temperature and does not produce toxic side reactions [4]. Choline chloride ionic liquid is a weak acid with low level toxicity and environmentally friendly. Choline chloride (ChCl) under the name IUPAC 2-hydroxy-N, N, N-trimethylethanaminium chloride or (2-hydroxyethyl) trimethylammonium chloride is one of the most widely used ammonium salts for DES formation because ChCl is cheap and can be easily extracted from biomass [6]. Initially, ChCl was used because when ChCl is mixed with most hydrogen bonding donors or metal halides, the physical properties of ChCl are almost always greatly increased compared to the use of other quaternary ammonium salts and the freezing depression tends to be one of the largest. An example is a mixture of ChCl: urea with freezing points of 303 °C and 135 °C, this mixture also shows lower viscosity than most other systems containing different ammonium salts and the conductivity is often higher when using ChCl [7].

2. Research Methodology

2.1. Materials
Raw material used in the research is bagasse. The chemicals are aquades (distilled water), choline chloride, sodium peroxide 3%, sulfuric acid 72%, cellulose from sulfuric acid (H₂SO₄) delignification 97%, potassium iodide (KI 20%), sodium thiosulfate (NH₄)₂HPO₄, starch indicator, Luff solution, Aquadest (H₂O).

2.2. Delignification Procedure by Using NaOH without Ionic Liquids
Bagasse powder is weighed 30 grams, and then put in a beaker, 150 ml of NaOH solution is added to the beaker containing powder bagasse. Then, the mixture is stirred equally until powder is soaked. The powder is soaked in NaOH 3% solution during a specified time. The powder that has been soaked with NaOH 3% is washed with water and dried at 105 °C for 16 hours before use.

2.3. Delignification Procedure by Using NaOH with Ionic Liquids
The bagasse powder is weighed 30 grams, and then put into a beaker. Then, 150 ml of NaOH 3% solution is added to the beaker containing bagasse powder. Then, the mixture is stirred equally to soak the powder for 90 minutes. The powder that has been soaked with NaOH 3% is then washed with water and dried at 105 °C for 16 hours before use. The powder is sifted, mixed with choline chloride ionic liquid, heated to a temperature of 130 °C for a specified time. After that, the powder is washed with water to remove ionic liquid to obtain cellulose. Wash it again with water, and then put it into the oven until the weight is constant. Cellulose, hemicellulose and lignin analysis are done with Chesson-Datta method.

2.4. Hydrolysis Procedure
An amount of 10 grams of cellulose from bagasse delignification is put into 250 ml Erlemeyer. It is then added with concentrated sulfuric acid as much as 10% of the sample weight that has been diluted by distilled water and choline chloride ionic liquid in a determined amount into Erlemeyer 250 ml. The erlenmeyer is then closed with cork and heated at 105 °C while stirred with a magnetic stirrer during a determined time. Then, pH of hydrolyzed sample is measured to achieve a pH of 7. After that, the hydrolysis is filtered using filter paper to obtain polysaccharide filtrate. Then, the glucose level is analyzed with Luff Schoorl method.
2.5. Analysis of Glucose
Weigh as much as 2 grams of sample and put it into a 250 ml measuring flask, add water and homogenize. Add 5 ml of half alkaline Pb-acetate and shake. Add one drop of (NH₄)₂HPO₄ 10% solution. Shake and match the contents of flask up to the mark with distilled water boundary, shake 12 times and filter. Pipette the filtered solution and put in a 500 ml Erlenmeyer. Add 15 ml of distilled water and 25 ml of Luff solution and some boiling stones. Connect the erlenmeyer with a cooler, heat over an electric heater to boil for 10 minutes. Heat continuously for 10 minutes, and then remove and cool. Once being cool, add 10 ml of KI 20% solution and 25 ml of H₂SO₄ 25% solution. Titrate it with 0.0990 N of tio solution with 0.5% starch solution as an indicator. Make blank solution by using 25 ml of water and 25 ml of Luff solution in the same way without the use of sample solution.

3. Results and Discussions

3.1. Effect of Choline Chloride (ChCl) Amount and Delignification Time to the Remained Lignin Level in Holocellulose
The process of bagasse delignification is conducted by using NaOH in choline chloride (ChCl) ionic liquid system in a wide variation of ChCl amounts: 10%, 15%, and 20% of bagasse weight. The result shows that the different number of ionic liquids affects the production of lignin. Figure 1 shows the relationship between levels of remained lignin in holocellulose with ChCl amount and delignification time. In the process of delignification, bagasse in ChCl system is expected to produce cellulose with low lignin level. Figure 1 shows that the lignin content decreases as the number of ChCl rises. Decreased level of lignin is caused by a long cooking time that triggers the base group degradation of cooker solution that attacks alpha and beta lignin, with the result that unstable degradation triggers condensation. Condensation causes the loss of lignin bond from hemicellulose and cellulose. Because ChCl is acid, acidic atmosphere tends to increase the molecular weight of lignin. This event causes the lignin to sediment and dissolve in water during the washing process. However, the ChCl number more than 15% makes remained lignin level in holocellulose constant on 90 minutes as a result of the addition reaction of hydroxyl group causing lignin not to dissolve in washing water [8]. Lignin produced is about 3.62%.

![Figure 1. The Effect Between ChCl and Delignification to Remained Lignin Level in Holocellulose](image)

3.2. Effect of Total Choline Chloride (ChCl) and Delignification Time towards Cellulose and Hemicellulose Levels
The following is a graph showing the relationship between levels of cellulose and hemicellulose and CHCl amount and timing delignification.
Figure 2. The effect of the amount of ChCl and Delignification Time Towards Cellulose Level

Figure 3. The effect of the amount of ChCl and Delignification Time Towards Hemicellulose

It can be seen in Figure 2 and Figure 3 that the levels of cellulose and hemicellulose increase with the rise of the number of ChCl and delignification time. Increased levels of cellulose and hemicellulose are caused by the lignin bond detachment from biomass due to a degradation of the alpha and beta lignin, so that cellulose and hemicellulose increase. However, the cellulose produced by ChCl amount is more than 15% due to degradation in polysaccharide groups, cellulose and hemicellulose, contained in the raw material. The highest cellulose level is 39.8% of the weight of bagasse, obtained in ChCl 20% and 90 minutes of time. While the highest hemicellulose level is 18.59%, obtained in 20% ChCl and 90 minutes.

3.3. Comparison of Remained Lignin levels in Delignified Holocellulose With and Without the Use of ChCl

The lowest remained lignin level in holocellulose produced using ChCl is about 3.62%, while the lowest remained lignin content in holocellulose produced without using ChCl is up to 18.99%.

Figure 4. Comparison of Remained Lignin levels in Delignified Holocellulose With and Without the Use of ChCl

Lignin level decreases significantly with the increase of ChCl amount and delignification time, whereas lignin level without ChCl only slightly decreases that can be seen in Figure 4. The significant decrease of lignin is resulted by the double work between ChCl and NaOH that detach the lignin bond from raw materials to increase the wasted lignin compound. The remained lignin level in holocellulose is less. However, the remained lignin level in delignified holocellulose uses NaOH without bigger ChCl. This happens without the help of ChCl.

3.4. Comparison of Delignification Process With and Without ChCL Towards Cellulose and Hemicellulose Levels

Figure 5 and 6 show the effect of the ratio between the use of ChCl 15% and without ChCl towards cellulose and hemicellulose levels. In the process of bagasse delignification, NaOH and ChCl work
together as a cooking solution to detach lignin bond. ChCl helps NaOH increase levels of cellulose and hemicellulose.

Figure 5. Comparison of Delignification Process With ChCl and Without ChCl Towards Cellulose Level

Figure 6. Comparison of Delignification Process With ChCl and Without ChCl Towards Hemicellulose Level

It can be seen in Figure 5 that the cellulose content increases significantly by using NaOH in CHCl ionic liquid systems, while the cellulose level obtained without ChCl does not increase significantly, even relatively constant. This is due to the acidic ChCl resulted in the degradation of the building compound of lignin that the cellulose level increases. While the level of cellulose obtained without ChCl increases slightly because NaOH is an alkaline base which has relatively small ability to dissolve organic and inorganic compounds.

In the study, researcher obtains cellulose level of 39.8% at 90 minutes with ChCl 20%, while the highest content of hemicellulose is 18.59% at 90 minutes with ChCl 20%.

3.5. Effect of Reaction Time Change and Concentration of Choline Chloride Towards Glucose Level

Bagasse hydrolysis process is conducted in the choline chloride ionic liquid system with ChCl variation of 10%, 15% and 20% of bagasse weight and variations hydrolysis time 30, 60 and 90 minutes. The highest number of glucose produced in the reaction time of 60 minutes with CHCl 20% by weight of bagasse is 39.4%. The analysis shows that the different number of ionic liquids affects the amount of glucose produced. Figure 7 shows the relationship between glucose levels with the amount of CHCl and hydrolysis time.

Figure 7. Effect of Reaction Time and Concentration of Choline Chloride Towards Glucose Level

As seen in Figure 7 that the glucose level increases with the increase of hydrolysis reaction time and concentration of choline chloride. In this study, for an increase of concentration of choline chloride from 10% to 20%, there is an increase in the percentage of glucose levels: 2.1% in 30 minutes, 2.7% at 60 minutes, and 2.4% at 90 minutes. As for the amount of choline chloride 20%, for an increase in hydrolysis time between 30 minutes to 90 minutes, there is a fluctuating glucose level. The more the increase of the reaction time, the more the glucose levels are generated and until at a certain time limit there will be obtained the maximum glucose level. This is due to the contact between the substances
that can react much longer and when the time is extended, the increase of glucose levels becomes very small even decreases. If reaction time is longer, it will be insoluble in water and therefore the breaking of polysaccharide chains into glucose cannot take place properly and can possibly damage the glucose produced as a result of continuous warming. ChCl is able to accelerate the hydrolysis reaction and increase the conversion of cellulose and lignocellulose into sugar. The ionic liquid reacts with water and can help bind hemicellulose in order that the hemicellulose is not taken away with cellulose to form good glucose compound and also because of its environmentally friendly fluid properties, it can reduce the concentration of sulfuric acid catalyst, making it safe for the environment and does not corrode the appliance.

3.6. Comparison of Hydrolysis Process Using Ionic Liquids and Without Using Ionic Liquids

Optimum glucose level is obtained at 60 minutes reaction time condition with the amount of glucose 39.4% in ChCl 20% by weight of bagasse. As for the hydrolysis process without the use of ionic liquids, the glucose level obtained at 90 minutes is 30.87% with a 10% concentration sulfuric acid. In this study, there is an increase in the percentage of glucose levels to be 6.4% at 30 minutes, 5.2% at 60 minutes, and 4.1% at 90 minutes with the use of 20% of ChCl bagasse weight as shown in Figure 7. It can be seen in Figure 8 that the glucose level increases with the increase of reaction time and the highest glucose level is obtained by using ionic liquids. Therefore, based on Figure 4, the optimum glucose level is obtained at 90 minutes of reaction time with glucose level of 39.4% and 15% of ChCl bagasse weight. As for the use of sulfuric acid 10%, glucose level is less in the same condition of 90 minutes that is 30.87%.

![Figure 8. Comparison of Glucose Hydrolysis Process Using Ionic Liquids and Without Using Ionic Liquids](image)

4. Conclusion

In bagasse delignification process with ChCl, cellulose level is best obtained when the ChCl use is 20% at 90 minutes where the result of cellulose level is 39.80%. The use of ChCl 20% of the bagasse weight is sufficient to the occurrence of degradation of the cooking solution base group that attacks alpha and beta lignin, which increases the cellulose level. The highest glucose level is 39.4%, obtained in 90 minutes hydrolysis time condition with 15% ionic liquid choline chloride concentration, whereas 30.87% glucose level is obtained without the use of choline chloride ionic liquid.

5. References

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