Hydrogen Production by Algae *Scenedesmus sp.* Biomass through Photosynthesis Process

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Abstract

The hydrogen production was studied using *Scenedesmus sp.* by PHM-S media under anaerobic and photosynthesis process. The Investigated of hydrogen gas using by Natural Gas Analyzer (NGA): HP (Hewlett Packard) 6890 with a molecular sieve 5A (CH_4, O_2. N_2, H_2) a thermal conductivity detector is used in a mesh-packed column. The results show highest value of hydrogen production in second experiment. It was obtained because the second experiment had longer for incubation time so the photosynthesis process was took longer then algae could produce more hydrogen gas. Interestingly, the hydrogen does not produce within a certain timeframe. We believe that this was related to the reaction enzymatic in algae that was mostly induced by the oxygen, an inhibitor of the hydrogenate enzyme, was reduced during anaerobic adaptation, resulting in an increase in hydrogen production.

Keywords: *Biomass; Scenedesmus sp.; Anaerobic Incubation; Photobioreactor; Hydrogen Production.*

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A. INTRODUCTION

The primary source of energy consumed worldwide remains conventional fossil fuels including coal, oil, and natural gas (Liu et al., 2012). Nevertheless that these conventional fuels are nonrenewable, with diminishing reserves and rising costs, is well known (Cai & Wang, 2013; Lei et al., 2012). Solar energy, hydropower, wind energy, geothermal energy, biomass energy, and ocean energy are among the currently identified renewable energy sources. The search for new fuel sources to replace fossil hydrocarbons has accelerated. Hydrogen is regarded as an ideal, non-polluting fuel for the future (Gest & Kamen, 1949).

Biomass is one of the promising renewable and neutral consider the carbon dioxide emissions. Biomass is the organic materials produced by reaction between carbon dioxide, water, sunlight, and other nutrients through photosynthesis (Sugie et al., 1984). There are several sources of biomass energy are being developed in several countries. The use of algae (microalgae) as a raw material has a bright prospect for microalgae easily cultivated and can produce more than other raw materials.

The production of biofuel from algae has received the most attention in recent years compared to other potential products. The conversion of plant storage carbohydrates (sugars and starch) into fuel was the foundation of the first generation of biofuel production (Manwell et al., 2010). The production of biofuels is one of the steps taken to increase access and reduce reliance on fossil fuels, particularly since

they are currently the only short-term alternative source of energy for mass transit until the advent of electro mobility. Based on the review, this study aims to analyze the principles of producing hydrogen in microalgae and identify some processes for producing hydrogen from various algae species.

B. METHOD

1. Microalgae Characteristic

Microalgae are thallophytes (plants without roots, stems, and leaves) that have chlorophyll as their part of photosynthesis pigment and lack a sterile protective cover of cells around the reproductive cells (Klass, 1998). Due to the presence of chlorophyll and the capacity for photosynthetic activity in a single algae cell, which makes it easier to generate biomass and conduct genetic and metabolic research effectively and more quickly than with conventional plants, they are unique among microorganisms (Kenichiro & Shigeki, 2010). The concept of using microalgae as a biofuel source is not new, and even though it is now being taken seriously due to the rising cost of petroleum and, more importantly, the growing concern about greenhouse gases emitted by the use of fossil fuels (Schubert, 2006).

Scenedesmus is among the most popular freshwater microalgal species in general, but identification is difficult due to the extremely diverse morphologies found within species. *Scenedesmus sp*. could exist as single cells, because they are more commonly found in coenobiums of four or eight cells within a parental mother wall (Brennan & Owende, 2012). Numerous studies have been conducted to optimize biomass productivity along with lipid content by varying the concentration of supplemental nutrients; currently, Scenedesmus lipid yield ever since enhancement has reached 60% dry cell weight, which is lower than some other algae (Singh & Sharma, 2012; Pulz, 2001).

2. Photobioreactor

However almost all biotechnologically significant parameters can be regulated and controlled in photo bioreactors, which also have a low risk of contamination, no CO2 losses, repeatable cultivation conditions, manageable hydrodynamics and temperature, and adaptable technical design (Pulz, 2001). A simple photo bioreactor was designed for laboratory scale with light supply system by using sunlight that has been efficient in supplying light energy for phytoplankton for optimally photosynthesis process. The light condition in reactor was faced towards the west or sunrise. The photosynthesis process carried out through the dark and light treatment in the column of photo bioreactor, the incubation period can trigger electron donor in the hydrogenate enzyme. This condition produced the maximum of colonies phytoplankton with scale of concentration was 10^8 cell/ml in 2 weeks, derived from the production of biomass algae.

3. Culturing of algae and growth condition

The determination of seeds in terms of availability of algae was found in Indonesia. One of algae seed found in Indonesia is *Scenedesmus Sp.* The seeds were taken from LIPI (The Indonesian Academy of Sciences) in Cibinong, West Java. PHM media was used as culture media and served as source of nutrients in culturing cell growth of *Scenedesmus sp.* while PHM-S was used as experiment media and served as pressure rate of oxygen production and glucose at photosynthesis process. The composition of PHM media containing KNO_3 (1mg), K_2HPO4 (0.2 mg), $MgSO_4$ (0.2 mg) were dissolved in 1 L of aquades with Fe solution (1 ml) and Tracer metal solution (5 ml) was added and mixed together until form a homogenized solution. The composition of PHM-S was made from the same substance as PHM media; at the same concentrations, all sulphates have been overtaken to chloride salts (S-deprivation).



Figure 1. Culturing of algae *Scenedesmus sp* for (a) 4 days; (b) 10 days

For two weeks, the culture has been incubated at room temperature under fluorescent light illumination of 18 Watt for 24 hours per day, as shown in figure 1. Through streaking the cell culture on, a single colony was obtained on PHM. The algae was grown in 300 ml volumetric each flask added to 100 ml of algae seed for the heterotrophic growth condition. It was cultivated at room temperature and was harvested in glass vessel each containing 10 ml and sealed with a rubber stopper followed by centrifugation in 2,000 rpm for another 20 minutes at room temperature then the PHM was removed by PHM-S. The algae washed in PHM-S by centrifugation in 2,000 rpm for another 20 minutes in the same temperature.

4. Analysis of Hydrogen Production

The anaerobic incubation condition was performed by dark treatment for 24 hours under dark treatment and the cells were allowed to incubate at room temperature. In the dark treatment, light condition and other equipment such as fan stirrer were being turn off and hose infuse linked the solution algae with a shelter gas closed by a slot infuse, this condition aims to solution algae in space experiment experienced state of anaerobic donor of electrons in photosynthesis. The purpose of donor electrons is to enable the electron donor hydrogenate enzyme to generate hydrogen gas at the light treatment. Hydrogen gas evolution was determined by analyzing gas phase by a Natural Gas Analyzer (NGA): HP (Hewlett Packard) 6890 with a molecular sieve 5A (CH4, O2. N2, H2) using a thermal conductivity detector

on a mesh packed column the temperatures of the injection system and detector were kept at 200oC, while the oven temperature was kept at 90oC.

Even during hydrogen analysis, helium gas has been used as a carrier gas. The term of hydrogen managed to evolve per time was used to calculate hydrogen production (%molH2). In this study, we made two experiment with different volume of algae and different algae density. The isolated algae was grown in 2,700 ml and 2,500 ml of PHM-S medium under sun light as described above for 64 hours (first experiment) and 300 hours (second experiment). Hydrogen gas was determined every 8 hours by algae density at 5.44 x 108 cell/ml and 2.16 x 108 cell/ml, respectively. Hydrogen evolution of cells at 16, 24, 32, 40, 48, and 56 hours of first experiment and at 108, 120, 216, 228, 240 and 288 hours of second experiment of incubation was measured.

C. RESULTS AND DISCUSSION

1. Measurements of a Algae Suspension

The density of algae *Scenedesmus sp.* was measured using microscopic and tabulated in Table 1. It can be seen from the table that the algae Scenedesmus sp. from first experiment shows highest value of algae density.

In this study the dark treatment was fixed variable at 24 hours, this condition to development of colonies algae when unable sunlight during one day. It can be concluded that the increasing of the total volume of algae during incubation time can enhancement the algae density.

Table 1. Description of algae Scenedesmus sp. and experimental values obtain from Natural Gas Analyzer (NGA)

No	Specifications	1st experiment	2nd experiment
1	Dark Treatment	24 hours	24 hours
2	Volume of algae	2,700 ml	2,500 ml
3	Algae density	5.44 x 10^8 cell/ml	2.16 x 108 cell/ml
4	Incubation Time	64 hours	300 hours
5	Total of Hydrogen Production	15.64 %molH_2	18.65 %molH_2

This table also shows highest value of hydrogen production in second experiment. It was obtained because the second experimant had longer for incubation time so the photosynthesis process was took longer then algae could produce more hydrogen gas.

2. Growth and Produce Hydrogen of Scenedesmus sp.

In the first experiment *Scenedesmus sp*. was incubation in 2,700 ml of PHM-S medium for 64 hours. Growth by optical density at 5.44 x 10^8 cell/ml was determined in several days of cultivation. Hydrogen evolution of *Scenedesmus sp*. was measured in cells at different time every 8 hours. In figure 3(a), we found the total of hydrogen gas production was 15.64 %molH_2 in cells grown for 64 hours (late-log phase cells) in PHM-S medium. At 64 hours of incubation, the hydrogen production of cells

decreased before algae cell died and turned bleach (a condition where algae change its color from green to white), as shown in figure 2.



Figure 2. The colour conditions of algae *Scenedesmus sp* for (a) before incubation; (b) after incubation time

In second experiment, algae were incubated in 2,500 ml of PHM-S medium for 300 hours with algae density was smaller than first experiment at 2.16 x 10^8 cell/ml. Figure 2(b) show the hydrogen productions of *Scenedesmus sp*. was measured in cells at the same time as first experiment and the highest total of hydrogen gas production was 18.65 %molH_2 in cells grown for 300 hours (late-log phase cells) in PHM-S medium. At 300 hours of incubation, algae condition was similar as the first experiment. It took a long time to produce hydrogen gas due to unfavorable weather (no sun light for several days) which affected the photosynthesis process and hydrogen production. The anaerobic incubation caused hydrogenase activity, which led to H2 evolution in the subsequent stage of light illumination. The duration of anaerobic incubation and the absence of sulphur as from medium had a significant influence on H2 evolution. Theoretically can be explained that for every production of hydrogen from algae has to be preceded by the reaction enzymatic in algae that does not produce hydrogen within a certain timeframe, then at subsequent intervals of new algae the hydrogen was produced. It is possible that oxygen, an inhibitor of hydrogenate enzyme, was reduced during anaerobic adaptation, resulting in an increase in producing hydrogen during first 2 hours after anaerobic adaptation. Due to the general decrease in electron and proton donors in cells, as well as the limitation of hydrogenate enzyme, incubation under anaerobic environments for further than 2 hours did not encourage higher hydrogen production (Rattana et al., 2012).

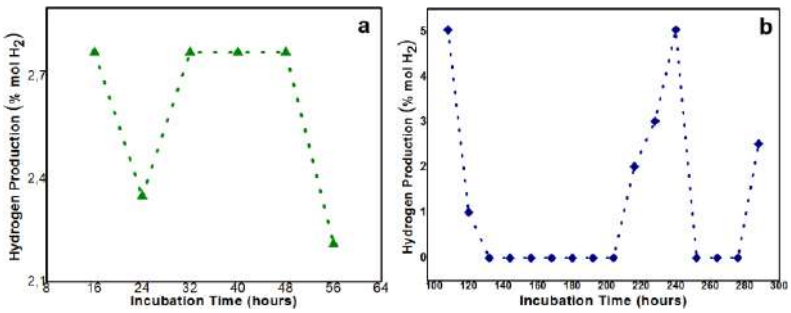


Figure 3. Hydrogen Gas Production of algae *Scenedesmus sp* for (a) first; (b) second experiment

D. CONCLUSION

In conclusion, it is critical to develop clean, renewable, and environmentally friendly energy led to concerns about global warming and environmental pollution brought on by the use of fossil fuels, as well as projections of a potential shortage of fossil fuels by the middle of the twenty-first century. There is still a lot of fundamental research to be done on the mechanism of hydrogen production by S-deprivation, as well as the cell metabolism and essential biochemistry that support this process. As a result hydrogen can be produced by using *Scenedesmus sp.* under anaerobic and photosynthesis process. The first experiment *Scenedesmus sp.* had a smaller hydrogen production, as analyzing phase by Natural Gas Analyzer (NGA). The most significant difference was the almost 10 times higher hydrogen production in the socond experiment as compared to the first experiment. According to the review analysis, the much improved hydrogen evolution in the second experiment could be attributed to an enzymatic reaction in algae that was primarily induced by the anaerobic adaptation, in which oxygen, an inhibitor of the hydrogenate enzyme, was reduced, leading to an increase in hydrogen production.

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