Microwave irradiation pre-treatment in third generation bioethanol production from tropical green algae

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Abstract. Through centuries the side effect of the massively growing of population is the energy demand and consumption. As a result of this, the world energy used from the non-renewable fossil fuel has reached 80%. To counter this, researchers have found alternatives for this fossil fuel; and bioethanol is one of the alternative and sustainable options. One source of bioethanol that is believed carrying high production potential is photosynthetic algae or green algae. In this study, Chlorophyta (Green Algae) was hydrolyzed using microwave irradiation. Operation Mode (OM) was varied to observe its effect during the hydrolysis stage. Further, hydrolysis time (t) was also selected as the variable in this study to examine the result of reducing sugar. From the result of this experiment, it is projected that the sugar produced from hydrolysis assisted by microwave irradiation would bring a high-quality bioethanol production. Also, from this study, it would exhibit the great potential of bioethanol from green algae as the fossil fuel substitute in order to create a more sustainable and environmentally friendly energy source.

1. Introduction

Nowadays, 80% of energy consumption in the world is from fossil fuel, which is in the form of non-renewable resources, then it is easy to become extinct [1]. In addition, the stock of sources is coming from natural gas, crude oil (petroleum), and coal, where it results in environmental problems, such as emission of greenhouse gases and economic concerns. Therefore, one of the main scientific tasks of the third millennium is to achieve an ability to exploit renewable energy sources in the chasing of minimal environmental impact. Most of the resources are coming from the natural occurrences, such as wind power, sunlight or solar system, biomass, geothermal energy, and hydrogen fuel cells; which the most promising in the use of transportation sector is the biomass, energy from the plants. A popular example of using biomass is burning trees in the use of cooking and warmth. But this process contributes a major unhealthy air to the atmosphere in the form of carbon dioxide. To overcome the effect, there are some modern approaches in using biomass energy to create sustainability and environmentally friendly energy sources, such as methane generation and production of alcohol for automotive fuel and power plant.

Biofuel is one of the biomass productions which is running in the automotive fuel and power plant sector [2]. In the process of production, the first idea of a scientist is using directly the food crops by abstracting the oils. However, the issues come up together with the mass production of biofuel which uses the food crops as a dish or as a fuel. Then, the limitation of the first generation can be handled by
the second production of biofuel. In the second production, the feedstock is using the non-food crops, such as wood, organic waste, and specific biomass crops in order to maximize the food crops for the food material of cooking. However, the issue is about the low-life cycle of non-food crops and using a greater area to plant. Therefore, the third generation of biofuel, engineered energy crops, is defined as the alternative because of the low cost, high-life cycle, and easy to be obtained. The process is to gain fuel by doing fermentation in order to get the bioethanol from the feedstocks.

Ethanol is produced mostly from a fermentation process using glucose derived from sugars [3]. In Australia, ethanol is made from a waste product such as sugar cane, starch production, and red sorghum [4]. However, those feedstocks are coming from the second-generation biofuel which has a complicated problem, such as low life cycle and greater area land used. So, in this research, biomass is produced from the tropical green algae which are massively produced and the easiest feedstock to be obtained in the environment. Green algae are included in the division of Chlorophyta where is having approximately 7500 species of eukaryotic, unicellular organisms [5]. These are storing the carbohydrate are in the form of starch and containing two forms of chlorophyll A and B which use to capture light energy to fuel the manufacture of sugars. To observe the concentration of sugars, scientist has developed many methods.

Based on previous studies, it was reported that ethanol production was obtained by waste cake hydrolysis [6]. In the hydrolysis process, α-amylase was used in a particular temperature in the range of 60-degrees to 100-degrees. It was a chemical component that may damage the bonding on the feedstocks. Also, the waste cake hydrolysis used bioreactor device which demanded a high cost. Thus, microwave irradiation has the advantage that no chemical other than water is needed to affect the sugar concentration and spend less cost of operation.

This study investigated the potential of tropical green algae by using various Operation Mode (OM) and Time (t) of the microwave in producing a bioethanol. The each of obtained results would be compared in order to observe which parameter would give larger amount of reducing sugar concentration. In another studied, there was a thing called gold nanoparticles which are applicable in various engineering field, such as biosensors [7]. Specifically, in order to replace the usability of fossil fuel on the automotive energy consumption, this research could be began by using third generation of biofuel, tropical green algae, as easy feedstock to be obtained that is assisted by microwave irradiation which nowadays, the number of dependency in fossil fuel on the engineering field concern is dramatically high. Therefore, this research which is running on the microwave-assisted biomass process; the feedstock would be observed and examined intensively in order to produce an alcohol to be applied in automotive energy consumption.

2. Method

2.1. Feedstock collection
Tropical green algae were collected from a small pond near the research centre and small pond in Bekasi city. Tropical green algae that was used for the feedstock did not have a specific species due to the environmentally habitat. The total mass that had been collected in one bottle was about 1 kg with water indeed and there were 3 bottles of feedstock collection. Green algae were washed by distilled water to remove from the dirt. The feedstock concentration that was used to be observed through experiment was 10 g/L as shown in figure 1.
2.2. Microwave irradiation hydrolysis
The feedstocks were observed using the method of microwave irradiation. Wet green algae (WGA) was hydrolyzed in a Sanyo EM-S1553 Microwave with 50 Hz frequency [8]. For microwave irradiation, the effect of time and power applied was studied extensively. The time (t) effects on reducing sugar yield was studied by varying the time duration of irradiation; 3 minutes, 8 minutes, and 12 minutes from the oven control panel. The power applied or the operation mode (OM) effects on reducing sugar yield were analysed by changing the output strength to 30%, 60%, and 100% of the maximum output power of 800 Watt (According to IEC 705 Test Procedures) [9]. Those all changing were done in order to improve the reducing sugar yield in the WGA solution. In all cases, reducing sugars, including glucose, xylose, and fructose, yield was determined at different time (t) and operation mode (OM) to find which the most reliable parameter of this experiment.

2.3. Glucose content analysis
The feedstocks’ reducing sugar were quantified using the DNS method which composed of 0.2% phenol, 0.05% sodium sulphite, 1% sodium hydroxide, and 1% dinitro salicylic acid [10]. The solution was produced by blending 200 ml of sodium hydroxide with concentration of 2 M with 10 grams of 3,5-Dinitrosalicylic acid. Sodium potassium tartrate of 300 grams was mixed with previous solution, then it was brought to 1 L with distilled water. DNS solution was reacted with each hydrolysate of microwave irradiation process for nine total parameters. Then, the reaction process needs to be heated up to 90-degrees Celsius for 5 minutes to ease the chemical reaction with DNS. They were examined by UV-Vis Spectrophotometer Optima SP-3000 Nano at the wavelength of 540 nm. The results were then multiplied by the factor of glucose standard content that had been examined experimentally which had a value of 0.4137.

The value was coming from the glucose standard on the Lab that was examined by DNS method also. It was treated in various concentration of glucose content. The obtained results were used to determine the trendline or conversion factor of the glucose standard.

2.4. Theoretical ethanol
The research about producing the ethanol from the lignocellulosic biomass was developed by the C.E. Wyman [11]. It is stated that in 1 g/L glucose content will produce 0.5111 g/L of theoretical ethanol. Thus, the theoretical ethanol of this research is gained by multiplying the conversion factor with the glucose content from previous observation. Then, it was plotted to find the highest result on various parameter, shown in the figure 3.

3. Result and discussion
3.1. Hydrolysis
The effect of variation power output on reducing sugar content were studied by treating hydrolysate at time range of 2 minutes to 6 minutes with increment of 2 minutes. Variation of the power output are 30%, 60%, and 100% which the maximum power output of the Sanyo EM-S1553 Microwave at 50 Hz
frequency is 800 W. In figure 2, it observes the glucose standard conversion factor which will be multiplied by the ABS result from the UV-Vis Spectrophotometer Optima SP-3000 Nano. ABS result is a term of absorbance (ABS) which measure amount of light that is absorbed by the sample; in this case is the microwave irradiation hydrolysate.

The obtained result would be used to find the glucose content in the hydrolysate. On the various time range, it then multiplied by 0.4137 to find the reducing sugar content which the value was obtained from previous experiment. The value of each parameter will be varying, shown in the figure 2. Look up to figure 2, the trendline between 2 minutes hydrolysate time compare with 4 minutes and 6 minutes hydrolysate time is inversely proportional. In 2 minutes-hydrolysate time, the trend is linear proportional with the maximum glucose content can be produced is 6.2055 mg/L of 100% power output. Otherwise, in 4 and 6 minutes-hydrolysate time, the trend is inversely proportional with the maximum glucose contents are 4.6886 mg/L of 30% PO and 6.2055 mg/L in the 30% PO, respectively. However, the experimental results, excluding the highest result, have own trendline which describe unique behaviour.

Four minutes hydrolysate time in range of 30% until 60% PO is the constant value of reducing sugar. It can be shown in the figure 2b that the glucose content produces 4.7 mg/L and 4.3 mg/L for 30% and 60% PO, respectively. According to the result of those two parameters, it can be averaged to be 4.5 mg/L. So, it can be concluded that for the range of PO 30% to 60% in 4 minutes hydrolysate time, it will not dramatically either increase or decrease. Otherwise, in hydrolysate time of 2 minutes and 6 minutes, shown in figure 2a and 2c, respectively; the result value is either linearly proportional or inversely proportional.

In figure 2b and 2c, as the increasing of hydrolysate time, the trend of the glucose content decreases. This occurs due to long-time duration of treatment process. It is speculated that longer microwave time will damage the molecule of the reducing sugar content. Furthermore, the glucose content will reach near zero if the hydrolysate time treatment is higher. As another studied, the hydrolysis was carried out on varies power rating of 550, 700, and 900 Watt during varies of time of 1, 2, 3 minutes [12]. As result on the other study, the higher power rating will affect to the production of glucose, since the result at
900W for 1-minute yields less of glucose production rather than at 700W for 2 minutes. So, it can be concluded that higher time treatment will produce less glucose. It is validated also in this research that the higher hydrolysate time, as in figure 2c, the reducing sugar content will decrease because hydrolysate cell was speculated to be destructed by microwave irradiation.

3.2. Theoretical ethanol
For the reference purposes, the theoretical ethanol conversion factor is obtained following the work of ref. [11], which is 0.5111. Then, the conversion factor is multiplied by the glucose content in order to get the theoretical ethanol. In figure 3a, it is shown that the trendline of theoretical ethanol is the same as the glucose content at figure 2a which behaves an increasing trendline. Whereas in 4 minutes and 6 minutes-hydrolysate time of is decreasing, shown in figure 3b and 3c. The highest theoretical ethanol through all the treatment is located on the 100% PO of 2 minutes-hydrolysate time, shown in figure 2a, and 30% PO of 6 minutes-hydrolysate time which the value of theoretical ethanol is 3.2 mg/L, shown in figure 2c.

As the highest is on 100% PO of 2 minutes hydrolysate time and on 30% PO of 6 minutes hydrolysate time, the other value of this time treatment is behaving oppositely, where in 2 minutes the slope is positive whereas in 6 minutes it is negative slope. In time treatment of 4 minutes for 30% PO and 60% PO, it results 2.2 mg/L and 2.4 mg/L, shown in figure 2b. However, it is not applicable for 100% power output due to the long-time treatment which causes the damage in the reducing sugar content where will cause the decreasing of theoretical ethanol.

As shown in figure 3, the trendline of three graphs is different as increasing of hydrolysate time. It shows that ethanol production through microwave irradiation either gives an increasing result or decreasing result based on the treatment to the hydrolysate. The behaviour is similar to the glucose content because the theoretical ethanol result is obtained by multiplying with the theoretical ethanol conversion factor, 0.5111 g/L which is following ref [11].
4. Conclusion
From the experiment, there are some important points needed to be highlighted. The results from this study proves that green alga is favourable for the third-generation bioethanol production. Here are few highlights based on the experimental result:

- The reducing sugar content is positively affected by the power output and hydrolysis time. In 2 minutes of irradiation time the trendline for ethanol theory is increasing, whereas in 4 minutes and 6 minutes is decreasing. The highest theoretical ethanol is 3.2 mg/L.
- Tropical green algae are feasible feedstock in bioethanol production and the microwave irradiation is an attractive method for future experiment.
- Furthermore, in order to get a high amount concentration of the theoretical ethanol, it is recommended to use a higher feedstock loading.

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