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Original Research Article

The Effect of a Mixture of Amylase and Cellulase Enzymes at Four Concentrations on Biogas Production from Rice Husk Waste under Liquid Anaerobic Digestion (L-AD) Conditions

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Abstract
Cellulose, hemicellulose, and lignin are some of the components found in rice husks, where cellulose plays an important role as a substrate for biogas fermentation. This study aims to determine the optimum concentration of amylase-cellulase enzyme mixture added to rice husks using the liquid anaerobic digestion (L-AD) method. Rice husks from the Rowosari area were chemically pretreated by soaking in 6% NaOH for 24 hours (control), then given biological pretreatment with varying enzyme concentrations of 9%, 12%, 15%, and 18%. The digestion process was carried out in batches using a 200 mL bioreactor, and the biogas volume was monitored every two days for 60 days. The total biogas yields at enzyme concentrations of 9%, 12%, 15%, and 18% were 5; 10; 11; and 13 mL/grTS, respectively, while the control reactor produced 9 mL/grTS. The highest production was achieved at an enzyme concentration of 18% with a maximum potential (A) of 13 mL/grTS, a production rate (U) of 1 mL/grTS·day, and a minimum biogas formation time (λ) of 1 day.

Keywords: amylase enzyme; biogas production; cellulase enzyme; liquid anaerobic digestion; rice husk;

1. Introduction

Fossil fuels have become the main source of the global energy supply, which is non-renewable because fossil formation takes millions of years (MacKenzie, 1998). Meanwhile, natural gas consumption in Indonesia during the period 2000-2014 increased at an average annual growth rate of 2.6%. Based on the R/P (Reserve/Production) ratio in 2014, natural gas reserves were recorded at 100.3 TCF and are estimated to be depleted within 37 years, or even sooner due to the continued increase in fossil energy production (Sugiyono, 2016). One way to conserve petroleum and other non-renewable energy sources is by utilizing biogas as an alternative fuel (Ichsan, 2014; Febriyanita, 2015).

Rice husks consist of organic components such as cellulose, hemicellulose, and lignin (Hsu, 1980). The decomposition of organic materials containing cellulose, hemicellulose, and lignin occurs gradually. Therefore, to accelerate the degradation process of the contained organic materials, pretreatment of the raw material is necessary (Taherzadeh, 2008). Chemical pretreatment can be carried out using NaOH, KOH, calcium, and other types of alkalis. Tests have shown that NaOH is the most effective in removing lignin for biogas production (Yang et al., 2009). This process is commonly known as delignification, which is the removal of lignin from complex compounds. Biological pretreatment with the help of microorganisms can also be used to process lignocellulose and improve enzymatic hydrolysis. Biological pretreatment for improving biogas production in anaerobic digestion (AD) includes fungal pretreatment, microbial consortium pretreatment, and enzymatic pretreatment (Tuomela et al., 2000).

One technology that can be developed for biogas production is Liquid Anaerobic Digestion (L-AD), which is a fermentation process of organic materials that requires a total solids (TS) content of less than 15% and the slurry form of the feedstock (Haryati, 2006). According to Yani and Darwis (1990), nutrient requirements in anaerobic digestion include carbon, nitrogen, hydrogen, and phosphorus. The most important nutrients are carbon and nitrogen, with an optimal C/N ratio of 20:1 to 30:1. A high C/N ratio in organic material will lead to low methane production, thus reducing biogas yield (Teghammar, 2013). Therefore, it is necessary to add organic materials with high nitrogen content such as livestock manure. On the other hand, if the C/N ratio is too low, ammonia will accumulate and may increase the digester pH to 8.5, which is toxic to methanogenic bacteria (Teghammar, 2013). In such cases, it is necessary to add other materials containing high carbon or fiber, such as grass, straw, rice husks, and leaves.

Rumen (ruminal contents) is partially digested food in the first stomach of ruminants, containing saliva, anaerobic microbes, cellulose, hemicellulose, protein, fat, carbohydrates, minerals, and vitamins (Van Soest, 1982). In the rumen of ruminant livestock, there are microbes consisting of protozoa, bacteria, and fungi (Sudaryanto, 2002). One important group of bacteria in the cattle rumen is cellulolytic bacteria. The biodegradation process of cellulose-containing materials is highly dependent on the ability of cellulolytic bacteria to produce cellulase enzymes with high activity (Asenjo, 1986).

Enzymes are biocatalysts produced by the tissues of living organisms, used to catalyze reactions in living organisms, and capable of increasing the rate of reactions occurring in tissues (Lehninger, 1990). Amylase enzymes found in human saliva act as catalysts in the hydrolysis of starch by water to form sugar (Widiasa, 2007). Amylase enzymes can be used as a pretreatment to improve biogas production from organic biomass (Liaquat et al., 2015). Meanwhile, cellulase enzymes found in animals such as goats, cattle, and termites can accelerate a reaction without affecting its outcome. Cellulase enzymes can be used as a pretreatment to increase biogas production from lignocellulosic biomass (Zhang, X. S.; Zhang, 2013). The activity of these enzymes must be tested under optimum conditions to obtain more accurate quantification results (Widiasa, 2007).

Several previous studies, such as that by Ziemiński et al. (2012), stated that pretreatment with enzymes was able to increase biogas production from sugar beet pulp by 19% and spent hops by 13%. Research by Syafrudin et al. (2017) also reported that enzymatic pretreatment could increase biogas production by 30% to 55%. Shandy (2017), in her study, stated that pretreatment using three variations of 6% NaOH resulted in the highest biogas yield, while pretreatment using enzymes increased yield by 11%. Based on this explanation, the researcher is interested in conducting a study by adding a mixture of amylase and cellulase enzymes to accelerate the rate of biogas production. The objectives of this research are to analyze the effect of adding a mixture of amylase and cellulase enzymes on biogas production from rice husk waste using the L-AD method and to analyze the rate of biogas production from rice husk waste with the addition of a mixture of amylase and cellulase enzymes through the L-AD method.

2. Methods

The data collection techniques used in this study included experiments, observation, documentation, and literature review. The equipment used consisted of a digital scale, 2-liter plastic bottles (biodigesters), plastic hoses, a pH meter, a stand, measuring cylinders, rubber stoppers, and clamps. The materials used included rice husks, cow rumen fluid, amylase enzyme, cellulase enzyme, urea, water, and NaOH. This research consisted of five stages: testing the total solids content of rice husks, preparation, operation, results analysis, and analysis of residual digester material. In the total solids testing stage, crucibles were dried at a temperature of 103-105°C for 1 hour, then cooled and stored in a desiccator. Samples of 25-50 g were placed in the crucibles, weighed, and dried at 103-105°C for 1 hour. They were then cooled in the desiccator and weighed until the weight decreased by 4% or 50 mg.

The preparation stage included checking the total solids content and moisture content of the rice husk organic waste (biowaste). The rice husks were then soaked in 6% NaOH for 24 hours, rinsed with water until reaching neutral pH, and dried under the sun. The operational stage involved mixing the rice husks with water, enzymes, urea, and rumen fluid. The samples were then placed into sealed reactors.

Measurements were taken every two days by channeling the gas into a measuring cylinder filled with water. During measurement, the reactor valve was opened, and the gas flowed into the measuring cylinder, with the change in volume being recorded.

After testing, the researchers analyzed the biogas volume data, which were presented in the form of a graph showing the relationship between biogas volume and time. The data analysis was conducted both graphically and theoretically based on the observed effects of chemical and biological pretreatment, as well as by comparing biogas volume calculations using Polymath 6.0 software. Additionally, the residual material from the digester was analyzed after the final biogas volume measurement. The data were analyzed using nonlinear regression tests with the aid of Polymath 6.0 software and Microsoft Office Word to produce descriptive analyses, graphs, and tables. The nonlinear regression analysis method was also used to determine the effect of total solids concentration on biogas production from agricultural waste using the liquid anaerobic digestion method.

3. Result and Discussion

3.1 Chemical and Biological Pretreatment in Biogas Production

This research aims to analyze the effect of various concentration variations of a mixture of amylase and cellulase enzymes on biogas production from rice husk waste, as well as to analyze the biogas production rate. The pretreatment of rice husk in this study was intended to optimize rice husk waste utilization by up to 30% (Syafrudin et al., 2017). The collected rice husk waste was then tested for its Total Solid (TS) content. The TS content analysis followed the standard APHA method, in which %TS represents the ratio of the dried sample weight to the wet sample weight. From the calculation, the %TS obtained was 93.4%. In this study, NaOH solution was used, which has been proven effective in degrading lignocellulosic biomass. Moreover, Zhong et al. (2010) stated that this pretreatment process aims to release more hemicellulose and make cellulose more available and easier to digest. Pretreatment of various lignocellulosic biomasses such as wheat straw, grasses, hardwood, and softwood using NaOH was also reported to reduce lignin content to less than 26% (Zhao et al., 2008).

Biomass that had undergone soaking according to the specified time and concentration was then subjected to a neutralization stage before being placed into the biodigester. Neutralization was necessary before the enzymatic hydrolysis stage to remove lignin and inhibitors (such as salts, phenolic acids, and aldehydes) (Menon and Rao, 2012). In this research, rice husk waste from Rowosari was used as raw material. First, the rice husk underwent chemical pretreatment with NaOH. It was soaked in 6% NaOH solution for 24 hours as the control variable. A total of 1.5 kilograms of rice husk was soaked in 45 liters of water. After 24 hours, the rice husk was washed with water to neutralize the pH and then sun-dried. Next, the reactor was prepared, and the dried rice husk was placed inside along with the enzymes (according to the variations in each reactor), rumen fluid, and urea. A total of 10 reactors were used, with each variable having two reactors, one of which served as a duplicate.

The reactors had to be tightly sealed, and anaerobic conditions were maintained inside to ensure the survival of decomposing microorganisms during the experiment (Syafrudin et al., 2017). Therefore, the reactors were tested beforehand by applying soapy water to possible leakage points. Leakage was indicated by bubble formation. The volume of biogas produced was observed every two days for 60 days. The gas volume was measured by channeling the gas from the reactor into a measuring cylinder filled with water through a hose. This method utilized the property of gas exerting pressure in all directions, so when the reactor valve was opened, the gas moved into the measuring cylinder, and the volume difference could be observed. Liquids and gases share the property of flowing. Any substance that can flow is called a fluid; hence, both liquids and gases are classified as fluids. In this study, gas measurement was carried out by channeling it into a measuring cylinder. The air pressure inside the measuring cylinder could be calculated using the following hydrostatic pressure formula:

$$P_a = P_o + P_h \quad (1)$$

$$P_a = P_o + (\rho \ x \ g \ x \ h) \quad (2)$$

Where P_o is the total pressure in Pascals (P_o), P_h is the hydrostatic pressure (Pa), P_o is the atmospheric pressure (atm), hhh is depth in meters, ρ is fluid density (1000 kg/m³), and g is gravitational acceleration (10 m/s²). If the measured water volume in the measuring cylinder was 15 ml, then using the formula above, the pressure was calculated to be 1,015 x 10^5 P_o or under standard conditions (STP), equivalent to 1 atm at 0°C. Thus, it can be concluded that the pressure inside the measuring cylinder during measurement was the same as the outside air pressure, approximately 105 P_o , equivalent to 1 atm, which is the normal atmospheric pressure. This observation aligns with Boyle's Law, which states that the product of pressure and volume of a gas in a closed space remains constant.

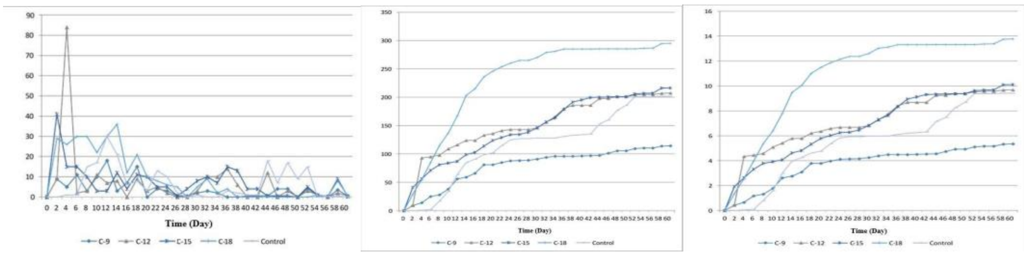
The experimental procedure for rice husk with different enzyme concentrations was as follows: each reactor contained a variation of enzyme concentration of 9%, 12%, 15%, and 18% of the reactor solution volume. The mixture of amylase and cellulase enzymes was prepared in a 1:1 ratio. A 9% enzyme mixture corresponded to 18 ml per reactor, with 9 ml of amylase and 9 ml of cellulase. A 12% enzyme mixture corresponded to 24 ml per reactor, with 12 ml of each enzyme. A 15% enzyme mixture corresponded to 30 ml per reactor, with 15 ml of each enzyme. An 18% enzyme mixture corresponded to 36 ml per reactor, with 18 ml of each enzyme. The biological pretreatment with amylase and cellulase enzyme mixture was carried out at the final stage, before the addition of cow rumen fluid. All solid materials were first loaded into the digester, followed by distilled water, then amylase enzyme, and finally cellulase enzyme.

3.2 The Effect of Amylase and Cellulase Enzyme Mixture on Biogas Production

In this study, the enzyme concentration variations used for biological pretreatment were 9%, 12%, 15%, and 18%. The aim of this research was to analyze the effect of a mixture of amylase and cellulase enzymes on rice husks in biogas production using the L-AD method. Based on the observations, it was found that the use of an 18% enzyme mixture produced more biogas compared to the 9%, 12%, and 15% enzyme mixtures, as well as the control. The reactors with enzyme mixtures of 9%, 12%, 15%, 18%, and the control produced cumulative biogas yields of 114.5 ml, 207.5 ml, 216.6 ml, 295.1 ml, and 201.8 ml, respectively.

Several factors influence biogas formation, including anaerobic conditions, feedstock, C/N ratio, acidity (pH), and total solids (TS) content. However, these factors were controlled by applying the same treatment to each reactor. This included checking for airtight conditions by submerging the reactor bottle in water to ensure no bubbles escaped, homogenizing the feedstock by mixing and stirring cow rumen fluid, measuring pH, and using rice husks that had undergone the same pretreatment. Biogas volume data were presented as daily and cumulative biogas yields over the 60-day research period, as shown in the following figure.

Figure 1(a) shows the differences in daily biogas yield from rice husks subjected to biological pretreatment with the addition of 9%, 12%, 15%, and 18% enzyme mixtures. In the reactor with a 9% enzyme mixture, the highest peak was reached on day 12 at 18 ml, after which biogas production gradually decreased. In the reactor with a 12% enzyme mixture, the highest peak was reached on day 4 at 84 ml, followed by a gradual decline in production. In the reactor with a 15% enzyme mixture, the highest peak occurred on day 2 at 41 ml, after which production steadily decreased. In the reactor with an 18% enzyme mixture, the highest peak was reached on day 14 at 36 ml, followed by a gradual decline thereafter. Figure 1(b) shows the effect of pretreatment using the enzyme mixture on cumulative biogas production. The results show that the reactor with an 18% enzyme mixture produced the highest cumulative biogas yield over the 60-day study period, amounting to 7,091.1 ml. In comparison, enzyme mixture concentrations of 9%, 12%, and 15% produced yields of 2,443 ml, 4,639.5 ml, and 4,483.2 ml, respectively. Figure 1(c) presents cumulative biogas yield per unit TS as influenced by the amylase-cellulase enzyme mixture in biogas production from rice husk waste. The highest cumulative yield per unit TS was obtained from the reactor containing an 18% enzyme mixture, reaching 331.21 ml/g TS. The 9%, 12%, and 15% enzyme mixtures produced 114.11 ml/g TS, 216.7 ml/g TS, and 209.4 ml/g TS, respectively, while the control reactor produced 167.63 ml/g TS. Therefore, the use of an 18% enzyme mixture as biological pretreatment for rice husk waste produced the most optimal biogas yield compared to 9%, 12%, and 15% mixtures.



(a) (b) (c)

Figure 1. The Effect of Enzyme Mixtures on Biogas Production

The addition of 18% amylase-cellulase enzyme mixture proved to be the most effective in increasing biogas production compared to the 9%, 12%, and 15% mixtures. The 9% enzyme mixture produced the lowest yield among all enzyme treatments. This is consistent with Dubrovskis et al. (2019), who reported that higher enzyme usage results in higher biogas production. The glucose production process in this study involved several stages, beginning with NaOH pretreatment. In the hydrolysis process, an optimal ratio of 1:1 was applied to maximize glucose production. Starch in cassava peels consists of two fractions that can be separated using hot water: amylose and amylopectin. During liquefaction with α-amylase, the enzyme breaks the bonds in the starch molecules (carbohydrates), producing shorter carbohydrate chains such as glucose, maltose, and various limit dextrins. Glucoamylase hydrolyzes α-glycosidic bonds in starch and oligosaccharides to produce glucose units. The results of this study indicate that mixing enzymes does not alter their performance in accelerating reactions. According to Poedjiadi (1994), enzymatic reactions work according to the substrate in the organism, and the reaction rate increases with increasing substrate concentration. Since the enzyme's active site can bind only a limited number of substrate molecules, increasing substrate concentration allows more enzyme-substrate interactions, thereby increasing the reaction rate.

According to Li, Jianchang (2017), in reactors with amylase enzyme addition, the highest gas production occurred on days 3-8, while in reactors without enzyme addition, the peak occurred on days 11-12. This is consistent with the present study, where reactors with enzyme mixtures generally reached peak gas production on days 3-8, whereas the control reactor peaked on day 12. Temperature is another factor influencing biogas productivity. Optimal temperatures facilitate bacterial growth, accelerating methane formation. Denta et al. (2015) compared digester temperatures, recording them during the biogas production process. Measurements included reaction temperature and ambient temperature, taken in the morning, noon, and afternoon. The study found that anaerobic digestion in all treatments occurred at temperatures of 31.7-34°C, with ambient temperatures ranging from 30.29°C to 31.33°C, conditions typical of Indonesia's tropical climate. Equal temperatures between the digesters and surrounding environment optimize biogas production. This aligns with the present study, where research was conducted during the day under hot weather, with ambient temperatures ranging from 32°C to 35°C, resulting in increased biogas production rates.

In this study, temperature measurements were taken every 10 days until day 40. The most optimal biogas production rate occurred with the 18% enzyme mixture, with a cumulative yield of 295.1 ml on day 60. On day 40, the final temperature measurement recorded biogas production of 285 ml, or 96.5% of the maximum yield, at 30°C. According to Al Seadi et al. (2008), temperatures of 30°C-42°C are classified as mesophilic, with a retention time of 30-40 days. This is consistent with the current research, in which mesophilic conditions were observed; at 30°C on day 40, maximum biogas yield was 96.5%. Compared to Al Seadi et al. (2008), who reported maximum biogas yield (100%) occurring between days 20-40 under thermophilic conditions (50°C), the results here are slightly lower. Al Seadi et al. (2008) also note that thermophilic microorganisms are more sensitive to disturbances and toxic compounds, whereas mesophilic systems are more robust but have slower reaction rates. Therefore, it can be concluded that the maximum biogas yield in this study was lower (96.5%) than that reported by Al Seadi et al. (2008), due to temperature effects on anaerobic digestion, which influenced the maximum production yield.

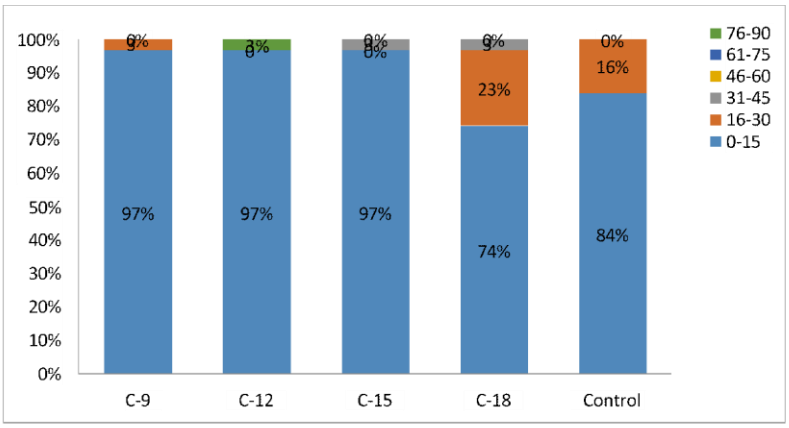
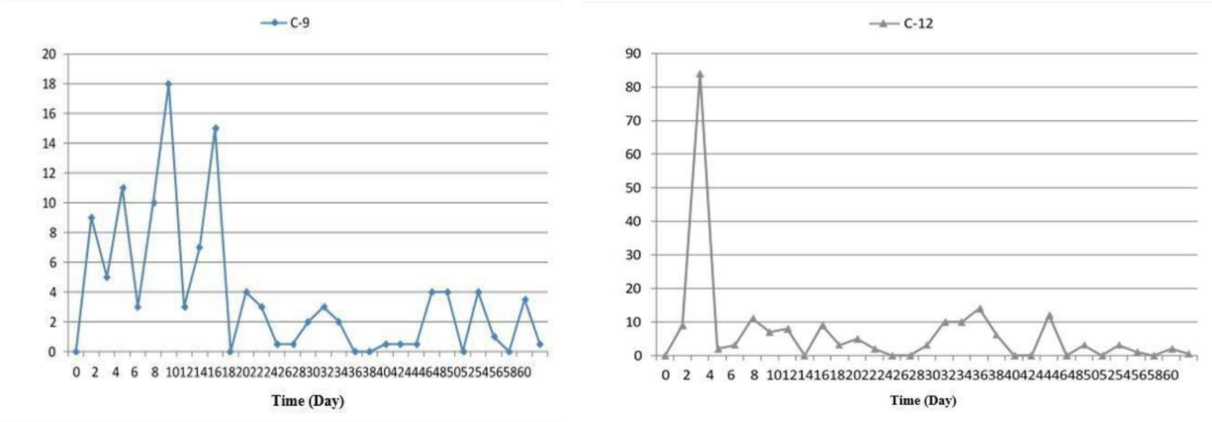


Figure 2. Range of Biogas Volume in Relation to the Effect of Amylase and Cellulase Enzyme Mixture

In Figure 2, the range of biogas production generated by the four reactors-namely the reactors with the addition of 9%, 12%, 15%, and 18% enzyme mixtures-can be seen. In the range of 0-15 ml of biogas produced per day, all reactors contributed. In the range of 16-30 ml of daily biogas production, this was generated by the reactors with 9% and 18% enzyme mixtures. In the 31-45 ml range, production came from reactors with 15% and 18% enzyme mixtures. Meanwhile, in the 76-90 ml range, production was observed in the reactor with a 12% enzyme mixture.

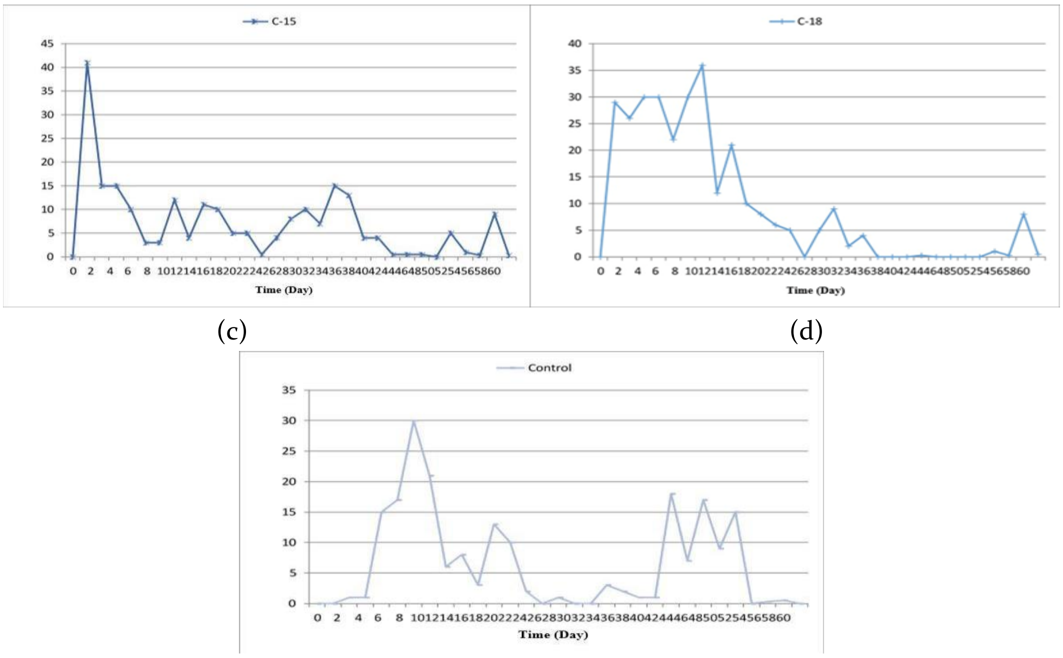
For the C-9 reactor with a 9% enzyme mixture, as shown in Figure 3(a), the highest biogas yield was recorded on the 12th measurement at 18 ml. By the 7th measurement, the yield had decreased to 3 ml. In the following days, biogas production fluctuated. The daily yield reached 0 ml on the 50th measurement, rose slightly, and then ended at 0.5 ml in the final measurement. For the C-12 reactor with a 12% enzyme mixture, as shown in Figure 3(b), the highest yield was recorded on the 4th measurement at 84 ml. In the subsequent days, production declined, fluctuating until it reached 0 ml on the 56th measurement, and finally ending at 0.5 ml. For the C-15 reactor with a 15% enzyme mixture, as shown in Figure 4(a), the highest yield was recorded on the 2nd measurement at 41 ml. The following day, the yield dropped to 15 ml and continued to decline, reaching 0 ml by the 50th measurement. Production then rose again on the 58th day to 9 ml, before decreasing to 0.3 ml on the final measurement. For the C-18 reactor with an 18% enzyme mixture, as shown in Figure 4(b), the highest yield was recorded on the 14th measurement at 36 ml. Production then continued to decrease, reaching 0 ml between the 38th and 52nd measurements, before increasing to 8 ml on the 58th day, and finally dropping to 0.5 ml in the last measurement.

In the control reactor, without the addition of amylase and cellulase enzyme mixtures, as shown in Figure 4(c), the highest yield was recorded on the 12th measurement at 30 ml. In the days that followed, production fluctuated, continuing until the 52nd measurement, and finally dropping to 0 ml at the end. Biogas production in the control digester was more fluctuating due to the natural growth phases of bacteria in methane production, as no enzyme catalyst was added. The gas generated tended to be unstable in the early days of the study because bacteria needed time to adapt to their environment and process the available substrate (Budiyono, 2014). The control digester produced gas for a longer period compared to digesters with enzyme mixtures, as substrates without pretreatment tend to take longer to produce biogas (Chandra et al., 2012). In this study, enzyme concentrations of 18%, 15%, and 12% yielded higher production than the control, while the 9% enzyme mixture produced less than the control. This indicates that the optimum enzyme concentration for biogas production is equal to or greater than 11%, consistent with the finding that the most effective enzyme mixtures were amylase and cellulase at 18%, 15%, and 12%. The biogas volume data are presented as daily and cumulative biogas production over the 60-day study period, as shown in the following figures:



(a) (b)

Figure 3. Daily Biogas Yield (a) with the Addition of 9% Enzyme Mixture; (b) with the Addition of 12% Enzyme Mixture



(e)

Figure 4. Daily Biogas Yield (c) with the Addition of 15% Enzyme Mixture; (d) with the Addition of 18% Enzyme Mixture; (e) without the Addition of Enzyme Mixture

3.3 Biogas Production Rate in Relation to the Effect of Enzyme Mixture

The following is the kinetics constant table from the study on the effect of amylase enzyme variation on biogas production and the relationship between experimental data and calculation results in the study on the effect of amylase-cellulase enzyme mixture variation. For a clearer visualization, the relationship between Experimental Data and Calculation Results in the Study on the Effect of Amylase and Cellulase Enzyme Mixture Variations are plotted below.

Table 1. Kinetics Constant in the Study on the Effect of Amylase Enzyme Variation on Biogas Production

Constant	9% Enzyme Mixture (ml)	12% Enzyme Mixture (ml)	15% Enzyme Mixture (ml)	18% Enzyme Mixture (ml)	Control (ml)
A, ml/grTS	4,93	9,913	10,954	13,39	9,519
U, ml/(grTS.day)	0,208	0,225	0,211	0,731	0,217
l, **day**	0,812	0,82	0,83	0,842	1,88

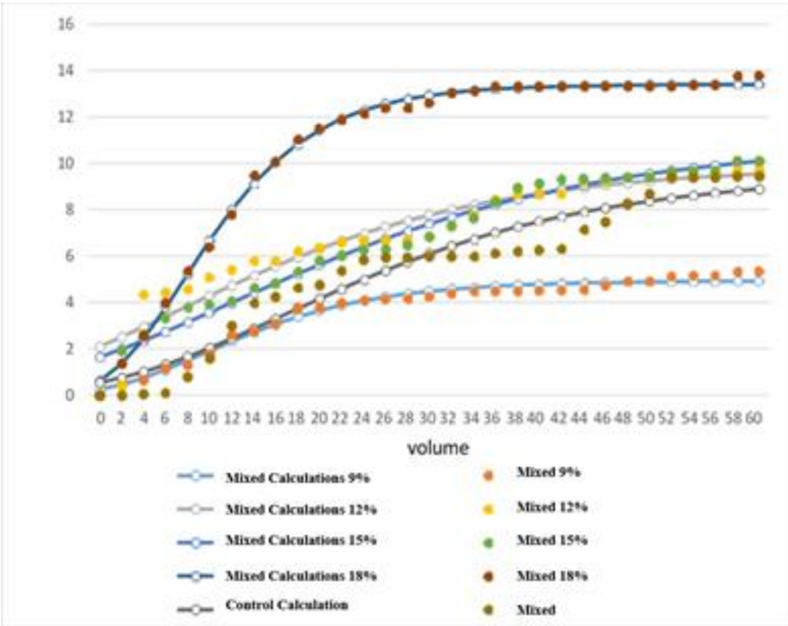


Figure 5. Relationship Between Experimental Data and Calculation Results in the Study on the Effect of Amylase and Cellulase Enzyme Mixture Variations.

From these data, it can be concluded that the use of varying mixtures of amylase and cellulase enzymes affects biogas production. Using the enzyme mixture resulted in a higher biogas yield compared to no enzyme addition (control), a faster biogas production rate, and a shorter time for biogas formation. The enzyme mixture concentration variation of 18% produced the highest biogas yield, namely 13.39 ml/g TS. This yield was higher than the biogas yields from lower enzyme mixture concentrations, namely 9%, 12%, and 15%. This indicates that pretreatment using an amylase and cellulase enzyme mixture affects biogas yield-the higher the enzyme concentration variation, the higher the biogas yield produced. This finding is consistent with research conducted by Syafrudin et al. (2018), where the use of enzymes increased biogas production from rice husk waste, with the highest concentration (11% enzyme) producing the maximum biogas yield compared to enzyme concentration variations of 5% and 8%.

Bacterial growth factors affect the gas volume in the digester. According to Kusnadi (2003), growth is the increase in cell number, volume, and size. The bacterial growth curve is divided into four main phases: lag phase, exponential (log) phase, stationary phase, and decline phase. These phases reflect the condition of bacteria in culture at a given time.

After calculations were performed using Polymath 6.1, the maximum biogas yield that could be produced and the day on which the reactor stopped producing biogas were determined. From the Polymath 6.1 calculations, the 18% enzyme mixture concentration variation produced the highest biogas production rate, namely 0.731 ml/(g TS·day). This rate was higher than that of other enzyme mixture concentrations 9%, 12%, and 15%. This occurred because, with the 18% amylase enzyme concentration variation, the reactor produced biogas in a shorter time compared to other variations, namely only until day 38, and in the following days, biogas production had already stopped at a volume of 0 ml. Thus, the biogas output was relatively stable or constant, producing a high production rate. The following is a graph of the biogas production rate with the highest result, namely at the 18% enzyme mixture concentration variation.

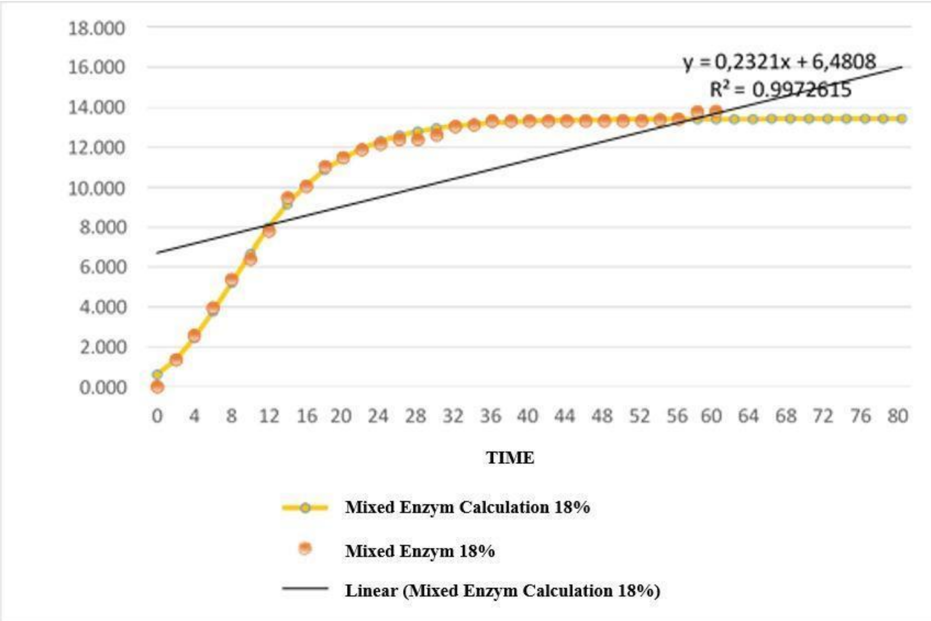


Figure 6. Calculated Data on the Effect of an 18% Amylase and Cellulase Enzyme Mixture on Biogas Production

Based on the calculation results using Polymath 6.1, it can be seen that at the 18% enzyme mixture concentration variation, which produced the highest biogas production rate, the maximum biogas yield obtained was 13.39 ml/g TS. According to the data in Figure 6, at the 18% enzyme mixture concentration variation, on day 60 the biogas yield reached 13.393 ml/g TS, and on day 80 it was still producing biogas at 13.397 ml/g TS. However, the biogas output no longer showed a significant increase in production. This indicates that the biogas yield from rice husk waste treated with an 18% enzyme mixture pretreatment had already become relatively stable and was no longer producing biogas. The data show that by day 60, biogas production had essentially stopped. This finding can be used to design an L-AD biodigester for rice husk waste with amylase-cellulase enzyme mixture pretreatment in continuous operation, with a retention time of 60 days.

3.4 Analysis of Enzyme Mixture on Amylase and Cellulase Enzymes

The addition of enzyme pretreatment plays an important role in improving the biodegradability of lignocellulosic biomass, such as rice husks. Amylase and cellulase act on different fractions of the substrate, thereby affecting the hydrolysis stage of anaerobic fermentation. Amylase primarily hydrolyzes starch into simple sugars, such as maltose and glucose, whereas cellulase catalyzes the breakdown of cellulose into cellobiose and glucose units, which can be easily fermented by anaerobic microorganisms (Lehninger, 1990; Zhang et al., 2013). The combined use of these enzymes is expected to increase the availability of soluble carbohydrates, thereby accelerating the subsequent acidogenesis and methanogenesis stages. Figure 7 showed Comparison between the Enzyme Mixture and the Amylase and Cellulase Enzymes.

Based on a comparison between individual enzymes and enzyme mixtures, cellulase had a stronger effect on biogas production than amylase alone or a mixture of amylase and cellulase. This finding can be attributed to the composition characteristics of rice husks, which are dominated by cellulose and hemicellulose rather than starch (Hsu & Luh, 1980; Taherzadeh & Karimi, 2008). As cellulose is the main biodegradable fraction, cellulase activity directly increases substrate accessibility for fermentative and methanogenic microorganisms. Similar observations have been reported by Ziemiński et al. (2012), who noted that cellulase-based pretreatment significantly increased the biogas yield from lignocellulosic residues compared to amylase-dominated systems. Although the enzyme mixture increased overall biogas production compared to the control, the results of this study showed that enzymatic synergy did not always produce an additive effect. This phenomenon can be explained by substrate limitations and enzyme-substrate specificity, where the availability of starch is insufficient to fully utilize amylase activity (Poedjiadi, 1994). Therefore, the effectiveness of enzyme mixtures is highly dependent on the biochemical composition of raw materials. These findings indicate that pre-treatment strategies dominated by cellulase or focused on cellulase are more suitable for rice husk digestion under Liquid Anaerobic Digestion (L-AD) conditions.

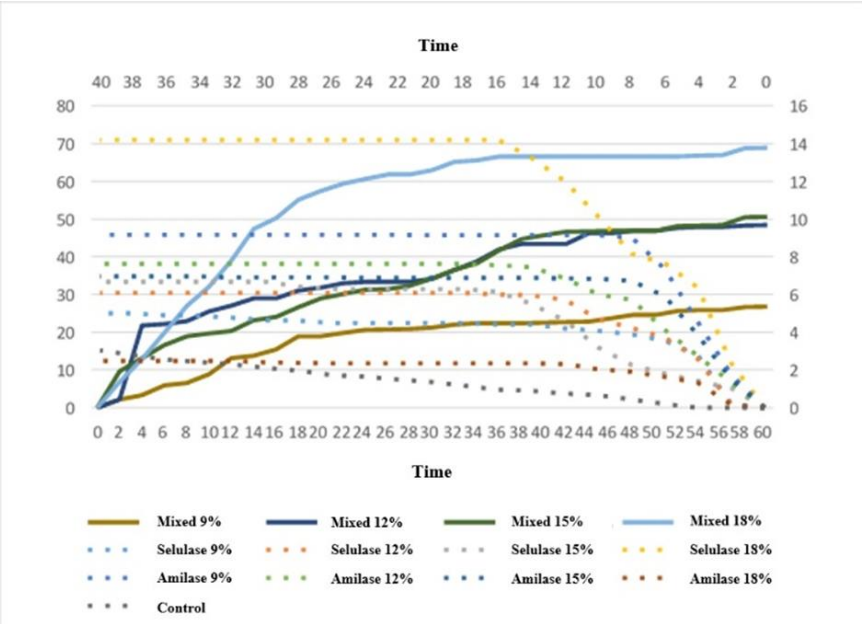


Figure 7. Comparison between the Enzyme Mixture and the Amylase and Cellulase Enzymes

3.5 The Quality of Biogas Produced From Rice Husk Feedstocks

The quality of biogas is primarily determined by its methane (CH_4) content, which reflects the efficiency of anaerobic digestion. In this study, although direct gas composition analysis was not performed, the use of a high concentration of rumen fluid inoculum (45%) indicated favorable conditions for the production of methane-rich biogas. Rumen fluid contains a diverse consortium of anaerobic microorganisms, including hydrolytic, acidogenic, acetogenic, and methanogenic bacteria, which are important for the complete conversion of lignocellulosic substrates to methane (Van Soest, 1982; Baba, 2013). The presence of cellulolytic and methanogenic microorganisms in rumen fluid increases the degradation of cellulose and hemicellulose, leading to increased production of volatile fatty acids (VFA) and methane. Studies show that Higher inoculum concentrations generally accelerate digestion kinetics and increase methane yield by reducing the microbial adaptation lag phase (Widiasa et al., 2007; Chandra et al., 2012). As a result, biogas produced from rice husk feedstock under these conditions is expected to have a relatively high methane fraction compared with systems with lower microbial densities. In addition, the application of enzymatic pretreatment prior to digestion indirectly contributes to improving biogas quality by increasing the proportion of biodegradable organic matter available for methanogenesis. Improved hydrolysis efficiency results in more stable digestion and reduces the accumulation of intermediate compounds, such as volatile acids, which would otherwise inhibit methane formation (Al Seadi et al., 2008; Syafrudin et al., 2017). Therefore, it can be concluded that the biogas produced in this study, particularly in reactors with higher enzyme concentrations, has favorable quality characteristics, with methane as the dominant component, making it suitable for renewable-energy applications. In addition, the application of enzymatic pretreatment prior to digestion indirectly contributes to improving biogas quality by increasing the proportion of biodegradable organic matter available for methanogenesis. Improved hydrolysis efficiency results in more stable digestion and reduces the accumulation of intermediate compounds, such as volatile acids, which would otherwise inhibit methane formation (Al Seadi et al., 2008; Syafrudin et al., 2017). Therefore, it can be concluded that the biogas produced in this study, particularly in reactors with higher enzyme concentrations, has favorable quality characteristics, with methane as the dominant component, making it suitable for renewable-energy applications.

4. Conclusions

This study shows that biological pretreatment using a mixture of amylase and cellulase enzymes significantly increases biogas production from rice husk waste under Liquid Anaerobic Digestion (L-AD) conditions. Among the tested enzyme concentration variations (9%, 12%, 15%, and 18%), the 18% amylase-cellulase enzyme mixture produced the highest cumulative biogas production and the fastest biogas production rate. The maximum biogas yield achieved was 13.39 ml/g DM, accompanied by the highest biogas production rate of 0.731 ml/(g DM·day) and the shortest lag phase (λ = 0.842 days), indicating improved hydrolysis efficiency and accelerated microbial activity.

The observed increase in performance at higher enzyme concentrations was associated with more effective hydrolysis of lignocellulose components, particularly cellulose, which is the dominant fraction of rice husk biomass. The combined application of chemical pretreatment using NaOH and enzymatic pretreatment facilitates lignin breakdown and increases substrate accessibility, thereby improving the microbial conversion efficiency during anaerobic digestion. Although the enzyme mixture increased biogas production compared to the control, the results showed that cellulase played a more dominant role than amylase because of the low starch content of rice husks. This highlights the importance of matching enzyme selection and concentration to the biochemical composition of the raw material.

These findings confirm that enzymatic pretreatment, particularly at an optimal concentration of 18%, is an effective strategy for increasing biogas yield and production kinetics from rice husk waste using the LAD method. The biogas produced is expected to be rich in methane because of the high concentration of rumen inoculum and favorable mesophilic operating conditions. This study provides a strong scientific basis for the application of enzyme-assisted anaerobic digestion as a sustainable approach for utilizing agricultural residues and supporting renewable energy production. Further research is recommended to evaluate the composition of biogas directly and to optimize the enzyme ratio for large-scale and sustainable digestion systems.

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