Preliminary study of acidic hydrolysis in third generation bioethanol production using green algae

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Abstract. In a mission to reduce the world’s dependency on non-renewable energy sources, through decades, researches are striving to find the best alternative energy substitutions. Considering the abundance availability of renewable sources, green algae are discovered as one promising candidate of future energy alternative. The aim of this research is to study the bioethanol production from green algae through acid hydrolysis. In this study, the acid hydrolysis was performed by utilizing hydrochloric acid (HCl) at concentration range of 0.5M-2.5M. Further, hydrolysis temperature was also varied at 50, 70 and 90°C. In this stage, a fixed hydrolysis period was set for 30 minutes. As acid pre-treatment is considered in this study as an effective way to convert carbohydrates into fermentable sugars, the sugars produced in this experiment would lead to an excellent grade of bioethanol produced. From the obtained result, green algae show a feasibly potential as the third-generation bioethanol feedstock. The highest theoretical ethanol value obtained by performing 2.5M of HCl at 90°C during retention time of 30 minutes.

1. Introduction

Presently, the uncontrollable population growth and the improvement of industrialization are the most considerable factors which highly contribute to world energy crisis. The inevitability of human dependency on non-renewable energy sources leads to the depletion of fossil fuels and climate change. Globally, the carbon emissions from the fossil fuel have been continuously increased since 1900 and in the massive scale, the carbon dioxide (CO₂) level on the atmosphere has also increased about 90% [1]. In this case, the industrial processes and transportation sector which mostly involves the petroleum-based fuel are the number one contributor which responsible around 78% of CO₂ from the total greenhouse gas emissions [1, 2].

The global energy concerns thus require the energy substitution that should satisfy the two major factors which are renewability and cost-effectiveness. Besides that, the future energy sources are expected to be chemically stable and viable to be used in ignition engine [3, 4]. Through the past decades, researchers are struggling to find the best alternative way to sufficient energy sources. Among all renewable energy candidates, bioethanol has outstanding feasibility. However, the first and second generation of bioethanol production do not yet meet the sustainability requirement [5]. And therefore, the third generation of bioethanol is introduced.
By relying on its effectiveness in the engine efficiency and reducing the carbon emission, bioethanol is projected to be a highly promising fossil fuel substitution in fulfilling future fuel demands. Algae is now becoming a reputable consideration to be used as the third generation of bioethanol production feedstock due to their rapid photosynthetic rate, easy cultivation, and high carbohydrates content [6]. More importantly, there will be no more fuel and food competition in the purpose of reducing the world addiction to non-renewable fuel [7].

In pursuing the goal of replacing the use of non-renewable energy sources, the study of this research aimed to figure out the reducing sugar number of green algae under the acidic hydrolysis. The feedstock was treated under the variation of concentration and hydrolysis temperature. The theoretical ethanol value resulted from the experiment projected to lead to the excellent grade of bioethanol that can be substituting the need of fuel sources within foreseeable future.

2. Method

2.1. Feedstock collection

Samples of green algae were collected from the local water fishpond in February 2019. The collected feedstock was then washed. The feedstock that is used in this experiment was 10 g/L.

2.2. Acid hydrolysis

In this stage, three sets of samples were prepared. Each sample consists of 1 g of wet feedstock and each sample was added by HCl with the concentration of 0.5 M, 1.5 M, and 2.5 M and then diluted in 100 ml of distilled water. After the feedstock added with acid, the samples were heated using a water bath at 50 °C for retention time of 30 minutes. After 30 minutes, the samples were taken, and they cooled down at room temperature. Besides that, 100 ml of 10 M sodium hydroxide was prepared and added to each sample in a purpose for neutralization. The optimum result in this variation of concentration then continued to the next variation of temperature which are 70 °C and 90 °C. The feedstock that is added by the acid with the variation of concentration are shown on the figures below.

![Figure 1. Acid hydrolysis.](image)

2.3. Reducing sugar content

In this study, the reading value of reducing sugar was investigated by performing DNS method [8]. The reagent solution that was made by mixing 2 M of 200 ml sodium hydroxide, 10 grams of 3,5-dinitrosalicylic acid and 300 grams of sodium potassium tartrate (Rochelle salt) with the final volume of 1 L.

The 1.8 ml of hydrolysate performed under different concentration and variation of temperature was centrifuged then transferred into the test tubes and added with 1.8 ml of DNS solution. Each test tubes were heated at 90 °C for a retention time of 5 minutes in a water bath. The reducing sugar content of this experiment was measured by measuring the color intensity of the solution using a wavelength of 540 nm in a spectrophotometer.

2.4. Theoretical ethanol analysis
According to the study of C.E. Wyman [9], the theoretical ethanol conversion factor from 1 g/L glucose content from the lignocellulosic biomass is 0.5111 g/L ethanol. Therefore, the theoretical ethanol value in this study was obtained by multiplying the glucose conversion factor (0.4137) of the glucose standard with the theoretical ethanol conversion factor.

3. Result and discussion

3.1. Effect of acid concentration

In this experiment, the feedstock was treated with the variation of concentration and temperature, in a fixed bath retention time which is 30 minutes. Figure 1 shows the reducing sugar content of the feedstock that is treated with the different HCl concentration at the range between 0.5M to 2.5M at the temperature of 50 °C. The graph resulted from this experiment has a positive trend, with the yield of sugar obtained when 0.5M of HCl added is 4.96 mg/L, 9.3 mg/L when 1.5M HCl was added and 20.89 mg/L when 2.5M of HCl was added to the feedstock solution.

![Figure 2](image)

**Figure 2.** Reducing sugar content of 0.5 M to 2.5 M of HCl at temperature of 50 °C.

As displayed in figure 2, it can be observed if the reducing sugar content is increasing in accordance to the increasing of HCl concentration added to the feedstock, with the highest reducing sugar obtained is 10.67 mg/L for 2.5M of HCl. Although the higher concentration added will obtain the better sugar yield, a very high acid concentration added to the feedstock solution is not suggested by considering the corrosive characteristic of acid which possible lead to the equipment hazard.

3.2. Effect of hydrolysis temperature

As it is mentioned before, the optimum result from the variation of concentration that is obtained was continued to the next variation of hydrolysis temperature. The feedstock which added by 2.5 M of HCl was brought into the next variation of temperature which are 70 °C and 90 °C. From figure 2, it can be observed if different hydrolysis temperature results different reducing sugar content obtained. For a fix bath time of 30 minutes and acid concentration of 2.5 M, 20.89 mg/L of sugar was obtained by setting 50 °C as the hydrolysis temperature. When the temperature performed is 70 °C, the sugar yield obtained is 31.44 mg/L and when the 90 °C used as the hydrolysis temperature, the reducing sugar content obtained is 36.19 mg/L. Therefore, the reducing sugar content that is obtained in this experiment is increasing accordingly to the increasing of hydrolysis temperature.
Figure 3. Reducing sugar content of 2.5 M of HCl at temperature of 50 °C, 70 °C, and 90 °C.

3.3. Theoretical ethanol content
As the parameter used in this experiment are the acid concentration and hydrolysis temperature, the graphs below show the theoretical ethanol content obtained from the experiment.

Figure 4. Reducing sugar content and theoretical ethanol of 2.5 M of HCl at temperature of 50 °C.

From figure 4, as it is displayed, the theoretical ethanol content is increasing along the increasing of the acid concentration performed at 50 °C for 30 minutes. For the 0.5M of HCl, the theoretical ethanol value obtained is 2.53 mg/L, 1.5M of HCl resulted 4.75 mg/L, and the highest theoretical ethanol value obtained resulted is 10.67 mg/L by performing 2.5M of HCl.

Figure 5 below shows the reducing sugar content and the theoretical ethanol value obtained by performing 2.5 M of HCl at the variation hydrolysis temperature of 50 °C, 70 °C, and 90 °C.
Figure 5. Reducing sugar content and theoretical ethanol of 2.5 M of HCl at temperature of 50 °C, 70 °C, and 90 °C.

The highest theoretical ethanol obtained in this experiment is 10.67 mg/L is when the acid concentration is used as the parameter is resulted from the feedstock that is added by 2.5 M concentration of HCl acid. Besides that, the highest theoretical ethanol obtained in this experiment when the hydrolysis temperature was varied is when the feedstock added by acid is 90 °C. Therefore, by performing the acid concentration (2.5 M) and hydrolysis temperature (90 °C) in which the highest theoretical ethanol value is obtained, the highest theoretical ethanol value resulted from this experiment is 18.50 mg/L.

However, although the graph of reducing sugar content and theoretical ethanol value of the effect of acid concentration and hydrolysis temperature show the positive trend, the value obtained in this experiment have the small theoretical ethanol value. This is speculated possibly to be occurred due to the small feedstock concentration and the abstain of mechanical pre-treatment in this experiment [10].

4. Conclusion
From this experiment, there are several conclusions that can be obtained. First, the highest reducing sugar content obtained from this experiment is 18.50 mg/L by performing 2.5 M of HCl at 90 °C during 30 minutes in which that can be concluded if the feedstock that is used in this experiment, which is green algae is feasible to be used as the feedstock in third generation bioethanol production. Second, the method used in this experiment, which is acid hydrolysis is highly promising method in third generation bioethanol production using green algae and third, the parameters used in this experiment which are the acid concentration and hydrolysis temperature are linear to the reducing sugar content. The high concentration of feedstock and the presence of mechanical pre-treatment are also strongly suggested to obtain the better ethanol quality.

Further research and study of the acid hydrolysis in bioethanol production using green algae is strongly encouraged as it carries high potential. Other than that, even though bioethanol production is not yet widely commercialized, it is assured if bioethanol would be a reputable future energy source in substituting the non-renewable energy sources.

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