ABDIMAS 24 (1) (2020): 1-7

ABDIMAS

Jurnal Pengabdian kepada Masyarakat
https://journal.unnes.ac.id/nju/index.php/abdimas/

Biogas Production Using *Aspergillus Nidulans* Isolated From Soil Teak Forest Kare, Madiun

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DOI: http://dx.doi.org/20567/abdimas.v24i1.XXX
Received : 20 November 2018; Accepted: 5 Agustus 2019; Published: 30 June 2020

Abstract
Energy is a difficult problem to deal with. The increase in energy demand is caused by increasing population growth. Therefore, the alternative energy needed is biogas. Biogas is a gas produced by the fermentation process of organic material by anaerobic bacteria. Organic material that can be used for the process of making biogas is bagasse. Biogas production process using bagasse will be pretreatment with cellulolytic molds, namely Aspergillus nidulans. The purpose of this study was to determine the effect of fermentation time and differences in substrate concentrations on the optimization of biogas production. The fermentation time used was 48 hours, 96 hours, 144 hours and 192 hours with mold concentration 0%, 20%, 40%, and 60%. Indicators to be measured were biogas pressure, biogas volume and C / N ratio. The results showed that the fermentation time and concentration of mold affected the biogas pressure and biogas volume. The most optimal biogas pressure was at 96 hours fermentation time with 60% mold concentration and 1,00249 atm while the most optimal biogas volume was 96 hours fermentation time with a concentration of 60% and 0.73 cm³.

Key words: Biogas,;Aspergillus nidulans;Bagasse

INTRODUCTION

The development of human civilization has resulted in increased energy needs, especially energy source from fossil fuels but current energy needs are inversely proportional to the world's available energy reserves. Fossil energy reserves are very limited, so we need alternative energy that can overcome these problems. One of the alternative energy that can be used as a substitute for fossil energy is biogas.

Biogas is a gas produced by the fermentation process of organic material by anaerobic bacteria. The principle of the process of making biogas is the existence of anaerobic decomposition of organic materials. Biogas produces gas which is mostly in the form of methane (CH4) and carbon dioxide (CO2) gas. The decomposition process of anaerobic is assisted by methane-producing microorganisms (Megawati and Control, 2014). Factors that influence biogas formation are temperature, anaerobic conditions, ingredient content, nutrient availability, and pH (Ramdiana, 2017). Organic material that can be used for the biogas manufacturing process is an agricultural waste. Examples of agricultural waste can be corn cobs, fruit skins, rice straws, and bagasse.

Bagasse is a lignocellulosic material that has not been used optimally. Bagasse contains cellulose, hemicellulose, and lignin with a percentage of 50% cellulose, 25% hemicellulose, and 25% lignin (Pujiati, 2018). The high cellulose content of bagasse can be used as a substrate mixture of biogas production. In the production of biogas, methane bacteria are also needed to produce methane gas. These methane bacteria can be obtained from livestock waste, one of which is cow dung. Cow dung has an elemental content of N as much as 26.2 kg/ton, P 4.5 kg/ton, and K 13.0 kg/ton (Riyanta, A. B., et al, 2017).

p-ISSN: 1410-2765; e-ISSN: 2503-1252

Based on research by Adityawarman, et al (2015), biogas production using cow dung produces methane gas (CH4) of 50% - 70% and carbon dioxide (CO2) by 30% - 40%. Maximum production in the study was achieved on day 20th. Riyanta, et al (2017) research on biogas production using a mixture of cow dung and bagasse produced a total volume of biogas 26.57 L / kg substrate. Agnesia et al (2017) also researched biogas production from rice husks using microbial consortium. The results of this study were the use of microbial consortium with 11% variation resulting in the highest biogas yield which is 667.5 ml. Whereas the use of 5% and 8% microbial consortium produced biogas yields of 462 ml and 480.5 ml. Hidayati (2016) conducted a study using inoculum concentration and bagasse hydrolysis duration which included 0 ml / g, 0.2 ml / g, 0.4 ml / g and 0.6 ml / g with 24 hours, 48 hours, 72 hours hour and 96 hours. Optimal results were at a concentration of 0.6 ml / g with duration of 72 hours. Based on previous research, efforts will be made to further optimize the production of biogas using biological pretreatment using cellulolytic molds isolated from Madiun Teak Forest's soil, namely *Aspergillus nidulans*.

Aspergillus nidulans or commonly called *Emericella nidulans* is a species of fungus that is included in the Ascomycota Phylum. In this study, *Aspergillus nidulans* mold acts as a biological pretreatment in the fermentation process of cellulose substrate in the form of bagasse which was used as an ingredient in the process of making biogas.

Based on the above problems, research can be done on the making of biogas from bagasse which is processed through biological pretreatment using *Aspergillus nidulans* molds. This research was expected to optimize biogas production so that it can be used as alternative energy.

METHODS

Research on optimizing biogas production using *Aspergillus nidulans* molds was conducted at Biology Laboratory 2, PGRI Madiun University and Sepuluh November Institute of Technology Surabaya. For the C / N test conducted at the Jengkol Sugar Research Center of PTPN X Kediri. This research was carried out for approximately 3 months.

RESEARCH DESIGN

The research design used was an experimental research design that aimed to determine the optimal biogas production using the fungus *Aspergillus nidulans*. This research was compiled using the factorial completely randomized design (CRD). The used factors were bagasse substrate fermentation with mold concentration of 20%, 40%, and 60% and the fermentation times were 48 hours, 96 hours, 144 hours, and 192 hours. Indicators to be measured in this study were biogas pressure biogas volume and C / N ratio. Measurement was done by the allotted time.

BIOGAS PRESSURE

Measurement of biogas pressure was done by looking at the number or value shown by the manometer U. The magnitude of the pressure value indicated by the manometer U shows the amount of biogas pressure produced. Biogas pressure can be calculated using the formula (Putra, 2017):

P = ρ.g.h + atmospheric pressure

Information :

P = Absolute Pressure (N / m2)

ρ = Density of liquid = 1000 kg / m3

g = Acceleration of gravity = 9.81 m / s2

h = water level

BIOGAS VOLUME

Biogas sample volume was measured by observing the change in the water on the U manometer (assuming: the biogas produced is the same as the change in gas-driven water in the hose). Biogas volume is calculated using the formula (Widyasmara, 2012):

V = π x r2 x t

Information:

V = biogas volume

Π = 3.14

r2 = circle radius

t = cylinder height

C / N content

Intake of levels of C and N was carried out at the beginning of the study on fresh material substrates. Measurements were made using the Kedjhal method.

TOOLS AND MATERIALS

The tools used in this study include test tubes, test tube racks, erlenmeyers, beaker glasses, measuring cups, micropipette, blue tips, autoclave, analytical balance, spatula, hot plate, petri dishes, stirring rod, electric stove, refrigerator, bunsen, lighters, digital scales, aluminum foils, plastic wrapping, sieve, infusion bottles, bed linen bottles, cotton pads,

gauze, dropper pipettes, ose needles, glass bottles, and a set of biogas reactors.

The materials used in this study are Potato Dextrose Agar (PDA), Nutrient Agar (NA), agar powder, CMC, aquades, chloramphenicol, griseofulvin, soil originating from Kresek Forest of Madiun, sugarcane bagasse, *Aspergillus nidulans* fungi, cow dung, and water.

RESEARCH PROCEDURE

The research procedure began with the isolation of mold using PDA media added with CMC then do mold propagation by the streak method. The results of pure molds were used as culture stock in physiological water as much as 700 ml for the pretreatment process. The pretreatment process was carried out in physical and biological ways. Physically was by chopping bagasse while the biological method was by adding *Aspergillus nidulans* molds to bagasse with concentrations of 20%, 40% and 60% then fermented for 48 hours, 96 hours, 144 hours, and 192 hours. After the substrate fermentation process, it was used to make biogas in a ratio of 2: 1

DATA ANALYSIS

Data obtained from the treatment of different concentrations and length of incubation producing experimental data on biogas volume, methane gas and carbon content. The data was analyzed using SPPS (Statistical Product and Service Solutions) with a Variance Analysis (ANOVA) test of 5% significance level to determine the effect of treatment. If an effect was obtained from the given treatment, the test would be continued with the Duncan test at 5% level to find out the significance. How to make data decisions from the ANAVA test is:

a. If P <α is obtained, H0 is rejected and H1 is accepted
b. If P> α is obtained, H0 is accepted and H1 is rejected

must be presented.

RESULTS AND DISCUSSION

Biogas pressure

Biogas pressure was measured by looking at the number that has been shown by the U manometer that was measured every day. The magnitude of the pressure value indicated by the manometer U had shown the amount of pressure and biogas production. Biogas pressure during the fermentation process increased and stagnated.

The total biogas pressure can be seen in the table below:

Table 1. Total Biogas Pressure (atm)

Fermentation time	Concentration			
	K1	K2	K3	K4
T1	1,00162	1,00162	1,00191	1,00201
T2	1,00143	1,00181	1,00210	1,00249
T3	1,00114	1,00152	1,00172	1,00210
T4	1,00123	1,00162	1,00162	1,00172

Table 1 shows that the highest total biogas pressure was found in the 96-hour fermentation treatment with a concentration of 60% (T2K4) which was 1,00249 atm, while the lowest total biogas pressure was in the 144-hour fermentation treatment with a concentration of 0% (T3K1) which was 1,00114 atm. Differences in fermentation time and concentration affected the total biogas pressure production.

Based on table 1, a histogram can be

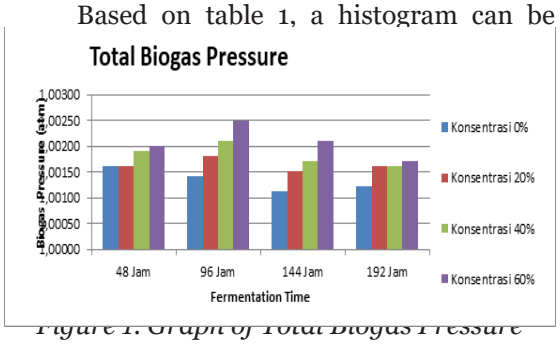
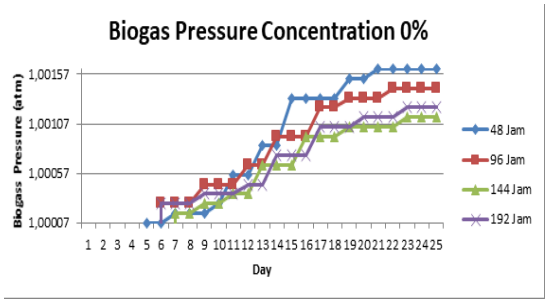
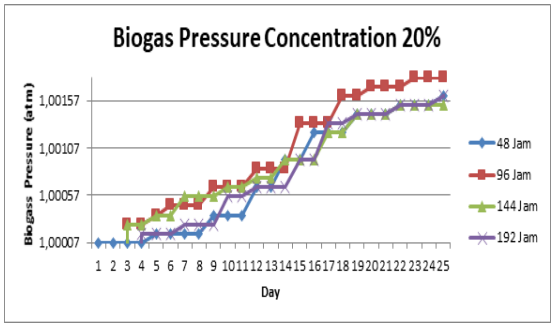
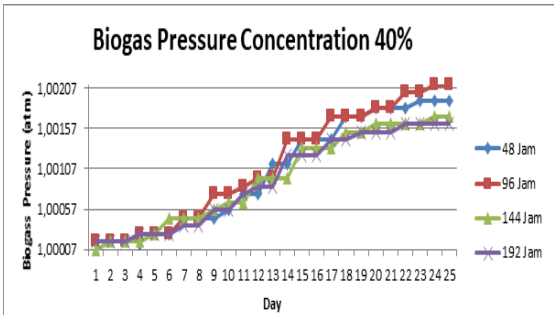
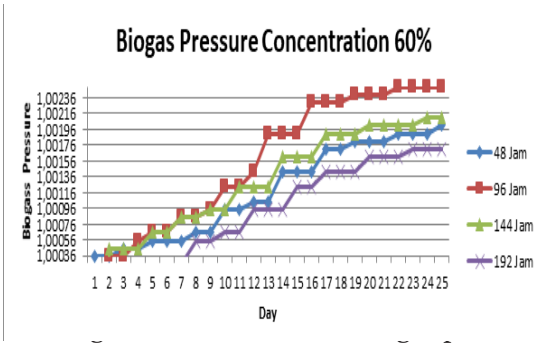


Figure 1. Graph of Total Biogas Pressure









for 25 days that continued to experience a significant increase. The highest biogas pressure was found in the T2K4 sample (96 hours fermentation time, 60% concentration) in the amount of 1,00249 atm. The increase was due to the high concentration of *Aspergillus nidulans* molds on the substrate and the fermentation time.

Based on the Anava test results obtained that the fermentation time and mold concentration have a significance value of <0.05 so that H0 is rejected and H1 is accepted, which means that there is an influence of fermentation time and the amount of concentration on biogas pressure. The higher the concentration of *Aspergillus nidulans* molds fed, the higher the biogas pressure increases. According to Usmana (2012) in his research on ethanol production, the more cellulase enzymes produced by molds, the ethanol yield obtained will also increase.

The duration of fermentation can also affect the biogas pressure production. Based on Figure 1 the 48 hour fermentation time shows the results of low biogas pressure. Then in 96 hours to 144 hours fermentation experiences a significant increase in pressure. According to Hidayati's research (2016), increasing gas pressure shows that mold is in the growth phase, wherein this phase mold metabolism produces optimal cellulase enzymes. Dwi (2011) also states that on the first day to the sixth day the increase in biogas yields is still slow because microorganisms are still in a phase of slow growth. This stage is the phase where microorganisms are in the process of adaptation. Then on the seventh to the 12th day, the biogas produced significantly increased. This is because microorganisms are located in the exponential phase.

During the 192 hour fermentation time the biogas pressure decreased and produced a lower biogas pressure compared to the other treatments. This can be caused by mold lacking nutrition, so mold cannot develop and many dies (Hidayati, 2016).

Biogas Volume

Biogas volume was the amount of gas that has been produced every day from digesters. The volume of the gas was measured by looking at the number that has been shown by the U manometer which was measured every day then calculated using the volume formula. The total biogas volume can be seen in the following table:

Table 2. Total Biogas Volume (cm^3)

Fermentation time	Concentration			
	K1	K2	K3	K4
T1	0,48	0,48	0,57	0,59
T2	0,42	0,54	0,62	0,73
T3	0,34	0,45	0,51	0,62
T4	0,37	0,48	0,48	0,51

Table 2 shows that the highest total biogas volume was found in the 96-hour fermentation treatment with a concentration of 60% (T2K4) in the amount of 0.73 cm^3, while the lowest total biogas pressure was in the 144-hour fermentation treatment with a concentration of 0% (T3K1), i.e. 0.34 cm^3. The difference in fermentation time and concentration affects the total volume of biogas produced.

Based on table 2, a histogram can be made as shown in Figure 2

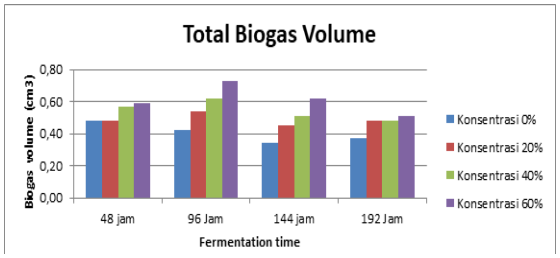


Figure 3. Graph of Total Biogas Volume

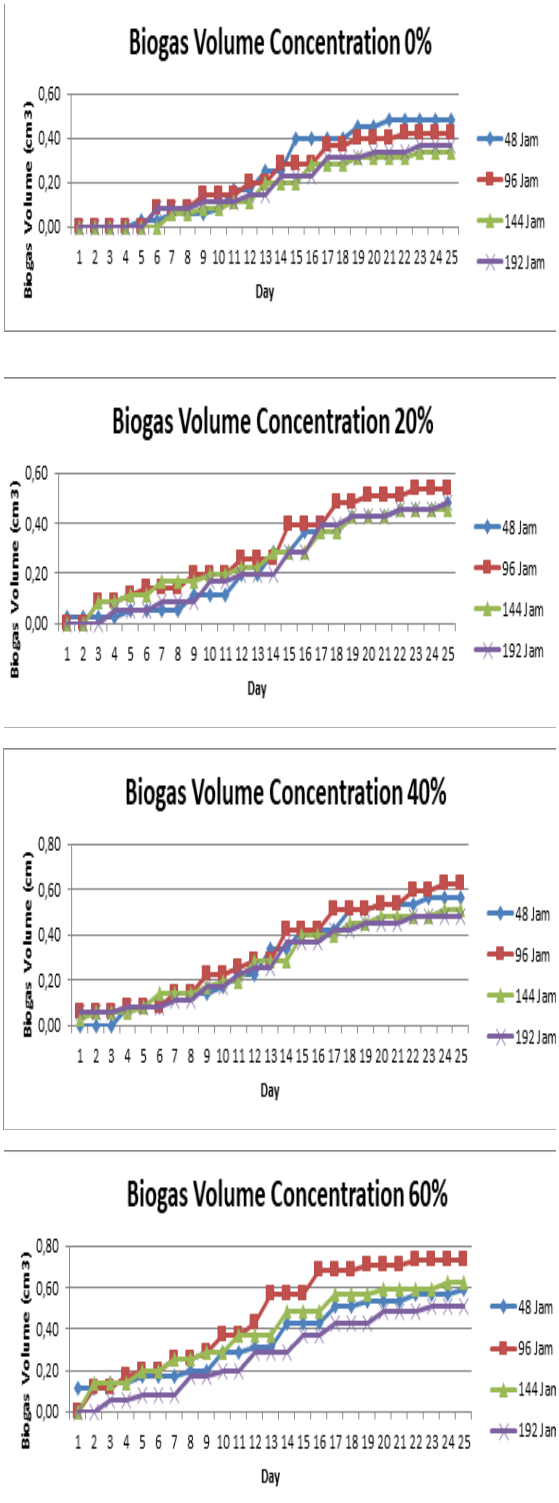


Figure 4. Graph of biogas volume

Figure 3 shows that the total volume of biogas produced had increased. The highest total biogas volume was found in the T2K4 sample (96 hours fermentation time, 60% concentration) which was 0.73 cm^3 while the lowest volume was in the T3K1 sample (144 hours fermentation time, 0% concentration) that was 0.34 cm^3. This biogas volume was the result of the accumulation of gas pressure produced over 12 days using the volume formula.

C / N RATIO

The results of the C / N ratio of bagasse substrate that has been pretreated using *Aspergillus nidulans* mold can be seen as follows:

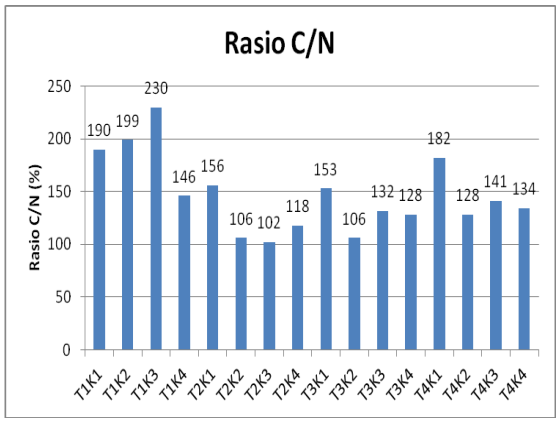


Figure 5. Graphical C / N Ratio

Based on figure 5 the lowest C / N ratio level was found in the T2K3 sample at 102% while the highest C / N ratio was found in the T1K3 sample at 230%. The C / N ratio of 102% produced a biogas volume of 0.282 cm^3 while at a C / N ratio of 230% produced a biogas volume of 0.226 cm^3. According to Wati (2016) in his research if the C / N ratio is too high it will be consumed very quickly by methanogenic bacteria to meet protein needs so that there is no longer a reaction with the remaining carbon. Vice versa if the C / N ratio is very low then nitrogen will be released and collected in the form of NH_4OH.

The analysis also shows that the bagasse was in a dry condition with a water content of 16.5% had a high content of element C which is 55.44% and a very low element N which was 0.28% so the C / N ratio became very high that was 198. Element C on this bagasse was needed by microbes as energy and methane formation. Therefore bagasse is very potential to be used as a biogas producer, but this high C / N ratio was very far from the optimum C / N ratio needed for biogas production (Saputra, 2010).

CONCLUSION

Based on the research that has been done, it can be concluded that the concentration of *Aspergillus nidulans* mold

and fermentation time affects the levels of C / N ratio, biogas pressure, and biogas volume. The highest pressure was found in the T2K4 sample (treatment within 48 hours of fermentation with 60% concentration of *Aspergillus nidulans* mold) of 1,00249 atm with 118% C / N ratio while the lowest was in the sample T3K1 (treatment at 144 hours of fermentation with 0% concentration of mold) of 1,00114 atm with a C / N ratio of 153%. The highest volume of biogas produced was also found in the T2K4 sample (treatment within 48 hours of fermentation with a concentration of 60% *Aspergillus nidulans* mold) of 0.73 cm3 with a 118% C / N ratio while the lowest was in the T3K1 sample (treatment at 192 hours fermentation time with 0% concentration) that was 0.34 cm3 with a C / N ratio of 153%.

ACKNOWLEDGEMENT

The author is grateful for the PPKPT research grant provided by RISTEKDIKTI, Dr. techn.Endry Nugroho Prasetyo, MT, Nurul Jadid, Phd as research partners so that this research can be completed well.

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