Biogas Production Comparison of Liquid Anaerobic Digestion (L-AD) Methods on Different Enzyme Addition

Syafrudin¹, W D Nugraha ¹, A Kahirunnisa¹, B S Ramadan¹*, M F Miftahadi ¹, S Yumaroh¹

¹ Department of Environmental Engineering, Faculty of Engineering, Universitas Diponegoro, Tembalang, Semarang, 50275, Indonesia

udin_syaf@yahoo.com

Abstract. Biogas, the gas generated from organic fermentation under anaerobic conditions, is an alternative way to combat the energy crisis. Rice husk is a biomass fuel, and as an agricultural country, Indonesia has not utilized this excessive waste opportunity. Bacteria break down organic matter without oxygen, called the Liquid Anaerobic Digestion (L-AD) method with the minimum condition of 10% total solids (TS) and 25% C/N ratio. Lignin content in rice husk could inhibit the degradation processes, hence pretreatment is a prerequisite. This study was designed to determine the optimum pretreatment concentration variation of amylase and cellulase enzyme (9%, 12%, 15%, 18%) in a persistent chemical condition (NaOH 6%). Within 40 days of observation, both biological pretreatments increased biogas yield contrary. The result showed that the pretreatment of a lower concentration of amylase enzyme (9%) produces a significant biogas yield of about 981 ml or 45.82 ml/TS. On the other way, higher cellulase enzyme concentration (18%) produced 1520 ml or 70.99 ml/TS. The rate of biogas production were (U) 1.27 (ml/gr/TS.day) for amylase and 4.60 (ml/gr/TS.day) for cellulose.

1. Introduction
As the consequences of population growth and world economic development, the world energy consumption is predicted to be over capacity [1]. Indonesia and world energy consumption rely on non-renewable energy sources (coal, natural gas, petroleum, lignite) [2]. One way to save these unsustainable energy sources is by using renewable energy alternatives [3]. The Indonesian government has also prepared regulations number 79 the year 2014, "National Energy Policy to Develop Alternative Energy Sources as a Substitute for Fuel Oil", which declared to reduce fossil fuel dependences and promote renewable resources.

Indonesia is an agricultural country with a large potential for rice husk availability. According to the Central Bureau of Statistics (2019), paddy production data for 2018 amounted to 56,573,774.00 ton, and it is predicted to produce 20% of rice husk, which equal to 11,307,554.80 ton [4]. Rice husks are a typical tropic biomass that convertible to clean energy source through biogas technology [5].

Biogas generated from organic matter fermentation under anaerobic conditions [2]. According to Mara (2012), biogas is a mixture of gases produced from organic material decomposition by natural methanogen bacteria [6]. Methane (CH₄) and carbon dioxide (CO₂) is the fundamental composition of biogas., but there
is a small amount of other gasses like hydrogen sulfide (H$_2$S), ammonia (NH$_3$), hydrogen (H), sulfur nitride, and water (H$_2$O) [7].

Lignin content in rice husk is the main inhibitor factor for biogas production due to the deceleration of microbe degradation [8]. Pretreatment processes are necessary to support the release of hemicellulose and cellulose [9].

2. Methodology

The rice husk is originated from Rowosari District – Central Java, Indonesia. For the initial step, it needs to be measured the total solid content (TS) using APHA method. Afterwards, it soaked by the 6% NaOH for at least 24 hours to remove the lignin. Later, rinse rice husk until it reaches a neutral pH level, which is indicated by the loss of mucus surfaces and dried the rice husk under sunlight.

The reactor size was adjusted to the amylase and cellulase enzyme variation (9%, 12%, 15%, 18%) with some rice husk mixed with water, enzyme, urea, and cow manure. The reactor was sealed to reach the anaerobic stage. The gas generation was examined once in two days through glass volume and calculated with the principle of Boyle Law.

3. Results and discussion

The research is purposed to analyze the rice husk biogas production effects by experimenting with several additional amylase and cellulase enzymes concentrations. To obtain an anaerobic condition, the reactor must be tightly-closed. It was observed once every two days for the total of 40 days. The gas volume is measured by flowing the gas in the reactor into a measuring cup containing water through a hose. This takes advantage of the natural gas, which is pressing in all directions so that the reactor valve is opened, the gas in the reactor will move into the measuring cup and the volume difference can be observed.

3.1. Biogas production effect by amylase enzymes concentration

The preliminary experiment of biogas production effects by the addition of amylase enzyme are appeared in figure 1. Biogas production with the addition of amylase enzyme in the pretreatment process is divided into four stages, namely: the lag stage (0$^{th}$-2$^{nd}$ days), the peak stage (3$^{rd}$-8$^{th}$ days), the sub-peak stage (12$^{nd}$-19$^{th}$ days), and the decreasing stage (after 20$^{th}$ days) [10]. In this study, these stages have quite different durations between all variations compared to previous studies. Figure 1 (a) shows how the lag stage difference between the addition of 9%, 12%, 15%, and 18% amylase enzymes. One of these differences is in the reactor with the addition of 9% amylase enzyme. On the 4$^{th}$ and 6$^{th}$ day of measurement, the measurement results reached the highest peak, namely 300 ml and gradually decreased until the 12$^{th}$ day. Whereas in previous studies, the yield value of biogas began to decline after the 20$^{th}$ day. This indicates the effect of differences in the percentage of enzymes on pretreatment.

Figure 1 (b) reveals the cumulative biogas production. It conveyed that the reactor with 9% amylase enzyme produced the highest biogas yield during the 40$^{th}$ days of the study at 981 ml. Whereas, with a concentration of 12% amylase enzyme, 15% amylase enzyme, and 18% amylase enzyme, the yield of biogas produce was 817 ml, 744 ml, and 263 ml, respectively.

Meanwhile, figure 1 (c) indicate the biogas cumulative yield each unit of TS due to the effect of amylase enzymes. The greatest cumulative biogas yield each unit of TS is produced by 9% amylase enzyme a reactor, which was equal to 45.81 ml/grTS. Then, for the variation with 12% amylase enzyme, 15% amylase enzyme, and 18% amylase enzyme produced biogas, namely 38.16 ml/grTS, 34.75 ml/grTS, and 12.28 ml/grTS, respectively. On the other hand, the control reactor produced 15.22 ml/grTS. Thus, the use of the 9% amylase enzyme produces the most significant biogas yield compared to other variation.
Based on previous research, the amylase enzyme would escalate the biogas yield production. It is recorded that these enzyme addition were able to generate methane yield of the lucerne pellets and birch pellets for about 19.95% and 22.9%, respectively [11]. Based on figure 1 (a), an increase in biogas yield has appeared on days 2-4 in all experimental reactors. This is because the addition of substrate can accelerate the effect of methanogenesis [10]. This study shows that the use of amylase enzymes helps degrade lignocellulose, thereby increasing biogas production.

3.2. Effect of cellulase enzymes on biogas production

Cellulase enzymes can be used as a pretreatment to increase biogas production from biomass containing lignocellulose. Anaerobic digestion in biomass containing lignocellulose is relatively slow, so the addition of cellulase enzymes is required to increase the rate of biogas production [12]. The use of cellulase enzymes as pretreatment in biogas production has been carried out in previous studies. In the production of biogas from corn waste, the use of cellulase enzymes as an efficient pretreatment increases the yield of biogas. Under optimal conditions, the cumulative biogas yield increases by 36.9% [13].

**Figure 1.** Effect of amylase enzymes on biogas production.

Based on previous research, the amylase enzyme would escalate the biogas yield production. It is recorded that these enzyme addition were able to generate methane yield of the lucerne pellets and birch pellets for about 19.95% and 22.9%, respectively [11]. Based on figure 1 (a), an increase in biogas yield has appeared on days 2-4 in all experimental reactors. This is because the addition of substrate can accelerate the effect of methanogenesis [10]. This study shows that the use of amylase enzymes helps degrade lignocellulose, thereby increasing biogas production.

3.2. Effect of cellulase enzymes on biogas production

Cellulase enzymes can be used as a pretreatment to increase biogas production from biomass containing lignocellulose. Anaerobic digestion in biomass containing lignocellulose is relatively slow, so the addition of cellulase enzymes is required to increase the rate of biogas production [12]. The use of cellulase enzymes as pretreatment in biogas production has been carried out in previous studies. In the production of biogas from corn waste, the use of cellulase enzymes as an efficient pretreatment increases the yield of biogas. Under optimal conditions, the cumulative biogas yield increases by 36.9% [13].
The results of observing the volume of biogas using variations in the concentration of cellulase enzymes can be seen in figure 2.

Figure 2. Effect of cellulase enzymes on biogas production.

Figure 2 (a) shows the difference in daily biogas yield in rice husks treated with biological pretreatment with the addition of 9%, 12%, 15%, and 18% cellulase enzymes. In the reactor with 18% cellulase enzyme addition, the highest peak was 430 ml when measuring on day 4. Then on the next research day, the biogas production gradually decreased.

Figure 2 (b) shows the effect of pretreatment using amylase enzymes on the cumulative biogas production. The results showed that the reactor with 18% cellulase enzyme produced the highest biogas yield during the 40 days of the study at 1520 ml. A concentration of 9% cellulase enzymes, 12% cellulase enzymes, and 15% cellulase enzymes yielded 535 ml, 650 ml, and 1042 ml biogas yields, respectively.

Figure 2 (c) shows the cumulative biogas yield per unit TS due to the effect of the use of cellulase enzymes on biogas production from rice husk waste. The highest number of cumulative biogas yield per TS unit is produced by a reactor containing 18% cellulase enzyme, 70.99 ml/gr TS. Then, for the 9% cellulase enzyme, 12% cellulase enzyme, and 15% cellulase enzyme produced biogas, namely 24.98 ml/gr TS 30.35
ml/gr TS, and 48.67 ml/gr TS respectively. In contrast, the reactor control produces biogas of 15.22 ml/g TS. Thus, the use of 18% cellulase enzyme as a biological pretreatment for rice husk waste produces the most optimal biogas yield compared to 9% cellulase enzymes, 12% cellulase enzymes, and 15% cellulase enzymes.

The production of biogas with the addition of cellulase enzymes can be classified into two stages. The first stage (days 0th to 9th) represents biogas' production from the hydrolysis process of easily degraded materials. The production of biogas with the addition of cellulase enzymes can be divided into two stages. The first stage (days 0th to 9th) represents the production of biogas from the hydrolysis process of easily degraded materials [12]. This is in accordance with the research conducted. Based on figure 2 (a), it can be seen that the peak of biogas production from four variations of enzyme enhancement, the peak occurs in the range of 0th-9th days.

4. Conclusion
Based on this research, it can be concluded that the addition of amylase and cellulase enzymes in the pretreatment of biogas production from rice husks can increase the yield of biogas produced. The addition of a 9% amylase enzyme resulted in the highest accumulation of biogas yield, namely 45.82 ml / grTS or 981 ml. Whereas the addition of cellulase enzymes, the highest accumulation of biogas yield was produced by variations with 18% of the enzyme, namely 70.99 ml/gr TS or 1520 ml. Both results were much higher than the process without the addition of enzymes with a total yield of 15.22 ml/grTS or 326 ml of biogas.

Acknowledgment
The authors would like to acknowledge the Faculty of Engineering, Universitas Diponegoro, for funding this research by RKAT scheme Fiscal Year 2020 Number 2496/STL08/UN7.5.3.2/PP/2020.

References
[1] Yan L, Yamei G, Yanjie W, Quan L, Zhiyuan S, Borui F, Xue W, Zongjun C and Weidong W 2012 *Bioresour. Technol* **111** 49–54
[2] Khaerunnisa G, Rahmawati I and Budiyono B 2013 *JTKI* **2(2)**
[3] Febriyanita W 2015 *Pengembangan Biogas dalam Rangka Pemanfaatan Energi Terbarukan di Desa Jetak Kecamatan Getasan Kabupaten Semarang* (Semarang: Unnes)
[4] Padiwiria A W 2006 *Teknologi penggilingan padi* (Jakarta: PT Gramedia Pustaka Utama)
[5] Junfeng L and Runqing H 2003 *Biomass Bioenerg* **25**(5) 483–499
[6] Mara M 2012 *DTM* **2**(1)
[7] Saputri Y F, Yuwono T and Mahmudshyah S 2014 *Jurnal Teknik POMITS* **1**(1) 1–6
[8] Matin H H A 2018 *E3S Web Conf.* **31** p 02007
[9] Fridia T 1989 *Pengaruh Cara Delignifikasi Terhadap Sakarifikasi Limbah Lignoselulosik* (Bogor: IPB)
[10] Li Y, Park S Y and Zhu J 2011 *Renew. Sust. Energ. Rev.* **15**(1) 821–826
[11] Dubrovsksis V, Plume I and Straume I 2019 *studies* **6** 8
[12] Speda J, Johansson M A, Odnell A and Karlsson M 2017 *Biotechnol. Biofuels* **10**(1) 129
[13] Wagner A O, Lackner N, Mutschlechner M, Prem E M, Markt R and Illmer P 2018 *Energies* **11**(7) 1797