High-pressurizing green algae in third generation bioethanol production

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Abstract. The effects of fossil fuel combustion are famously concerning. 70% of the global production of carbon monoxide is accounted from transportation sector; the sector in which fossil fuel is popularly being continuously used. As one alternative fuel, bioethanol is a renewable fuel which have attracted many researchers in the pursuit of lowering dependency on fossil fuel and its negative effects to the environment. In third generation bioethanol production, green algae are deemed to carry high potential as the feedstock due to its ampleness, especially in a tropical country such as Indonesia. In this study the ability to produce simple sugars of green algae was observed through high-pressurization treatment. Different treatment pressures were achieved through varying temperature set in the autoclave: 110 and 130oC. The recorded pressure was up to about 180 kPa gage, and this treatment was also compared with non-pressurizing production method. Samples were analysed for reducing sugars content through DNS method. Since simple sugars are essential in the fermentation stage, the produced sugars from green algae are the indication of potential as the feedstock in bioethanol production. The impact of this study supports the progress of fossil fuel-to-biofuel scheme in Indonesia, and the outcome from this preliminary study can be used as the reference for further studies.

1. Introduction
In transportation sector, the progress in technological development is visible through the betterment of vehicle engine mass-production. It is inarguable that the advancement has gradually established a better civilization, however, this comes with a price. Paired with the rapid growth of global population, the development of vehicle production consequently results for a higher energy demand. Focusing on the transportation sector, the rising fossil fuel consumption results a huge challenge to handle: sustainability and environmental issues. According to International Energy Agency, IEA, in 2017 global crude oil consumption reached over 4,400 Mtoe, and it is projected to be steadily rising [1]. Global demand projection on crude oil is shown in figure 1, which displays an increasing demand until 2040 under New Policies Scenario. Moreover, our dependency on fossil fuel is inseparable, which worsens the effect of global warming due to the trapped greenhouse gases in the atmosphere. The harmful compounds resulted from in-cylinder combustion of fossil fuel, including NOx, CO and particulate matters, degrade the air quality. On the environmental side, the global warming develops a higher global temperature and sea level, as well as upsetting the balance of the ecosystem [2].
The popularity of bioethanol as an alternative fuel for internal combustion engine has grown over time. Many studies and researches have reported the advantages of bioethanol as alternative fuel, including its resistivity to engine knocking due to high octane number. Moreover, the use of this renewable fuel can lower CO₂ emission as high as 80% than the conventional petrol [3–5]. Despite of the advantages, a challenge rise from this is the sustainability of the resources. The main concern on first generation is the topic of fuel versus food supply. Meanwhile, bioethanol production through second generation follows the natural life-cycle of crops, which is still relatively deemed as not fully sustainable [2]. This is when the third generation is coined to encounter the issues from the other routes. Algae as bioethanol production feedstock is advantageous in its high production rates due to fast life-cycle, high carbohydrate content and contributes in absorbing CO₂ [2, 6]. Since the principle of bioethanol production is in the fermentation of sugars, a reliable feedstock treatment supports the production. Treating feedstock under high pressure and temperature using autoclave is one beneficial method to break complex sugar over the conventional acid treatment. This is mainly because acid hydrolysis reduces the equipment lifetime, corrosion and pollutes the environment [4, 7]. In this study, production of reducing sugar from green algae using autoclave was investigated, as an effort in the preparation prior fermentation stage. The main objective of this study is to observe the effect of the method through the amount of reducing sugar detected.

2. Methods

2.1. Green algae collection
The green algae sample was collected from a local pond in Bekasi, West Java. Upon collection, the sample was put into a jar then washed using distilled water to clean any impurities. The cleaned sample was further dried using drying towel prior entering the next stage.

2.2. Mechanical pre-treatment
The green algae sample was further treated mechanically. The process was started by placing the sample in a glass beaker then dried in a furnace for 2 hours at 1200°C. The dried green algae sample was then brought into mortar for grinding process. The end result of this stage is a powder form of green algae.

2.3. Autoclaving
Autoclaving process in this study was aimed to degrade the main structure of the green algae, which by the work of high pressure and temperature. Prior this stage, feedstock was prepared by mixing the...
dried green algae into flask of 100 ml distilled water. The autoclave process was set in variation of autoclave temperature of 110 and 130°C. Substrate loading was also varied at 1 and 5 g/L. Figure 1 shows the flash filled with the substrate loading prior the autoclave process.

Figure 2. Green algae loading in autoclave process.

Further, the effect of drying the feedstock was also investigated by comparing the result from autoclave process with the wet and non-grinded feedstock. In this study, the autoclave timing was set to a fixed value of 15 minutes. The pressure was observed to reach as high as 180 kPa of gage pressure.

2.4. Reducing sugar assay
The hydrolysate resulted from autoclave process was tested for its fermentable sugar, or reducing sugar, content. In this study, the reducing sugar assay was performed following method by Miller [8]. The solution was prepared 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 then mixed with the mixture. The final volume was brought to 1 L with distilled water.

Upon reducing sugar determination, the DNS solution was first reacted with the hydrolysate after autoclave process. The reaction was set by supplying heat using water bath at 95°C for 5 minutes. The reducing sugar content was read through the absorbance value through detection of color intensity at 540 nm wavelength using UV-Vis Spectrophotometer Optima SP-3000 Nano.

2.5. Theoretical ethanol determination
The potential of bioethanol production was determined through theoretical calculation. Following ref. [9], each g/L lignocellulosic sugar would produce 0.5111 g/L of ethanol. This study used the value as the conversion factor from the detected sugar from the experiment to predict the potential ethanol production.

3. Results and discussions

3.1. Effect of autoclave temperature
In observing the effect of autoclave temperature, the substrate loading was set fixed at 1 g/L and using the feedstock after mechanical pre-treatment (in powder final form). Figure 3 shows the concentration of reducing sugar produced at different autoclave temperature. At 130°C of autoclave temperature, the reducing sugar was detected the highest, at 9.72 mg/L. Despite the linear trend, the lower autoclave temperature does not produce sugar far than that of the higher temperature, which is at 7.65 mg/L of sugar produced.
Figure 3. Reducing sugar and theoretical ethanol vs. tested autoclave temperature.

Following the detected sugar content, the theoretical ethanol concentration displays the similar linear trend. This is expected since the potential ethanol concentration was calculated using the conversion factor directly by taking the empirical sugar concentration. The highest is seen at 130°C autoclave temperature, that is 4.98 mg/L. Meanwhile, 110°C autoclave temperature would potentially produce 3.91 mg/L of ethanol.

3.2. Effect of substrate loading

The substrate loading was varied at 1 and 5 g/L of mechanically pre-treated green algae. To proceed this, the autoclave temperature was fixed at 130°C. This approach was considered after observing the reducing sugar concentration at 130°C that shows the optimum amount of sugar produced. The result of autoclaving different substrate loading is shown in figure 4. Observing the detected sugar concentration, there is a significant jump on the sugar concentration resulted from 1 g/L of substrate to 5 g/L of substrate. From 5 g/L substrate, 94.73 mg/L of sugar is produced. Meanwhile, 1 g/L of dried, grinded algae yielded only 9.72 mg/L.

Accordingly, the contrast value jump is also observed on the calculated theoretical ethanol concentration. Figure 4 shows that 5 g/L substrate would produce bioethanol as high as 48.42 mg/L. In comparison with potential ethanol produced from 1 g/L loading, the potential ethanol from 5 g/L loading
is an improvement in about 100-fold. An optimization study using response surface methodology also reported that substrate loading plays the most significant factor in producing reducing sugar [10].

3.3. Effect of substrate treatment
The variation of pre-treatment to the green algae was also investigated in this study. Pre-treatment of green algae was varied by comparing the wet and dry sample. In weighing wet sample, flask was placed on top of a weighing scale filled with few mL of distilled water, and green algae was then added to increase 0.5 grams and 3 grams of total weight. The flask was then topped to reach 100 mL, which leads to 5 g/L and 30 g/L of total loading respectively. The dry sample was taken from a powder form of green algae, which treatment is described in the method section.

![Graph showing reducing sugar and theoretical ethanol concentration](image)

**Figure 5.** Reducing sugar and theoretical ethanol resulted from substrate of treatment variation.

Figure 5 shows the obtained reducing sugar from various substrate treatment after autoclave process at 130°C for 15 minutes. Wet sample of 5 g/L was observed to not producing any trace of reducing sugar. In contrast, it shows that 5 g/L of dry algae yielded remarkably higher than that from the wet loading at the same weight. Increasing the load of wet algae to 30 g/L does not substantially improve the sugar content, as it only produces 2.34 mg/L of sugar. Following the trend, the potential ethanol concentration of 30 g/L wet loading could only produce 1.19 mg/L of ethanol. The mechanical pre-treatment and drying process are believed to help the structure breaking process of the algae, dissolving more sugar into hydrolysate during autoclave. Study by Zeng et al. [11] also reported that by decreasing the substrate size of corn Stover it enhanced the cellulose-to-glucose conversion to more than 1.5-fold. The overall improvement in sugar production in this study is supported by the study of Cao et al. [12], who reported the enhanced sugar conversion after comparison with non-autoclave hydrolysis.

4. Conclusion
Green algae as feedstock was investigated in this study by means of high-pressurizing (autoclave). This study observed the effects of autoclave temperature, substrate loading and substrate treatment to the resulted reducing sugar concentration. Further, the potential of ethanol production was computed theoretically. This study concludes that high autoclave temperature with high substrate loading result a high sugar concentration. This is suggested from 94.73 mg/L of sugar resulted from autoclaving 5 g/L of substrate loading at 130°C. This value is translated as 48.42 mg/L of theoretical ethanol. Further, dried and grinded substrate enhances the sugar extraction during autoclave than that of wet substrate. Increasing the load of wet substrate does not result any meaningful improvement on the sugar yield.

Based on the results of this study, green alga is one potential feedstock for third generation bioethanol production. Further research is recommended to explore the potential of green alga in bioethanol
generation. Physicochemical properties, as performed by ref. [13], is advisable to perform on the produced ethanol.

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References
[1] International Energy Agency World Energy Outlook 2018 International Energy Agency.
[2] S R Chia et al. 2018 Sustainable approaches for algae utilisation in bioenergy production,” 1st Int. Conf. Bioresour. Technol. Bioenergy Bioprod. Environ. Sustain 129 838–852.
[3] M Balat 2007 Global Bio-Fuel Processing and Production Trends Energy Explor. Exploit. 25(3) 195–218.
[4] H B Aditiya, T M I Mahlia, W T Chong, H Nur and A H Sebayang 2016 Second generation bioethanol production: A critical review Renew. Sustain. Energy Rev. 66 631–653.
[5] A H Sebayang et al. 2016 A perspective on bioethanol production from biomass as alternative fuel for spark ignition engine RSC Adv. 6(18) 14964–14992.
[6] C Yoo, S-Y Jun, J-Y Lee, C-Y Ahn and H-M Oh 2010 Selection of microalgae for lipid production under high levels carbon dioxide Suppl. Issue Recent Dev. Biomass Convers. Technol. 101(1) S71–S74.
[7] H M Kim, S G Wi, S Jung, Y Song and H-J Bae 2015 Efficient approach for bioethanol production from red seaweed Gelidium amansii Bioresour. Technol. 175 128–134.
[8] G L Miller 1959 Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar Anal. Chem. 31(3) 426–428.
[9] C E Wyman 1994 Ethanol from lignocellulosic biomass: Technology, economics, and opportunities Spec. Issue Biotechnol. Convers. Lignocellul. 50(1) 3–15.
[10] A H Sebayang et al. Optimization of Reducing Sugar Production from Manihot glaziovii Starch Using Response Surface Methodology Energies 10(1) 35.
[11] M Zeng, N S Mosier, C-P Huang, D M Sherman and M R Ladisch 2007 Microscopic examination of changes of plant cell structure in corn stover due to hot water pretreatment and enzymatic hydrolysis Biotechnol. Bioeng. 97(2) 265–278.
[12] W Cao, C Sun, R Liu, R Yin and X Wu 2012 Comparison of the effects of five pretreatment methods on enhancing the enzymatic digestibility and ethanol production from sweet sorghum bagasse Bioresour. Technol. 111 215–221.
[13] A Warra 2019 Analyzing Physicochemical Properties of Wild Grapes (Lannea Microcarpa) Seed Oil Indones. J. Comput. Eng. Des. 1(1) 37–43.