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To cite this article: M S A Abobaker *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **230** 012009

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Design and performance of bioreactor for fermentative biogas production from marine microalgae

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Abstract. Novel application of marine microalgae as a new source of renewable energy production, by using anaerobic digestion route, is presented in this paper. Traditionally, biogas was produced from animal manure. Microalgae however, has a potential application to be fermented and converted into biogas. This work describes the design of bioreactor to produce biogas. The bioreactor was made of stainless steel tube of 250 ml with a thickness of 2 inches, and surrounded by a water jacket with the height of 10 cm, diameter of 8 cm. The temperature was controlled using thermocouple and data logger. The bioreactor contains stirring system to maintain good mixing. On the top side, a hose is used as biogas outlet and the gas pressure was measure. Testing of bioreactor was done using *Dunaliella salina* microalgae as main substrate and tofu wastewater as additional substrate. The anaerobic digestion was maintained from 7, 14 and 21 days, and the biogas produced were collected. The methane content analyzed by using gas chromatography and despite its low methane production, it is found that microalgae is potentially used as biogas feed.

1. Introduction

Recent years experienced renewable energy production from biomass, including biofuels and biogas [1]. Most biomass derived from in-soil crops (agriculture), and is still limited utilization of biomass derived from aquatic plants or organisms (aquaculture) such as microalgae. The abundant cultivation of microalgae relative to other biomass, has potentially made microalgae as good alternative for biofuel production [2].

Microalgae could grow 5-10 times faster making the production rate higher compared to terrestrial biomass [3]. Moreover, microalgae can be grown in arid zones such as deserts or coastal lands [4]. The use of fast-growing microalgae species for anaerobic fermentation for the production of biogas, might allows the replacement of natural gas resources. Actually, the search for anaerobic fermentation of algae biomass dates back more than 50 years ago [5]. A number of research projects have been rolled out and intensified; early research efforts culminated in the late 1970th and 1980th as a result of the first oil crises, species included investigation which took many macroalgae such as *Macrocystis*, *Gracilaria*, *Hebonia*, *Olva*, *Laminaria* and *Sargsum*. Recently, attention has been highlighted in the use of algae to generate energy and obtain biogas by identifying micro algal strains with striking properties [6] and also advancing and deepening in micro-algae cultivation [7] and advancing



microalgae harvesting using membranes [2, 8-9]. This paper reported the potential of production of methane from the microalgae *Dunaliella salina* species, where it is considered to be one of the microalgae strains that produce a high quantity of methane using tofu substrate. The use of a customized laboratory scale bioreactor is also presented.

2. Materials and Methods

2.1. Design of bioreactor

The bioreactor structure is designed from stainless steel tube with a thickness of 2 mm surrounded by a jacket. The bioreactor tube has a volume capacity of 250 ml with a diameter of 5 cm and a height of 12.5 cm. The jacket surrounding the bioreactor is made of stainless steel and through it is passed water is increased temperature through the heater when the need to increase the temperature inside the bioreactor. Double jacket has a height of 10 cm diameter 8 cm so that the distance between the reactor tube and double jacket is 1.5 cm. There is a thermocouple on the inside of the bioreactor to measure the temperature of the substrate where the thermocouple will be connected to the data logger. The temperature control system is performed using a double jacket with a heat conducting medium of water that is circulated from the heater. The reactor also contains stirring system using a magnetic stirrer. At the tube cap, there is a hose as the biogas output passing through the manometer to measure gas pressure, to include gas collection tubes as shown in Figures 1 and 2 below.

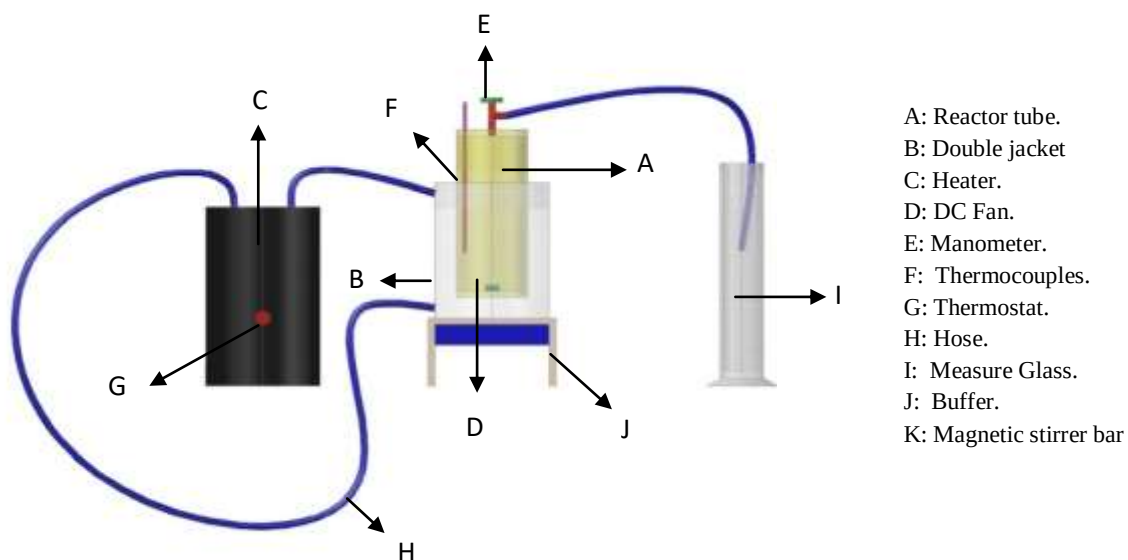


Figure 1. Bioreactor system for fermentative biogas production.

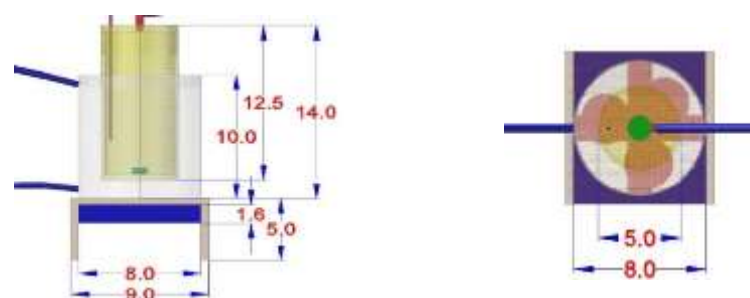


Figure 2. Schematic view of bioreactor (*left*: front view; and *right*: upperside view).

2.2. Fermentation test

The substrate used for biogas production consists of dry microalgae from *Dunaliella salina* species obtained from Brackish Water Aquaculture Center at Situbondo, Indonesia. Tofu liquid waste is obtained from local factory in Malang. Meanwhile, activated sludge was obtained from PT. Amerta Indah Otsuka, Pasuruan.

Substrate was prepared by weighing 1 gram of microalgae using an analytical scale, then measuring the tofu wastewater that had been raised to pH of 6-8 by 100 ml and 20 ml of activated sludge with a measuring cup. Microalgae, tofu liquid waste, and activated sludge are then introduced and mixed into the bioreactor simultaneously. The bioreactors were then operated for 7, 14, and 21 days, measured as Reactor 1, 2 and 3, respectively, under anaerobic conditions under stirring condition. During the anaerobic digester process, biogas will be produced which will then be measured in volume and CH₄ levels on desirable days of 7, 14 and 21. Several parameters were measured, i.e. pH and temperature of the substrates, temperature and pressure of biogas, volume of biogas and methane content.

2.3. Biogas volume analysis

The biogas volume analysis generated from the anaerobic digester process is carried out by measuring the COD (Chemical Oxygen Demand) substrate value on days 0, 7, 14, and 21. From the data, the COD value is calculated which is calculated in 3 times, namely from day 0 to 7, 0 to 14, and 0 to 21. From the results of the COD reduction can be calculated how much volume of gas is produced by using the following formula [7]:

$$V_g = [(COD_0 - COD_n) \times V_s] \times 0.44 \text{ m}^3 \quad (1)$$

where:

V_g : Gas volume (L)

V_s : Substrate volume (L)

COD_0 : COD on day 0 (mg / L)

COD_n : COD on day n (7, 14 and 21) (mg / L)

0.44 m^3 : The biogas volume for every 1 kg decrease in COD

2.4. Determination of CH₄ level

The determination of CH₄ gas content was done by using Flame Ionization Detector for Gas Chromatography (GC-FID). The measurement of CH₄ levels was carried out on the 7th, 14th, and 21st days by flowing gas through a hose connected to the reactor, which then entered the gas cylinder. The gas sample in the gas storage tube was taken using a syringe. The sample then was injected into the septum and into the sampling valve, and then entered into gas chromatography and the data is analyzed in peak form and interpreted in shape area.

3. Results and Discussion

3.1. Temperature and pressure

The reaction rate of biogas formation is influenced by temperature because temperature plays an important role in regulating the course of bacterial metabolic reactions in anaerobic bioreactors. Ambient temperature that exceeds the tolerance limit of microorganisms will cause protein and essential cells to be damaged so that the cell will die. Similarly, if the temperature is less than the tolerable limit, it will cause nutrient transportation to be hampered [10, 11]. The temperature observed is the temperature in the bioreactor tube as a place for the formation of gas by using a thermocouple connected to the data logger.

Pressure is one indication that gas is formed. The gas formed will accumulate in the bioreactor during the fermentation process. In making biogas, the pressure usually increases with the length of the fermentation time [12]. The reactor used has a fixed volume so that when more gas is produced

and fills the room, it will cause pressure increase from inside the bioreactor. In this study the pressure was observed every 24 hours to find out whether or not gas was formed.

Figure 3 shows the temperature and pressure profiles during fermentation of microalgae substrate. The pressure graph shows that each reactor has different pressures where Reactor 2 has the highest-pressure profile with a range of 0.05 to 0.2 bar or equivalent to 5000-20.000 N/m². In contrast, Reactor 3 for 21 days fermentation has the smallest pressure profile with a value range of 0-0.03 bar or equivalent to 3000 N/m² where the pressure starts to be measured on day 9. At Reactor 1, the measured pressure ranges from 0.025-0.06 bar. Of the three reactors showed a linear relationship between fermentation time and biogas pressure.

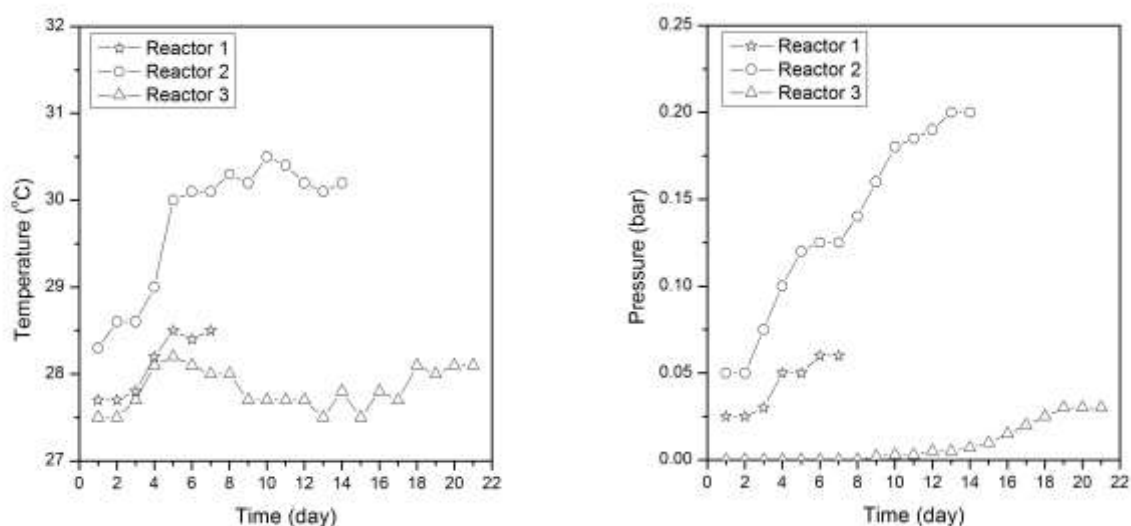


Figure 3. Biogas temperature profile (left) and pressure profile (right) during fermentation time.

3.2. Chemical oxygen demand (COD) and biogas volume

Chemical Oxygen Demand or chemical oxygen demand is the amount of oxygen needed to oxidize substances in a substrate chemically. The COD value in this study is an indication that the degradation of organic matter by bacteria could then produce a certain amount of gas. COD testing was carried out on the substrate before fermentation, after fermentation for 7 days, 14 days and 21 days which is presented in Figure 4 (left). From these data shows that the relationship between COD value and fermentation time is inversely proportional while the relationship between COD reduction and fermentation time is directly proportional.

The biogas volume was calculated by decreasing the COD value, by using Equation (1). It was explained that the greater of COD reduction, indicating that organic matter which is degraded to organic acids also greater, then organic acids are converted to methane gas [13]. Therefore, a decrease in COD indicates biogas production which can produce a certain volume of gas [14]. In addition to being influenced by the COD value, the volume of gas produced also depends on the length of fermentation time because the longer the time used, the more organic material can be consumed by bacteria and converted into gas in the digester. The relationship between biogas volume and fermentation time in this study is presented in Figure 4 (right).

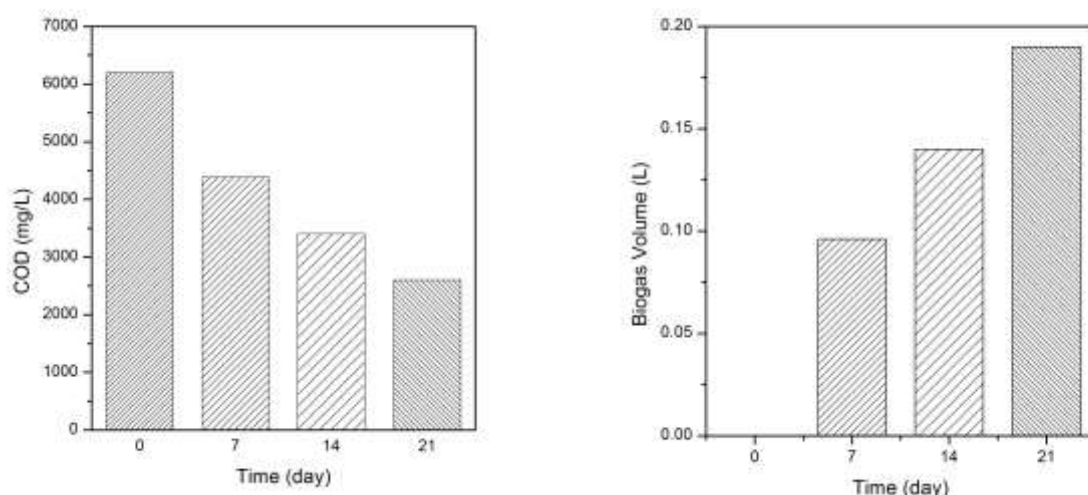


Figure 4. Relationship of COD value (*left*) and biogas volume (*right*) with fermentation time.

As shown, the longer the time used for fermentation, the greater the volume of biogas produced. In this study, the smallest volume is obtained at 7 days fermentation with a COD value that is greater than the 14th and 21st days. On the 21st day, the highest biogas volume is obtained, which on the 21st day has the smallest COD value. This shows that the volume of biogas is inversely proportional to the COD value and is directly proportional to the time of fermentation. The decreasing COD shows that organic matter has been degraded into organic acids, organic acids are then converted by methanogenic bacteria into methane, CO₂ and other gases.

3.3. Methane levels

Methane is an important component in biogas due to a high heating value which acts as a fuel. This compound is produced from the methanogenesis stage where organic acids are converted by methanogen bacteria into methane and a number of other compounds such as carbon dioxide, ammonia, hydrogen sulfide, oxygen, hydrogen, and nitrogen. In this study, the composition of gases obtained includes CH₄, CO₂, and air with other gases were characterized by using GC-FID [15, 16] and summarized in Table 1.

Table 1. Composition of biogas produced.

Time (Days)	Substances	Peak Area (GC-FID)	Concentration (%)
7	CH ₄	-	0
	CO ₂	1815	-
	Air (other gases)	1613801	-
14	CH ₄	93783	0,056
	CO ₂	1608348	-
	Air (other gases)	-	-
21	CH ₄	2064	4,57
	CO ₂	72316	95,43
	Air (other gases)	1552037	-

As shown, the Reactor 1 showed methane content of 0% or no methane gas had been obtained. During the 7 days of fermentation, the bacteria have not reached the methane or methanogenesis formation stage, but have gone through the stages of hydrolysis and acidogenesis because they have produced gases such as CO₂ and air or other gases. All gases normally produced simultaneously at constant rate, when hydrolysis, acidogenesis and methanogenesis processes works properly [17] CO₂ and air then cause pressure in the reactor increase. The degree of acidity (pH) of the substrate at 7 days dropped to 6.8. The decrease in pH value is caused because the substrate has passed the acidogenesis reaction where there is a modification of simple compounds resulting from hydrolysis to organic acids. Methane gas began to form in fermentation for 14 days with a very small value of 0.06%. The presence of methane gas indicates that the methanogenesis reaction can be achieved within 14 days.

In Reactor 2, the highest pressure is obtained, this is due to the presence of CO₂ and other gases that are formed. This causes the pressure inside the reactor to increase to 0.2 bar. Acidity (pH) is obtained on a 14-day substrate lower than pH on 7 days of fermentation which is 6.6. This is because the process of acidogenesis and acidogenesis in Reactor 2 lasts longer until finally to the stage of methanogenesis. At 21 days fermentation (Reactor 3) there was an increase in substrate to 6.8 and methane content of 4.57% which was the highest methane content compared to fermentation for 7 days and 14 days. The results show positive contribution of *Dunaliella salina* microalgae as a source of biogas [18]. The methane content however is still lower than others, due to two folds. First, the reaction time. 21 days fermentation may not sufficient enough to allow the methanogenesis process completed, and longer fermentation time is needed. Secondly, the anaerobic digestion in this study was conducted at average temperature lower than 30°C. Previous studies show that the optimum temperature of 35-38°C required to achieve higher gas methane production [19]. The next study is required to test the biogas production of *Dunaliella salina* at this optimum temperature as well as different biological substrates.

4. Conclusion

Based on the results of the study, the following conclusions can be drawn. *Dunaliella salina* dried microalgae with tofu liquid waste can be used as a substrate in making biogas as a development of renewable energy. Temperature is an important and fundamental factor affecting the volume and quantity of gas produced. The fermentation time affects the amount of biogas volume and methane content produced where the highest biogas volume and methane content are found at the time of fermentation for 21 days with a value of 0.19 L biogas volume with methane content of 4.57%. Further research is needed to determine the optimal yield of biogas from *Dunaliella salina* and tofu liquid waste by increasing the substrate size and C/N ratio, extending the fermentation period as well as maintaining fermentation temperature.

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