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Alternative bioenergy through the utilization of *Kappaphycus alvarezii* waste as a substitution of substrate for biogas products

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Abstract. Biogas is one of the renewable energy resources which are a to be developed by providing some sufficient renewable substances and manufactured from the fermentation process of organic substances metabolized by anaerobic bacteria. In this research, *Kappaphycus alvarezii* seaweed waste from carrageenan processing and contents of rumen were used. This research aims to comprehend the carrageenan processing waste of macroalga *K. alvarezii* can be used as alternative source generating biogas. The research method is P0 (100 % of the contents of rumen), P1 (75 % of the contents of rumen and 25 % of seaweed waste), P2 (50 % of the contents of rumen and 50 % of seaweed waste), and P3 (25 % of the contents of rumen and 75 % of seaweed waste), and P4 (100 % of seaweed waste). The result showed that according to the quality determination of biogas based on SNI (Indonesia National Standard) 8019:2014, the *K. alvarezii* seaweed waste from carrageenan processing can be utilized as the alternative source of manufacturing biogas and got the methane gas resulted from the comparison method is P2 (50 % of the contents of rumen and 50 % of seaweed waste), with value of 58.61 %.

1. Introduction

The need for fuel has increased along with population growth, regional development and energy crisis in the world. The scarcity of fuel caused by significant rise of world's oil prices has pushed the government to invite people to deal with energy. Furthermore, many studies have been conducted in order to find alternative energy sources as renewable resources [1]. An increase in the population's demand for energy, the depletion of world's oil reserves and the problem of fossil fuel emissions put pressure on every country to immediately produce and use renewable energy [2]. Indonesia has various natural resources which can be utilized as energy. Natural resources in the form of oil, gas, coal, geothermal heat, water and so forth. They can be used in various development activities directly or exported to earn state revenues. These materials are not renewable. The use of non-renewable resources causes crisis problems. One of the signs of energy crisis occurring lately is scarcity of oil fuels, such as kerosene, liquefied petroleum gas, gasoline and diesel fuel.

Scarcity happens because the need for fuels is very high and keeps on increasing every year. On the contrary, crude oil as the raw material for making fuel is limited and it takes time for the fuel to be produced [3]. Hence, alternative energy is necessary for preserving oil reserves at the present time.

Biogas is one of energy sources that can be developed by providing raw materials available in the nature in sufficient amount (renewable condition). Renewable energy sources are relatively easy to obtain, require low operating expenses, and do not cause any waste problem thus oil shortage can be overcome.

A biogas is a flammable gas derived from the process of fermentation of organic materials by anaerobic bacteria that do not need oxygen to survive and reproduce. Generally, all types of organic materials like rumen contents and animal's urine suitable for simple biogas system can be processed to produce biogas [4]. Biogas can be used to produce methane as a substitute for fuels, especially kerosene, and can be used for cooking. On a large scale, biogas can be used as the material for power plant. In addition, the process of biogas production will produce residue of cattle's rumen contents that can be used directly as organic fertilizers for crops or agricultural cultivation. More importantly, it can be used to reduce the dependence on non-renewable fuels [5].

One of the commodities in Indonesian waters that is potential to be developed is seaweed. Seaweed is widely used in and profitable for the industries of food and beverage, personal care, cosmetics, animal feed, steel, ceramic, paint, ink, mining, coal briquette and asphalt, paper and pulp, textile, fertilizer and medicine (pharmacy) as food fibers [6]. Seaweed can be processed into various food and will produce waste (unused), so what is available can be utilized. According to Alamsjah and Prayogo [7], seaweed waste is usually left accumulating at landfills, but it has the potential to be processed into biogas. An example of seaweed is *Kappaphycus*, that can be extracted into carrageenan. The waste from the processing of carrageenan is still underutilized. According to Saputra *et al.* [2], biogas may come from various organic matters such as cattle's rumen contents, feces, scrap paper and aquatic plants (e.g. hyacinth, filamentous algae), seaweed and seaweed waste. The C/N ratio in the making of biogas should be noted. The C/N ratios for *Kappaphycus* and carrageenan waste are 43.98/L and 55.01/L, respectively.

Cattle's rumen contents are the most suitable starter in observing the use of biogas because they have C/N ratios of between 11 and 30 [8]. Another important thing found in the substratum of rumen of a cow is bowel bacteria producing methane. The bacteria in the bowel of ruminants can break down organic compounds using fermentation, so that the waste processing in anaerobic animals can produce gases consisting of methane (CH₄) and carbon dioxide (CO₂). The substrate in cattle's rumen contents consist of bacteria that produce methane in the stomach of ruminants. The bacteria in the bowel of a ruminant help with fermentation process, thus the biogas production in digester's body can be done faster. In addition, fresh rumen contents are easier to process compared with long and/or dried rumen contents during the drying time [9].

2. Methodology

A research method was used to solve a problem, which may be done by data collection through observation, survey and experiments [10]. The method used in this research was descriptive method. Descriptive method is a planned attempt to express new facts, corroborate a new theory, or even deny the existing research results. The study was done by observing and comparing the amount and volume of methane and carbon dioxide and the C/N ratio resulting from each research treatment.

The materials used in this study were 10,000 g of carrageenan processing waste of *Kappaphycus*, 10,000 mL of fresh rumen contents and 4,000 mL of water. Into each treatment was added 200 mL of water. The following was used in this research: P0 = 1,000 mL of rumen contents + 0 g of carrageenan processing waste (100 % : 0 %); P1 = 750 mL of rumen contents + 250 g of carrageenan processing waste (75 % : 25 %); P2 = 500 mL of rumen contents + 500 g of carrageenan processing waste (50 % : 50%); P3 = 250 mL of rumen contents + 750 g of carrageenan processing waste (25 % : 75 %); and P4 = 0 mL of rumen contents + 1000 g of carrageenan processing waste (0 % : 100 %). The materials used in the preparation of anaerobic fermentation process were carrageenan seaweed waste (substrate), rumen contents (co-substrate) and water. The addition of water as one of the materials was aimed to meet the water levels required for biogas production.

The number of bioreactor or digester used in this research was 20. There were 5 treatments and they were repeated 4 times. The volume of a bioreactor was 1,500 mL, and the bioreactor was made from plastic, with an airtight cover, faucet and hose. The cover was connected to the faucet, that was connected to the hose. The hose was connected to top gas in the form of a plastic bag fastened with rubber band and cling wrap. The substrate consisted of cattle's rumen, carrageenan waste and water. Cow's rumen and carrageenan waste were mixed at a ratio as specified. Every tube digester will contain filling materials (processed carrageenan, seaweed and rumen contents) and water at 80% of the fermenter volume [11].

The fermentation system in the biogas production in this research used a closed system (batch fermentation) for 21 days of fermentation. For every treatment, four tests were conducted. The biogas produced filled the space in the bioreactor, then moved toward the hose to be accommodated at the gas on the top. The gas to be accommodated was directly analyzed according to the length of time of fermentation. According to Angraini *et al.* [12], the length of time of fermentation required to produce methane (CH₄) was 21 days. The biogas was analyzed by considering the levels of methane parameters, volume of gas and carbon dioxide. The biogas sample was extracted into plastic bag, then the volume of the biogas was measured using a volume meter and the levels of methane (CH₄) and carbon dioxide (CO₂) was measured using gas chromatography. The analysis of C/N ratio was conducted by measuring the C-organic content and the total nitrogen of the sample.

The analysis of C-organic was conducted in this research using cremate method. Included in the procedure of C-organic analysis was the weighing of an empty cup (a) of each sample treatment at about 1 g. Then, the sample was put into an oven for 4 hours at a temperature of 105 °C. Then, the cup was cooled in desiccator for 15 minutes. The sample and the cup were weighed again after being removed from desiccator (c). In the next step, the cup that contained the sample was put in a furnace (600 °C for 4 hours), then the cup was incorporated into a desiccator after being removed and weighed for the final measurement (d). The calculation for this analysis used the equation below:

$$\% \text{ of water content} = \frac{b-c}{b-a} \times 100\% \quad (1)$$

$$\% \text{ of ash content} = \frac{d-a}{b-a} \times 100\% \quad (2)$$

$$\% \text{ of organic substrate} = 100 - (\% \text{ of water content} - \% \text{ of ash content}) \quad (3)$$

$$\% \text{ of C-organic} = \% \text{ of organic substrate} \times \frac{58}{100} \quad (4)$$

Note: $\frac{58}{100}$ is the conversion of organic substrate to carbon

The analysis of the total nitrogen in this research used Gunning method. This analysis was conducted in three stages, namely destructor, distillation and titration. The destructor stage was aimed to destroy material (sample) through the addition of K₂SO₄ and CuSO₄. One g sample was put into Kjeldahl squash and added with 10 mL of H₂SO₄, 5 g of K₂SO₄, 0.3 g of CuSO₄ and stones boiling. Next, the mixture was heated on an electric heater, firstly at low heat and then at high heat. Warming ended after the solution became colorless. As much as 100 mL of ddH₂O, 1 g of Zn and 50 mL of 45% NaOH were added to the Kjeldahl squash. Then, the Kjeldahl squash was mounted in the stage distillation. The mixture was distilled until the volume of distillation reached a value of 75 mL.

The titration was started by pouring 75 mL of distillate on titration squash, added with 50 mL of 0.1 N HCl and PP indicator. The titrant used was 0.1 N NaOH. Titration was carried out to figure out the volume of NaOH needed to neutralize sample. The levels of N (%) can be calculated using the equation below:

$$N (\%) = \frac{\text{Volume of NaOH blank} - \text{Volume of NaOH sample}}{\text{massa} \times 1000} \times N \text{ NaOH} \times 14.008 \times 100\% \quad (5)$$

The C/N ratio could be calculated after obtaining the C-organic level and the total nitrogen value. The C/N ratio can be calculated using the equation below:

$$\text{Ratio of C/N} = \frac{C\text{-organik}}{N\text{-total}} \quad (6)$$

3. Results and discussion

This research yielded the methane levels, C/N ratio (before and after fermentation), pH and temperature value (before and after fermentation). The C/N ratio of rumen contents to seaweed waste was 41.04:36.5. The organic carbon of rumen contents was 28.15 and the total nitrogen was 0.68, while the organic carbon of seaweed waste was 26.80 and the total nitrogen was 0.73. The measurement of the biogas production in this research was conducted in terms of quality (levels of methane in biogas) and quantity (biogas volume). The methane levels and the biogas volume produced in each of the rumen contents and seaweed carrageenan waste are displayed in table 1.

Table 1. The average levels of methane (%) and biogas volume (mL) from the comparison of rumen contents and seaweed waste.

Treatment	Methane Level (%)	Biogas Volume (mL)
P0 (100 %:0)	40.70 ± 2.97	198 ± 183.85
P1 (75 %:25 %)	56.51 ± 5.67	409 ± 74.95
P2 (50 %:50 %)	58.61 ± 2.56	1182.5 ± 109.60
P3 (25 %:75 %)	56.50 ± 7.07	1306 ± 113.14
P4 (0:100 %)	44.35 ± 2.47	746.5 ± 160.51

Note: P0 (100 % : 0 %) = 1000 mL of rumen contents + 0 g of carrageenan processing waste; P1 (75 % : 25%) = 750 mL of rumen contents + 250 g of carrageenan processing waste; P2 (50 % : 50 %) = 500 mL of rumen contents + 500 g of carrageenan processing waste; P3 (25 % : 75%) = 250 mL of rumen contents + 750 g of carrageenan processing waste; P4 (0 % : 100 %) = 0 mL of rumen contents + 1000 g of carrageenan processing waste.

Table 1 showed that there was a dynamic of methane levels for each ratio of rumen contents to seaweed waste in all treatments. Treatment P2 yielded the highest average levels of methane (58.61%), while the highest average value of biogas volume was yielded by treatment P3 (1306 mL). If the levels of methane and the biogas volume were related, the following equation was obtained:

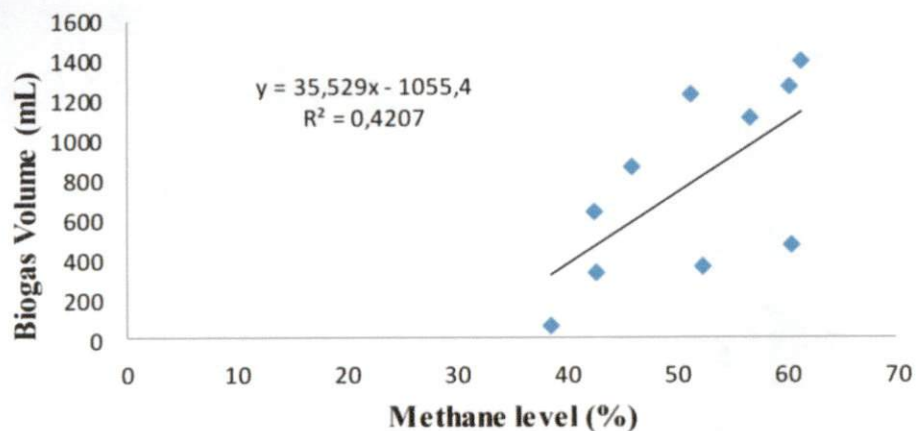


Figure 1. The relation between the methane levels and biogas volume in each ratio of rumen contents and seaweed.

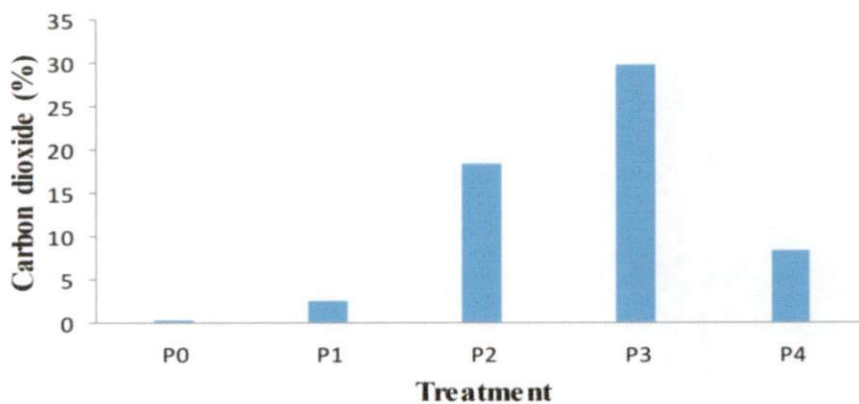


Figure 2. The average levels of carbon dioxide (%) in each ratio of rumen contents and seaweed waste.

Note: P0 (100 % : 0 %) = 1000 mL of rumen contents + 0 g of carrageenan processing waste; P1 (75 % : 25 %) = 750 mL of rumen contents + 250 g of carrageenan processing waste; P2 (50 % : 50 %) = 500 mL of rumen contents + 500 g of carrageenan processing waste; P3 (25 % : 75 %) = 250 mL of rumen contents + 750 g of carrageenan processing waste; P4 (0 % : 100 %) = 0 mL of rumen contents + 1000 g of carrageenan processing waste.

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The biogas production can also be linked with the C/N ratio. The C/N ratios in the beginning and the end of the treatments for 21 days of fermentation are shown in Figure 3.

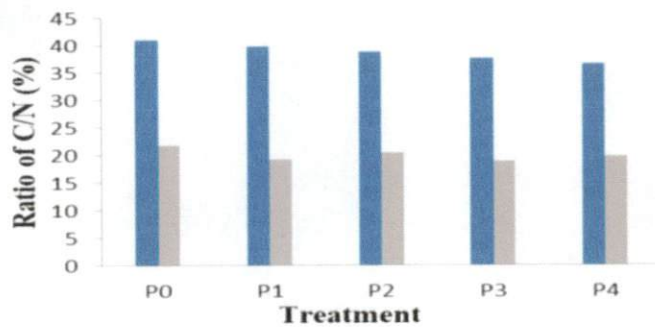


Figure 3. The C/N ratio in the beginning and the end of fermentation on every comparison of rumen contents and seaweed waste.

Note: P0 (100 % : 0 %) = 1000 mL of rumen contents + 0 g of carrageenan processing waste; P1 (75 % : 25 %) = 750 mL of rumen contents + 250 g of carrageenan processing waste; P2 (50 % : 50 %) = 500 mL of rumen contents + 500 g of carrageenan processing waste; P3 (25 % : 75 %) = 250 mL of rumen contents + 750 g of carrageenan processing waste; P4 (0 % : 100 %) = 0 mL of rumen contents + 1000 g of carrageenan processing waste; ■ ratio of C/N in the beginning of fermentation; ■ ratio of C/N in the end of fermentation

The C/N ratio decreased with research observation. The C/N ratios at the beginning of fermentation for P0, P1, P2, P2, P3 and P4 were 41.04 %, 39.84 %, 38.69%, 37.58 % and 36.5 %, respectively. Furthermore, C/N ratios at the end of fermentation for P0, P1, P2, P2, P3 and P4 were 21.85 %, 19.25 %, 20.4 %, 18.9 % and 19.7 %, respectively. In addition, the pH value and temperature before and after fermentation changed. The changes are presented in figure 4 and 5.

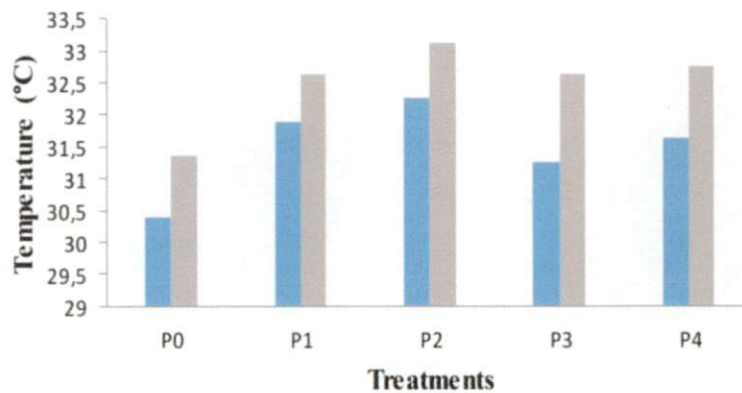


Figure 4. The average temperature in the beginning and end fermentation on every comparison of rumen contents and carrageenan processing waste.

Note: P0 (100 % : 0 %) = 1000 mL of rumen contents + 0 g of carrageenan processing waste; P1 (75% : 25%) = 750 mL of rumen contents + 250 g of carrageenan processing waste; P2 (50% : 50%) = 500 mL of rumen



contents + 500 g of carrageenan processing waste; P3 (25 % : 75%) = 250 mL of rumen contents + 750 g of carrageenan processing waste; P4 (0% : 100%) = 0 mL of rumen contents + 1000 g of carrageenan processing waste; average temperature in the beginning of fermentation; average temperature in the end of fermentation.

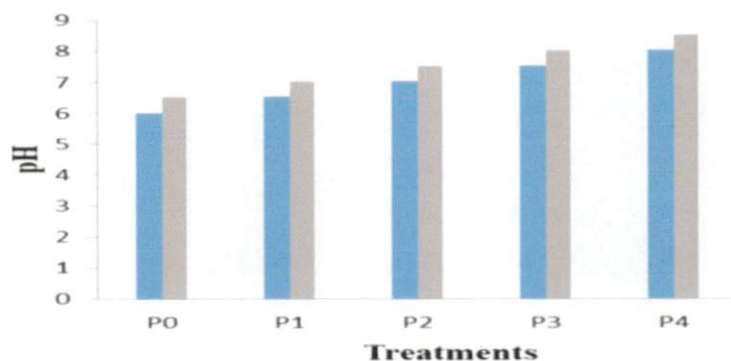


Figure 5. The average pH for the beginning and the end fermentation on every comparison rumen contents and carrageenan processing waste

Note: P0 (100 % : 0 %) = 1000 mL of rumen contents + 0 g of carrageenan processing waste; P1 (75 % : 25 %) = 750 mL of rumen contents + 250 g of carrageenan processing waste; P2 (50 % : 50 %) = 500 mL of rumen contents + 500 g of carrageenan processing waste; P3 (25 % : 75 %) = 250 mL of rumen contents + 750 g of carrageenan processing waste; P4 (0 % : 100 %) = 0 mL of rumen contents + 1000 g of carrageenan processing waste; ■ average pH in the beginning of fermentation; ■ average pH in the end of fermentation.

The pH and temperatures rose at the end of fermentation, whereas temperature in the beginning of fermentation ranged 30.38–31.63 °C, which increased to 31.38–32.75 °C at the end of fermentation. Meanwhile, the pH in the beginning of fermentation ranged at 6-8 and increased to 6.5-8.5 at the end of fermentation. In the anaerobic fermentation process to form biogas, bacteria have a very important role in reorganizing organic matter. All reactions during fermentation involved bacteria since the stage of hydrolysis, acidogenesis, acetogenesis until methanogenesis. The process of biogas formation must be in anaerobic condition, which means that the digester must be airtight and no air can come into it. The processing of organic compound contained in waste into methane and carbon dioxide does not require oxygen. If there is a leak in the digester during the fermentation, methane will not be formed. In general, the process of biogas making uses cow dung because cattle dung is the most suitable starter for the formation of biogas [9]. In this research, the starter used was rumen contents, while a substrate used were carrageenan processing waste. According to Triwisari [13], reports that the main compositions of carrageenan processing were cellulose (26.72 %) and carbon (46 %). Different organic contents can affect the formation of biogas and methane [14]. According to Anggraini *et al.* [12], the methane content in biogas produced on a reactor depends on the type of feeds, input composition, fermentation time and reactor capacity. The highest methane was produced in P2 (50 %:50 %) with 500 mL of rumen contents and 500 g of carrageenan processing waste. This may happen because the raw materials of carrageenan processing waste was not degraded by bacteria completely, while rumen contents as a source of methane which have organic components were degraded by the bacteria. Organic component is a source of nutrients for bacteria to produce biogas. According to Saputro [15], Mara and Ida [16], add that organic component affects bacteria productivity in producing

biogas. The biogas volume produced was different from the levels of methane but was still influenced by the ratio of rumen contents to carrageenan processing waste. The measured biogas volume was influenced by the length of fermentation. In this research the duration of time required for fermentation was 21 days. The duration of time applied in this research was referred to the research of [12], which states that the length of fermentation time required to produce the highest amount of methane (CH₄) recorded is 21 days.

According to Mara and Ida [16], the main factor affecting the difference in the biogas volume produced is the physical properties of material caused by water content and acidity of media (pH levels). The ratio of the rumen contents to carrageenan processing waste and the addition of water will result in different properties in the blending condition of each composition. Furthermore according to Budiharjo [17] claims that the increase in the volume of biogas volume is an indication that the system has been running well and stable. According to Calzada *et al.* [18], Indarto [19], the composition and the volume of biogas depends on the different characteristics of the substrate, while the highest volume of biogas is not always offset by high levels of methane. According to Yulistiawati [20] says that the lowest C/N ratio in the substrate will produce biogas with characteristics such as low level of CH₄ and H₂ and high level of CO₂ and N₂. According to Indonesia National Standart [21] reports that biogas generally consists of CH₄ (40–70 %) and CO₂. According to Soerawidjaya [22], biogas consists of CH₄ (50–80 %), CO₂ (20–50 %) and several other gases such as H₂, CO, N₂, O₂ and H₂S. The results of the test conducted on methane to find out the ratio of rumen contents to carrageenan processing waste in this treatment showed 58.61% of methane, thus this level of methane met the standard amount of biogas. Biogas production was also linked to the change in C/N ratio that showed a decline during fermentation (21 days). The decrease of C/N ratio shows that nutrition has been used well. The C/N concentration declined with fermentation time. The decline in carbon and nitrogen was the result of biodegradation of organic matter by bacteria originating either from rumen contents or carrageenan processing waste. It is used as a source of energy for the growth of bacteria and the formation of biogas. This phenomenon was reported by Yani and Darwis [23], who states that the decrease in the C/N ratio means that carbon and nitrogen are used as nutrients by microbes to grow and develop.

Furthermore, according to Siallegan [24], shows that the reduction in C/N ratio can cause the production of biogas to stop, which in this case, the C/N ratio cannot help bacteria to produce biogas. This means that this substance can be used as fertilizer. The C/N ratio allowed for an ingredient to make solid fertilizer ranges at 12-25 [25]. The process of fermentation also affects the temperature and pH [26, 27, 28].

According to Sofyan [29], the enzyme of bacteria will change the substrate into a product and yield heat. The release of the heat involved an exothermic reaction for organic matter decomposition to the process of biogas formation in the acidifying stage. Material decomposition will result in acids, carbon dioxide and heat. The process of organic matter decomposition takes place in anaerobic condition, thus the system will produce heat, and consequently, the temperature in the reactor increases and affects the microorganisms and microbes in the fermentation process. The other thing that changed during the fermentation process is pH. Anaerobic biodegradation to organic matter will be influenced by the environment in biodigester. Acid environment is appropriate for anaerobic biodegradation. The degradation by bacteria will be affected by pH. Furthermore, [17,30,31] conclude that pH will increase after the fermentation. Bacteria can live at pH of 6.5-8.5 (optimally at pH of 7.0-8.0). This is due to the fact that the acid production will convert into ammonia and alkali compound. Afterwards, pH will decline again and become stable at the end of fermentation. The role of pH is important because such compounds produce acids and alkalis, such as organic acids and ammonium ion for the degradation of organic compounds in digester. According to kresnawaty *et al.* [32] states that at the beginning of biogas formation, acid bacteria will be active and cause pH in the digester to decrease. Then, methanogen bacteria will use the acid as a substrate, and the pH increases. This is supported by Sejati [28], who explains that the acid formed in the acidification will be used by methanogen bacteria as a substrate for producing methane and others. Subsequently, it will increase pH in digester. This

indicates that in the process of biogas production, pH is regulated naturally. The pH values impact on the activity of methanogen bacteria, thus the bacteria are very vulnerable to acid condition.

4. Conclusion

The results of the research show that according to the requirement of quality of biogas of SNI (Indonesia National Standard) 8019:2014, *K. alvarezii* waste from carrageenan processing can be utilized as an alternative source of manufactured biogas. The highest amount of methane gas resulted from the ratio method was that in P2 (50 % of the rumen contents and 50 % of seaweed waste), amounting approximately 58.61 %.

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