Development of lignocellulose-based bioethanol from chrysanthemum flower waste (Chrysanthemum sp.)

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Abstract. The fuel crisis has given a sign that the Indonesian fossil energy reserve is low. Conversely, fuel consumption in the country has been increasing from year to year. The limited resources of fossil energy cause the need for renewable energy development and energy conservation. One form of renewable energy is biomass energy. The biomass energy source can be derived from waste biomass plantations and agriculture. Chrysanthemum flower waste is biomass used as a raw material in bioethanol manufacturing because it has a high cellulose content. This study aims to determine the conditions of the hydrolysis and fermentation processes that produce the highest amount of bioethanol and determine the quality of bioethanol from chrysanthemum waste. The method used in this research was acid hydrolysis with a variation of sulfuric acid concentration at 0.5%, 1%, 1.5%, and 2% with a deviation of hydrolysis time at 60 minutes and 90 minutes. The type of yeast in the fermentation process was Saccharomyces cerevisiae at 5%, 10%, 15%, and 20% w/w, with fermentation times used were 3, 5, and 7 days. Based on the research that has been done, the highest ethanol content was 13.53% produced in the hydrolysis treatment using 1% H2SO4 concentration with 60 minutes of hydrolysis time and five days of fermentation time with 10% yeast content. The test results for the quality of bioethanol from chrysanthemum flower waste were ethanol content of 13.53%, density 0.981 g/mL, a specific gravity of 0.981, API gravity of 12.711, and a calorific value of 10,422.2 kcal/kg.

1. Introduction
The level of world oil production is decreasing while the consumption of petroleum and fuel oil is increasing. In the future, it is expected that the trend of energy development will shift from fossil-based energy to new renewable energy, one of which is bioethanol [1]. One of the efforts and solutions to reduce dependence on energy sources derived from fossils and provide alternative solutions to the above problems is developing renewable energy sources. Bioethanol is an alternative energy source that can reduce pressure due to high world oil prices. Bioethanol can be used as a gasoline or premium mixture to minimize the use of gasoline. Bioethanol is expected to replace fuel oil in motor vehicles and can reduce air pollution previously caused by motorized vehicles.

Lignocellulose can be used as an alternative material for making bioethanol [2,3]. Lignocellulosic waste is a second-generation raw material in the manufacture of bioethanol. The availability of abundant lignocellulosic waste materials does not threaten the balance of food, and feed availability makes it an ideal raw material source for producing ethanol. Therefore, it is necessary to develop bioethanol from materials that are not a food source for the community, namely lignocellulosic materials.

Chrysanthemum is one of the ornamental plants that have high economic value [4]. The many types, shapes, and colors of chrysanthemums make chrysanthemums very popular in the community. Indonesia
is one of the chrysanthemum exporting countries. When viewed from the production position, the number of chrysanthemum stalks and non-chrysanthemum ornamental plants in 2011-2015 in Indonesia shows that chrysanthemum commodities always occupy the highest rank. Production of chrysanthemums in 2015 reached 442,698,194 stalks, while the tuberose commodity was only 116,687,423 stalks, the rose commodity was 188,302,152 stalks, and the gladiolus commodity was 2,552,060 stalks [5].

The high production and demand for chrysanthemums resulted in a high amount of waste generated. Chrysanthemum is a commodity that has a short shelf life and is easily damaged. Therefore, the waste generated from these flower commodities is quite a lot. Chrysanthemum flower waste is a lignocellulosic material available in large quantities and has not been used optimally in Indonesia. At the same time, the cellulose content of chrysanthemum flowers is quite a lot and can be used as raw material for bioethanol. This study used a sample in the form of chrysanthemum crowns because the cellulose content of chrysanthemums, especially chrysanthemum crowns, was relatively high, namely 24.51%. Moreover, the lignin content was lower than other parts of chrysanthemums, such as stems and leaves, so that the chrysanthemum crowns were more easily hydrolyzed.

2. Materials and method

2.1. Materials

2.1.1. Tools. The tools used in this research include beaker, Erlenmeyer, measuring cup, measuring flask, test tube, pipette, fermenter bottle, cup, stirrer, blender, analytical balance, oven, autoclave, sieve, spectrophotometer, aluminium foil, pycnometers, electric stoves, pH-meters, thermometers, filter paper, a series of fermentation equipment, a series of distillation equipment.

2.1.2. Materials. Waste of chrysanthemum crown, H$_2$SO$_4$, yeast, aquadest, NPK, urea, granulated sugar.

2.2. Method

Making bioethanol from chrysanthemum crown waste (CCW) in this study used several variations of treatment. The hydrolysis process used variations of 0.5%, 1%, 1.5%, and 2% H$_2$SO$_4$ concentrations with hydrolysis times of 60 minutes and 90 minutes. The choice of concentration variation is because of all the catalysts; weak sulfuric acid (0.5-1.5%) is the most widely used in the industrial world. It can hydrolyze most sugars from hemicellulose by 75-90% [6]. In addition, the choice of concentration variations was based on previous studies using hydrolysis times of 60, 90, 120, 150, and 180 minutes with concentrations of H$_2$SO$_4$ of 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% [7].

2.2.1. Lignin-cellulose analysis and CCW preparation. Lignin and cellulose tests were carried out on the crown and leaves of chrysanthemum flowers. At the beginning of the study, the measurement of lignin and cellulose aimed to determine the part of the chrysanthemum flower used based on the levels of lignin and cellulose in the chrysanthemum flower. Tests for the lignin and cellulose content of chrysanthemum flowers were conducted at the Integrated Research and Testing Laboratory, Gadjah Mada University. CCW is dried by drying in the sun for 24 hours. After that, the dry samples were checked for lignin and cellulose content. For the preparation stage, CCW was dried using an oven at a temperature of 105°C to a constant weight. Then the sample was blended until smooth and filtered using a sieve.

2.2.2. Hydrolysis process. Acid hydrolysis was carried out at a temperature of 100°C with variations in the concentration of H$_2$SO$_4$ as much as 0.5%, 1%, 1.5%, 2%, and with variations in time of 60 and 90 minutes. The material is then neutralized and sterilized. Neutralization is used by using a solution of NaOH or H$_2$SO$_4$ to balance a pH that is too acidic or basic. Sterilization is carried out by heating at high temperatures.
2.2.3. Fermentation process. Before the fermentation process, the hydrolyzate was neutralized and sterilized at 121°C for 15 minutes. Then the fermentation was carried out at room temperature using fermentation time with variations of 3, 5, and 7 days. The usual fermentation time for making bioethanol is 3-14 days [8,9]. Fermentation is carried out under anaerobic conditions because Saccharomyces cerevisiae grows better in aerobic conditions. Saccharomyces cerevisiae will ferment sugar much faster in anaerobic conditions [10]. The yeast used in this study was yeast grown in a growth substrate. The growth substrate consisted of 1000 mL of distilled water added to 100 g of granulated sugar (10% sugar concentration) prepared in a beaker [11]. However, only 100 mL of distilled water was added in this study, and 10 g of sugar was added because the inoculum requirement was not too much. The fermentation process used various starter concentrations of Saccharomyces cerevisiae, 5%, 10%, 15%, and 20%. In addition to the addition of the starter, the fermentation process also added supporting materials, namely NPK and urea. Urea serves as a nutrient for yeast. Urea added as much as 0.5% of the sugar content in the fermentation solution. Adding 0.1% NPK of the sugar content in the fermentation solution serves as a nitrogen source for yeast to grow correctly.

2.2.4. Distillation process. After the sample has been fermented, the fermented liquid is then distilled using a series of distillation equipment. After the fermentation is complete, it can continue the following process, namely distillation. However, before entering the distillation process, the sample was pasteurized by heating at 80°C for 10 minutes to kill bacteria [12]. Distillation or distillation is a method of separating chemicals based on differences in the speed or ease of evaporation of a material. In distillation, a mixture of substances is boiled to evaporate, and this vapor is then cooled back into liquid form. The substance with the lower boiling point will evaporate first. The application of this process is based on the theory that in a solution, each component will evaporate at its boiling point. Ethanol or ethyl alcohol is a chemical compound with a boiling point at a temperature of 70-78°C. The distillation process is maintained at around 79-81°C because the bioethanol has evaporated at that temperature, but the water does not evaporate [13].

3. Result and discussion

3.1. Lignin and cellulose content
Lignocellulose is a component of plant cell walls. Lignocellulosic materials generally consist of cellulose, hemicellulose, and lignin. Cellulose is naturally bound by hemicellulose and protected by lignin. The presence of lignin binding compounds makes lignocellulosic materials difficult to hydrolyze [14]. The test results are shown in table 1.

| Flower parts | Component | Content (%) |
|--------------|-----------|-------------|
| Crown        | Lignin    | 12.14       |
|              | Cellulose | 24.51       |
| Leaves       | Lignin    | 23.03       |
|              | Cellulose | 21.55       |

The cellulose content in the chrysanthemum crown was 24.51%, and in the chrysanthemum leaf, 23.03%. Cellulose can be converted into products of higher economic value such as glucose, ethanol, and animal feed by hydrolyzing cellulose with the help of cellulase as a biocatalyst or by acid/base hydrolysis. The lignin content of the chrysanthemum crown was 12.14%, and the chrysanthemum leaf was 21.55%. Lignin is the main part of plant cell walls which is the most abundant polymer after cellulose. The hydrolysis process at high temperatures can help release lignin from cellulose and
hemicellulose and break down lignin into smaller particles. The release of lignin from the cellulose and hemicellulose matrix causes the cellulose and hemicellulose to be hydrolyzed faster to increase the sugar content [15].

From the lignin and cellulose testing results, it can be seen that the content of chrysanthemum crown cellulose is greater than the chrysanthemum leaf cellulose content. Still, the chrysanthemum crown lignin content is smaller than the chrysanthemum leaf lignin content. Based on this, it can be concluded that the sample to be used is CCW. The higher the cellulose content, the better the material because cellulose will be converted into reducing sugar. In addition, the lower the lignin content, the easier the sample is to be hydrolyzed.

3.2. Flower waste preparation
In this study, the chrysanthemum crowns were sorted dried first to obtain a constant dry weight. The purpose of drying is so that the chrysanthemum crown is easier to process in the following process. After drying, the chrysanthemum crown then goes through a size reduction process using a blender. The dried flowers will make it easier to blend the flowers into powder. This size reduction cannot remove lignin but aims to reduce the size of the substrate and increase the surface area of the absorption area to increase the contact between the substrate and the acid so that it is easier to hydrolyze.

3.3. Hydrolysis using sulfuric acid
Hydrolysis is the process of breaking down polysaccharides in lignocellulosic biomass, namely cellulose and hemicellulose, into their constituent sugar monomers. This process aims to break lignin bonds, remove lignin and hemicellulose content, damage cellulose's critical structure, and increase the material’s porosity [6]. Damage to the crystalline structure of cellulose will facilitate the breakdown of cellulose into glucose. In addition, hemicellulose also decomposes into simple sugar compounds: glucose, galactose, mannose, hexose, pentose, xylose, and arabinose.

The hydrolysis process in this study used the dilute acid hydrolysis method with H₂SO₄. The advantages of acid hydrolysis are faster reactions, more reducing sugars, the temperature can be conditioned with various ranges, and it is cheaper than enzymes [15]. Dilute acid hydrolysis also has the disadvantage of producing certain compounds that can reduce sugar levels in fermentation. These compounds can be acetic and phenolic acids, which are the degradation of lignin.

The hydrolysis process was carried out at a temperature of 100°C by varying the sulfuric acid concentration and the hydrolysis time to find the right combination of concentration and hydrolysis temperature to produce the most reducing sugar. Hydrolysis experiments with sulfuric acid were carried out with a concentration variation of 0.5%; 0.1%; 0.15%; and 0.2%. The choice of concentration variation was based on previous research by Sun and Cheng [6]. Of all the catalysts, weak sulfuric acid (0.5-1.5%) is the most widely used in the industrial world because it can hydrolyze most of the sugars from hemicellulose by 75-90%. The time variations used in this study were 60 and 90 minutes.

In the hydrolysis process, the variable observed was reducing sugar. Before testing the reducing sugar on the sample, a glucose standard curve was first made. The glucose standard curve expresses the relationship between glucose concentration and optical density (OD) at a wavelength of 540 nm. The method used to test this reducing sugar is the Nelson Somogyi method. This standard curve is made to determine the equation in calculating reducing sugars and the absorbance range that can be used. If the absorbance is less than the standard curve, it means less reducing sugar. If the absorbance exceeds the standard curve, the reducing sugar is high, and dilution must be included in the standard curve. The standard curve that has been created can be seen in figure 1.
The standard curve produces an equation to calculate the reducing sugar of the sample. The results of the reduced sugar content of the sample are then plotted into a graph shown in figure 1. The results of the reducing sugar analysis show that the reducing sugar content ranges from 6.36% to 27.35%. At 0.5% H$_2$SO$_4$ concentration, the value of reducing sugar content at the time of hydrolysis of 60 minutes was 25.52%, while at the time of hydrolysis of 90 minutes, the level of reducing sugar was 15.08%.

The results showed that the reducing sugar content ranged from 6.36% to 27.35% (sig. p<0.05) for the different treatment concentrations of H$_2$SO$_4$. The difference in H$_2$SO$_4$ concentration has a significant effect on the reducing sugar content of the hydrolyzed samples. The difference in hydrolysis time also has a considerable impact on reducing sugar content. The graph above shows that the reducing sugar content increases with increasing H$_2$SO$_4$ concentration and decreases after reaching its optimal point, namely at 1% H$_2$SO$_4$ concentration with the highest reducing sugar content. In experiments with 0.5% and 1% H$_2$SO$_4$ concentrations, it was found that the reducing sugar content was relatively high when compared to the 1.5% and 2% concentrations which had a little reducing sugar content. It means that the concentration of H$_2$SO$_4$ affects the level of reducing sugar obtained. The higher H$_2$SO$_4$ concentration
will provide more opportunities for cellulose and hemicellulose to be hydrolyzed into simple sugars. Still, if the \( \text{H}_2\text{SO}_4 \) concentration exceeds the optimal point, the sugar content will decrease. The optimal threshold for this hydrolysis is a treatment with \( \text{H}_2\text{SO}_4 \) at a 1% concentration. It is called optimal because the highest reducing sugar content at that concentration is 27.35%, and after passing the 1% concentration, the reducing sugar content decreases. The decrease in sugar levels at more than 1% \( \text{H}_2\text{SO}_4 \) occurs because \( \text{H}_2\text{SO}_4 \) is too high. Hydrolyzes cellulose and hemicellulose into glucose, resulting in glucose being further converted through furfural and hydroxymethylfurfural, forming formic acid [16].

Hydrolysis time also affects reducing sugar content. Hydrolysis time of 60 minutes gave a higher reducing sugar content than reducing sugar content at 90 minutes of hydrolysis. It shows that the optimal hydrolysis time is 60 minutes. If the hydrolysis time is too long, the glucose will be hydrolyzed to hydroxymethylfurfural and further react to form formic acid to reduce sugar content.

3.4. Fermentation

After the hydrolysis process, the simple sugar compounds will be fermented by microorganisms to produce ethanol. The fermentation process is carried out by preparing 50 mL of a hydrolyzate solution that has been sterilized into an Erlenmeyer. The hydrolyzate solution used is the result of hydrolysis, which has the highest reducing sugar content. Then the prepared starter is added to the hydrolyzate solution. This fermentation experiment was carried out with various starter concentrations of 5%, 10%, 15%, and 20% of the weight of the feed. Increasing the starter volume will accelerate the fermentation, especially when a high-grade substrate is used. But if the starter volume is excessive, it will result in the loss of the ability of bacteria to live so that the death rate of bacteria is very high.

In addition to the addition of the starter, the fermentation process also added supporting materials, namely NPK and urea. Urea serves as a nutrient for yeast. Urea added as much as 0.5% of the sugar content in the fermentation solution. Adding 0.1% NPK of the sugar content in the fermentation solution serves as a nitrogen source for yeast to grow appropriately after adding the starter and supporting materials (urea and NPK). The fermentation process is carried out at a temperature of 30°C. Fermentation temperature affects the length of fermentation because the temperature of the fermentation environment influences microbial growth.

Fermentation is carried out under anaerobic conditions. \textit{Saccharomyces cerevisiae} grows better in aerobic conditions; \textit{Saccharomyces cerevisiae} will ferment sugar much faster in anaerobic conditions. After the fermentation is complete, it can continue the following process, namely distillation. However, before entering the distillation process, the sample was pasteurized by heating at 80°C for 10 minutes to kill bacteria [12]. The fermentation process results in ethanol with the average levels obtained can be seen in figure 3.

![Figure 3](image-url)  
**Figure 3.** a) The relationship between fermentation time and average ethanol content; b) The relationship between starter concentration and average ethanol content.
The graph above shows that the ethanol content increased from the 3rd day to the 5th day from the 3rd day's ethanol content of 5.55% (v/v) to 13.53% (v/v) on the 5th day. The increase in ethanol content is due to the activity of microbes growing by multiplying to increase the alcohol produced. At the time of 5 days, the maximum microbial proliferation. Meanwhile, the ethanol content decreased on the 7th day of fermentation to 13.25% (v/v). The decrease in ethanol content was due to the nutrients used by microbes to breed had been exhausted. The microbes consumed alcohol, which was indicated by the formation of acetic acid. This process can be seen by the appearance of air bubbles during fermentation. Based on figure 3, it can be seen that the highest bioethanol content was produced in 5 days with an ethanol content of 13.53% (v/v), where five days was the optimal point of fermentation. So it can be concluded that the longer the fermentation time, the bioethanol content will increase until a specific time limit and then decrease.

Tests with variations in concentration were also carried out to determine the concentration of starter capable of producing the highest levels of ethanol in this study. The fermentation time for 5 minutes was chosen because the highest ethanol content was obtained during the fermentation time in the previous test. In the 5% starter concentration treatment, the 8.71% ethanol content increased to 13.53% at the 10% starter concentration treatment. It happens because, at a fixed fermentation time, a starter with more concentration is used. The more microbes that convert glucose into ethanol, the more ethanol produced will have high levels. However, after the starter concentration exceeded 10%, the ethanol content decreased. In the 15% concentration treatment, the ethanol content decreased to 12.39%, and in the 20% concentration treatment, the ethanol content decreased to 11.08%. It is due to more Saccharomyces cerevisiae than the available nutrients, so Saccharomyces cerevisiae uses more of these nutrients to survive rather than converting sugar into alcohol.

The interaction between the concentration of starter fermentation and fermentation time dramatically affects the levels of bioethanol produced because the results of the 2-way ANOVA test show a sig value of 0.002 so that sig. p<0.05. It means that the different concentrations of starter (5%, 10%, 15%, 20%) and fermentation time (3, 5, 7 days) greatly determine the level of bioethanol formed from CCW.

3.5. Bioethanol quality analysis

The calculation of the ethanol content of bioethanol obtained bioethanol with levels of 13.53% (v/v). The ethanol content produced in this study was much smaller than the standard bioethanol content, which should have been 94%. However, this value is more significant when compared to the results of bioethanol production in previous studies for bioethanol production from lignocellulosic sources from rice straw and reeds [7,11].

From the results of the analysis and calculations, it can be seen that the density of bioethanol obtained is 0.981 g/mL, where the density exceeds the absolute bioethanol density of 0.789 g/mL, so it can be concluded that the bioethanol is still not pure. It is because bioethanol still contains a lot of water so that the density becomes greater. Theoretically, the density of water is greater than the density of ethyl alcohol, where the density of water is 1 g/mL. Hence, the volume of water that includes ethyl alcohol also affects the density value of ethyl alcohol. The distillation causes the impurity of bioethanol carried out only simple distillation, not multilevel distillation, and the lack of accuracy in maintaining the stability of the distillation temperature so that the steam that comes out is not only bioethanol but mixed with water. The density of the fuel is expected to affect the rate of fuel consumption significantly. The greater the density, the more fuel consumption will increase or be more wasteful. A large density will produce a small calorific value, causing low quality. Density is affected by temperature. The higher the temperature, the lower the density, and vice versa, the lower the temperature, the higher the density, so the quality worsens.

Specific gravity is the density of fuel divided by the density of water at the same temperature. The specific gravity value is used to calculate the API gravity value. The specific gravity of bioethanol produced in this study was 0.981. It shows that bioethanol from CCW does not meet the quality requirements of bioethanol, which should have a maximum specific gravity of 0.8215. Higher specific gravity will make the fuel challenging to ignite, so the quality of the fuel is lower. The specific gravity value that is still above this threshold may be due to impurities in bioethanol.
The calorific value is inversely proportional to the density and specific gravity. In materials with enormous specific gravity, the calorific value will be smaller, and vice versa. The calorific value obtained in this study is 10,422.2 kcal/kg. An immense calorific value will cause it to burn more quickly so that the quality is better.

4. Conclusion
The most optimal hydrolysis and fermentation treatment to obtain bioethanol with the highest ethanol content of 13.53% was hydrolysis for 60 minutes with 1% H₂SO₄ concentration and fermentation for five days with 10% yeast concentration. The quality of bioethanol from CCW is as follows: a) ethanol content of 13.53%; b) a density of 0.981 g/mL; c) specific gravity of 0.981; d) gravity API of 12.711; e) calorific value of 10,422.2 kcal/kg; and the physical appearance of bioethanol is clear, transparent, without sediment.

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