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INFLUENCE WATER CONTENT OF BIOGAS SUBSTRATE MADE BY DAIRY COWS FAECES AND RICE STRAW ON THE QUANTITY AND QUALITY OF SLUDGE

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Abstract

Biogas is an alternative to livestock waste management, however, it leaves a sludge which is contains organic composition that can be used as a solid organic fertilizer. This research aims to know the effect water content of biogas substrate consisting of dairy cow faeces and rice straw to the quantity and quality of sludge. The water content will influence C/N ratio, which is highly dependent on the dry material. Then it can affect the organic materials fermentation. This experimental design using a Completely Randomized Design with three water content treatments, namely, P1 = 60%, P2 = 75%, and P3 = 90. Each treatment has six replicates. The sludge parameters measured were chemicals (N, P, K, Ca, Mg and CEC, heavy metal, microelement) and biological (Salmonella sp. and E.coli). Biological parameters were analysed descriptively. The results showed that the 60% provides higher quantity of sludge compare to 75% and 90%. However, although 60% water content provides chemical parameters concentration similar to 75%, exclude phosphor, but higher count of E. coli and Salmonella sp. than that of 75%. Finally, 90% water content resulted in lower quantity, lower all chemical parameters and higher meters. Therefore, 75% water content provides the best result in quantity and quality of sludge, so it can be used as the best alternative for biogas production in batch system digester.

***Key words**: dairy cows faeces, rice straw, sludge, solid organic fertilizer.*

INTRODUCTION

Biogas production was influenced by various factors, such as pH, temperature, dry matter content, oxygen, C/N ratio, agitation, time and activity of microorganisms in a digester. Dry matter is a critical factor for biogas because other factors heavily depend on it. Therefore, the gas production can be measured by the total of dry matter contents in the substrates (Deublein and Steinhauser, 2008). Dry matter is inversely proportional to water content. Thus moisture also is an essential factor to be considered in biogas.

The C/N ratio depends on the water content. The ratio is calculated by the level of dry matter content measured in the number of water contained in the substrate. C/N ratio of units representing organic matter compounds in the carbon and nitrogen element comparisons. Microorganisms use both of these elements in their metabolic processes. Carbon is used as an energy source, while nitrogen is used in protein synthesis. If the nitrogen content is too much and the carbon content is small, then the gas production will decrease. As a result, there is much nitrogen in the mud. Conversely, if the nitrogen content is low and the carbon content is high, the nitrogen will quickly run out, and gas production stops. Dry matter content or moisture also participates in microorganism activity. Each type of microorganism has a water capacity (*aw*) for its activity and life. When it is optimal, the activity of microorganism is also optimal. The effect will also be better for gas production. Thus, biogas processes strongly influenced by the content of dry matter or moisture content. The optimum dry matter content ranged from 7-9% (Panjaitan and Pemayun, 1985) and 25% (Deublein and Steinhauser, 2008).

There are two several ways for making biogas, namely dry fermentation and wet fermentation. Dry fermentation is carried out on a substrate which has a water content less than 85%, whereas wet fermentation is carried out on a substrate with a water content of 85% or more (Deublein and Steinhauser, 2008). However, the dry matter content of the substrate should not be less than 5% (Werner et al., 1989). Therefore, the water content of the substrate should not be more than 95%. Also, the total substrate material content should be more than 30% (Waltenberger and Krichmayr, 2013). Accordingly, the water content used in the study ranges from 60 to 90%. Under that matter, hence research aims to know the influence of water content of substrate on quantity and quality of sludge which result from making of biogas from a mixture of dairy cow faeces and rice straw.

MATERIALS AND METHODS

The material in this research was used dairy cow faeces and rice straw. Faeces of the dairy cow were obtained from Dairy Production Laboratory, Faculty of Animal Husbandry of Padjadjaran University, while rice straw was carried out from Cianjur, West Java. This experimental study was performed based on Complete Randomized Design (CRD) with three substrate water content treatments, there are P1 = 60%, P2 = 75%, and P3 = 90%. Each treatment has six replicates.

Before starting the experiment, the materials preparation should be done. Faeces dairy cows and rice straw were dried bypassed it with the wind until both materials have 60% water content. Then both of materials are taken samples for analyzed the content of organic carbon and nitrogen. Organic carbon was analyzed by Walkley and Black method, while nitrogen was analysed by Kjeldahl method. Based on the results of the analysis, the calculation of C/N ratio is presented in Table 1. By using C/N ratio 30 (Kurnani et al., 2012), the proportion of dairy cow faeces and rice straws for 1 kg of biogas substrate with 60% water content, there are 0.85 kg and 0.10 kg respectively.

Table 1. Organic carbon and nitrogen concentration of dairy cow faeces and rice straw

Materials	Carbon (%)	Nitrogen (%)	C/N Ratio
Dairy cow faeces	43.16	1.77	24.38
Rice Straw	45.92	1.06	43.32

The biogas substrate was collected then it was incubated in a plastic bag for seven days. After the water content reaches 30% on the substrate, it will be extracted using hot water (90°C). The extraction uses 12 liters of water per 1 kilogram of the substrate. After that, the residue was diluted with water according to the treatment up to a total volume of 150 liters. Each substrate is fed into a 200-liter volume digester. Biogas process will finish in 30 days. The parameters are the quantity and quality of residual sludge of biogas manufacture. The quality of sludge consists of chemical parameters of N, P, K and Cation Exchange Capacity (CEC), heavy metals (As, Hg, Pb and Cd), microelements (Fe, Mn, and Zn) and biological parameters of *E. coli* and *Salmonella sp.*. Chemical parameters were analysed using Spectrophotometer, and the data were analysed statistically using SPSS 17, while the biological parameters were identified by plate count method and the result was described by the descriptive method.

RESULTS AND DISCUSSIONS

Effect of substrate water content on sludge quantity

The quantity of sludge from the biogas manufacture of dairy cow faeces and rice straw varies according to the water content of the substrate. The ANOVA results show that the water content of the substrate affects the sludge quantity produced. To know the differences between the treatments on the sludge quantity deeply, the result of the Tukey-test showed in Table 2.

Table 2. Effect of substrate water content on the quantity of the sludge

Treatment	Sludge (%)	Significance ($\alpha<0.05$)
P3	0.10	a
P2	0.24	b
P1	0.35	c

The results of this study showed that the water content of P1 produced sludge of 0.35% which was significantly higher than other treatments, there are P2 (0.24%) and P3 (0.08%) respectively. It can be seen that P2 significantly has a higher sludge than P3. Based on that, it can easily to understand that the total sludge is directly proportional to the substrate dry material.

Effect of substrate water content on the sludge nitrogen

The analysis results of nitrogen content in biogas sludge from dairy cow faeces and rice straw showed that the reactions gave varying results, and then a variety analysis test was conducted to determine the effect (ANOVA). The analysis shows the water content of the substrate influences sludge nitrogen. The effect of treatment was determined using Tukey-test, which showed in Table 3.

Table 3. Effect of substrate water content on the sludge nitrogen

Treatment	Mean	Significance (0.05)
P2	3.47	a
P1	3.25	ab
P3	3.05	b

Percentage of nitrogen in sludge is higher than other minerals. The result of percentage nitrogen in sludge biogas from dairy cow faeces and rice straw after implementation water content showed variance effect. The Tukey test in Table 3, showed that the substrate produces the different effect on the nitrogen sludge. It turns out that the water content in biogas manufacture P2 (75%) gives the highest nitrogen sludge (3.47%). This is because the most of the nitrogen (99%) is present in the sludge, while the residue became a gas, then flew into the air (Apandi, 1979). It is also suggested that about 17% of the total nitrogen are present in the water-soluble form, NH_3, and the remainder is organic nitrogen form. Thus, at 90% water content treatment, most of the nitrogen is released, and therefore the nitrogen in sludge is lower than other water content treatments.

Moreover, the loss of nitrogen occurs through two levels of decomposition, namely aerobic and anaerobic. The aerobic process occurs very quickly, resulting in high calorie and loss of nitrogen in the form of ammonium and nitrate. After the oxygen supply is reduced, the condition will change to anaerobic and facultative anaerobic. In anaerobic conditions, reduced nitrogen release in the compounds form (Ismawati, 2003). Although there are variations of nitrogen, the sludge biogas accomplishing the international standards with the value is greater than or equal to 2.3%.

Effect of substrate water content on the sludge phosphor

The content of phosphor sludge biogas varies from 1.04% to 1.34%. The result of ANOVA showed that the treatment of water content of biogas manufacture from dairy cowsfaeces and rice straw gave a real effect to phosphor sludge biogas content. This analysis was continued with Tukey-test, and the result is presented on Table 4.

Table 4. Effect of substrate water content on the sludge phosphorus

Treatment	Mean	Significance (0,05)
P2	1.34	a
P1	1.11	b
P3	1.04	b

The content of phosphorus in sludge biogas varies from 1.04% to 1.34%. The result of analytical statistic shows that sludge biogas from dairy cow faeces and rice straw gives an effect to the phosphorus content of sludge. P2 with water content 75% can produce 1.34% phosphor higher than 1.04% phosphor in P3. Water content 60% and 90% give similar phosphor content. The content of phosphor in sludge is related to the nitrogen content in the substrate resulted from biological degradation. The microorganisms will be involved with phosphor to build up their bodies, such as cell nuclei and protoplasm. The process involves an enzyme called phosphatase (Stofella and Kahn, 2001).

Effect of substrate water content on the sludge potassium

The analysis of the potassium content of the sludge also varied from 1.23% to 1.27%. To find out a variety of treatment effects analysis of variance (ANOVA) test was conducted. The result of the variance analysis showed that the water content treatment had a significant effect on the potassium content of the sludge. Accordingly, the test is continued with the Tukey-test whose results are presented in Table 5.

It can be seen in the Table 5, P2 with water content 75% results in 1.53% potassium, higher than P1 and P3, whileP1 and P3 have asimilar percentage at approximately 1.2%.

Table 5. Effect of substrate water content on the sludge potassium

Treatment	Mean	Significance (0,05)
P2	1.53	a
P1	1.27	a
P3	1.23	b

The analytical statistic result is significantly differentiated of all treatments. The high number of potassium in the P2 (75%) is caused by the condition of the acidity degree is more suitable for bacterial growth. Already composed at 75% water content, the acidogenic bacteria proliferate to produce volatile fatty acids. These acids can change the pH of the substrate being acid, and in this situation, potassium will release in inorganic potassium form, which is useful for microorganisms in the later fermentation stage. From these data can also be known potassium in sludge biogas is lower than that contained in liquid organic fertilizer (1.76%). It is easy to understand because the element of potassium in the substrate comes washed out during the extraction. Furthermore, the content is lower than that in liquid organic fertilizer, and potassium content is still by SNI 19-7030-2004 (0.20%).

Effect of substrate water content on calcium content of the sludge

ANOVA test was conducted to know the effect of treatment on calcium level. Apparently the treatment of water content in the manufacture of biogas from dairy cows faeces and rice straw affects the calcium sludge content. Therefore, the test is continued with a Tukey test whose results are presented in Table 6.

Table 6. Effect of substrate water content on the sludge calcium

Treatment	Calcium Content	Significance (0,05)
P2	1.26	a
P1	1.21	ab
P3	0.82	c

The Tukey-test results showed that the P2 with 75% water content also produced a calcium content (1.26%) higher than the P3 with 90% (0.82%) water content, but no different from the calcium content of the P1 with 60% (0.21%). Calcium content of 60% water content treatment is not different with the result of 90% water content treatment.

Calcium plays a role in cation exchange, so it has an important role in determining the quality of a fertilizer. In the substrate, most of the calcium comes from rice straw. In the early decomposition, the faeces of dairy cows and rice straw are degraded by microorganisms through enzymatic processes. Calcium in the substrate is utilized by microorganisms as micronutrients and is returned into inorganic calcium when degradation is complete. Inside of substrate, Ca act as a buffer to keep the acidity (Diaz and Savage, 2007). At 75% water content, the process of organic matter degradation is better because the growth of acidogenic bacteria is faster, then resulting more enzymes to degrade crude fibre.

Effect of substrate water content on the sludge magnesium

The average magnesium sludge content ranges from 1.04% to 1.41% in each 90% and 75% water content treatments. To find out the influence of treatment of water content on the sludge biogas made by dairy cows faeces and rice straw on variation magnesium content was conducted by ANOVA. The results of the variance analysis showed that the water content treatment affected the magnesium sludge content. To know the effect of treatments, Tukey-test results are presented in Table 7.

Table 7. Effect of substrate water content on the sludge magnesium

Treatment	Mean	Significance (0,05)
P2	1.41	a
P1	1.26	ab
P3	1.04	b

According to the data in Table 7, it is known that in the 75% water treatment, magnesium content is (1.41%) higher than the 90% (1.04%) water treatment, but no difference with the 60% water content. Like calcium, magnesium also plays a role in cation exchange in the soil, which is an essential indicator in determining the quality of solid fertilizer. This element is mostly derived from rice straw. In the rice straw, magnesium binds to amylopectin and hemicellulose (Insam and Bertoldi, 2007). Therefore, the availability of magnesium is influenced by the biological straw degradation of rice through enzymatic processes. At 75% water content, microbial activity is higher than 60% and 90% water content in biogas. In general, the microbial activity is higher with water conditions >90%, but in the fermentation process, it can be changed, because other factors can influence the temperature and pH fermentation. In the biogas, fermentation takes place under thermophile anaerobic conditions and acidic pH, so that is only high temperature and acid resistant microorganisms who can survive at this stage. These microorganisms will break down the crude fibre into volatile fatty acids. In this condition, magnesium is released in an inorganic form that will be used by other microorganisms or plants that fertilized with this organic sludge from biogas production.

Effect of substrate water content on cation value of exchange capacity of the sludge

The results showed that biogas sludge water content treatment of dairy cows faeces and rice straw produced average CEC in varied from 31.56 cmol/kg up to 35.88 cmol/kg. To find out whether the variation is influenced by the water content of the substrate at the time of biogas manufacture, the Tukey test was performed the results, and there are presented in Table 8.

Table 8. Effect of substrate water content on the cation exchange capacity

Treatment	Mean	Significance (0.05)
P2	35.42	a
P1	34.88	a
P3	31.56	b

The Tukey-test results in Table 8 show that the 75% water content produced CEC value (35.42 cmol/kg) higher than the 90% (31.56 cmol/kg) water content, but it has a similar result with CEC in 60% water content treatment. Treatment of 75% water content is also not different with 60% moisture treatment. The CEC value is an indicator of nutrient availability that can be absorbed by plants. This value is related to the content of calcium and magnesium and other elements. Also, the value is also related to the degree of organic matter degradation. As the degradation progresses, the particle size of the substrate will decline, so the surface area will increase and can affect to increase the CEC value. Increased surface area of the substrate, usually occurs in the water content of 75%, because the growth of acidogenic bacteria will faster, followed by occurred in 60% and 90% water content.

Effect of substrate water content on heavy metal and other elements contents

The heavy metal content of As, Hg, Pb, and Cd is so small that it cannot be detected by an Atomic Absorption Spectrophotometer. This is due to the initial content of heavy metals in the substrate components (dairy cowsfaeces and rice straw) which is also too small. Similarly with the microelements Fe, Mn, and Zn. The microelements have a low concentration, for example, Fe, Mn and Zn with 90 ppm, 110 ppm, and 40 ppm respectively. The content of these elements is lower than the solid organic fertilizers required. According to the Minister of Agriculture No.70/Permentan/ SR.140/10/2011, namely the value maximum is 500 ppm, 5000 ppm, and 5000 ppm.

Effect of substrate water content on *Escherichia coli*

The number of *E. coli* bacteria on sludge biogas of dairy cow's faeces and rice straw is varied, according to substrate water content, such as consecutive 0.26 x 102 CFU/g for water content 60%, 0.10 x 102 CFU/g for water content 75%, and 0.35 x 102 CFU/g for water content 90%. To determine whether this variation is due to water content, ANOVA is performed. The analysis results showed that the water content treatment significantly affected the number of *E. coli*. Therefore, further testing is done by using Tukey-test which results are presented in Table 9.

Based on the Tukey test result, it was found that the treatment of 90% substrate water content gave *E. coli* (0.35 x 102 CFU/g) significantly higher than *E. coli* due to water content of 75% (0.10 x 102 CFU/g) and water content 60% (0.26 x 102 CFU/g). The lowest number of *E.coli* resulted in 75% water treatment.

Table 9. Effect of substrate water content on the number of *E. coli*

Treatment	Mean	Significance (0.05)
P3	0.35	a
P1	0.26	b
P2	0.10	c

This is due to a better fermentation process at water content. The acidogenic bacteria play a role in the acidogenesis stage grows and develop rapidly, and also produce more organic acids. As a result, pH sludge biogas decreases, so the atmosphere becomes more acidic and being the inhibits for *E. coli* growth. In addition, acid-producing bacteria metabolism increases in temperature 60-70^{o}C. This temperature is not suitable for *E. coli*. The stage of acidogenesis gradually reaches anaerobic conditions faster than other treatments, so *E. coli* bacteria which is gram negative and anaerobic facultative bacteria more quickly decreased.

Effect of substrate water content on *Salmonella sp*.

The number of *Salmonella sp.* have varies from an average of 0.09 x 102 CFU/g to 0.21 x 102 CFU/g. To know whether the water content of 60%, 75% and 90% effect on the number of bacteria *Salmonella sp.* was conducted by ANOVA. The analysis results revealed that the water content treatment affected the number of *Salmonella sp.* in sludge biogas from dairy cowsfaeces and rice straw. Accordingly, the test is continued by using the Tukey-test whose results are presented in Table 10.

Table 10. Effect of substrate water content on *Salmonela sp*.

Treatment	Mean	Significance (0.05)
P3	0.21	a
P1	0.14	ab
P2	0.09	b

Tukey-test results in Table 10 provides information that there are significant differences in the effect of treatment on the number of *Salmonella sp.* bacteria. In the Table it clearly stated that the 90% water treatment resulted in the number of *Salmonella sp.* (0.21x102 CFU/g) higher than 60% (0.14x102 CFU/g) and 75% (0.09x102 CFU/g) water contents. Conversely, *to E. coli* bacteria, *Salmonella sp.* is an anaerobic bacteria. Thus it is disturbed by reducing a substrate oxygen level. However, *Salmonella* sp. bacteria cannot stand at 72^{o}C in 15 seconds. Because the temperature of the biogas process can reach temperature >70^{o}C, regarding that in a long time total of *Salmonella sp.* bacteria will decrease rapidly.

CONCLUSIONS

It can be concluded that the water content of the substrate 75% produces medium the quantity, and quality of sludge biogas better than 90% (P<0.05), but produces sludge biogas quality which almost equal to 60% water content. This finding suggests that for obtaining a good sludge from the biogas manufacture made from dairy cows faeces and rice straw using batch type digester, it is recommended to use substrate with water content of 75%. Chemical composition of the resulted sludge meets the standard quality of solid organic fertilizer.

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