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Original Article

Enhancement of biogas production from bagasse biomass through *Aspergillus flavus* pretreatment

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Abstract

Population growth in Indonesia is directly linked to energy consumption and environmental waste production, including organic waste. Abundant organic waste must be exploited to develop alternative energy sources that can fulfil the needs of the community, such as biogas production. This study intends to investigate the potential of the fungus *Aspergillus flavus* in the pretreatment of bagasse as a mix for biogas production. The biological pretreatment technique for bagasse substrate with the influence of fermentation time and concentration is implemented as a substrate combination for biogas production between bagasse and cow dung. The results indicated that the highest biogas pressure occurred after 48 h of fermentation at a concentration of 40% (T_1F_3) of 1.00230 atm, while the lowest pressure occurred after 192 h of fermentation at a concentration of 0% (T_4F_1) of 1.00123 atm. The maximum amount of biogas was produced after 48 h of fermentation, when the concentration reached 40% (T_1F_3) and the volume reached 0.68 cm^3. The lowest number of biogas was produced after 192 h of fermentation, when the concentration was 0% (T_4F_1) and the volume was 0.37 cm^3. Based on the research, it can be concluded that the addition of organic waste, including bagasse and the pre-treatment process with *Aspergillus flavus*, can increase biogas production by around 40%. The long-term goal of this research is to find a mold formula that can increase biogas production, especially biogas production that uses more organic material.

Keywords: Biogas, *Aspergillus flavus*, pretreatment, bagasse, pretreatment

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Introduction

Population expansion results in an increase in energy consumption. The depletion of energy sources and the high cost of energy have required the creation of new technologies and solutions to address these needs. This population growth affects the amount of trash in the ecosystem, including organic waste. Agricultural and livestock byproducts are considered organic waste. There is a lot of organic waste that can be used as a raw material to produce biogas and other renewable energy sources(Ohnmacht et al. 2021). Biogas is one method of digesting waste, such as agricultural, livestock, and industrial waste, that can produce alternative energy (Asparuhov and Simeonov 2012; Eswanto, Ilmi, and Siahaan 2018; Lemma, Ararso, and Evangelista 2020; Mirmohamadsadeghi et al. 2021; Pujiati, Dewi, and Setiawan 2020; Pujiati, Ratna, and Dewi 2020; Singh et al. 2021). Biogas dominated by methane, a flammable gas. Biogas has a beneficial environmental impact because less CO2 is released during combustion than during photosynthesis(Mehta et al. 2018). The major source of methane production by spoilage bacteria is the decomposition of organic matter. Methanogenesis by methanogenic bacteria is dependent on the availability of critical materials for their metabolism, such as carbon (C) and nitrogen (N). Carbon and nitrogen are macromolecules that play structural and functional roles in the cell components of bacteria. Carbon is mostly used by microorganisms in the form of carbon dioxide (CO2), but it also exists in other organic forms (glucose) and serves as a major constituent in cellular structures. Nitrogen in the forms of NH3, NO3, N2, and organic forms is used to make amino acids, coenzymes, and nucleic acids (Widadri, Mangunwardoyo, and Ambarsari 2019)

Cow manure is used as a raw material in the biogas production process because it includes methanogenic bacteria. The literature reports that cow dung manure contains between 1.6 and 23.5% cellulose, 1.4 and 12.8% hemicellulose, and 2.7 and 13.9% lignin. Cow dung's % cellulose content makes it an appropriate feedstock for the extraction of cellulose for the production of biomaterials based on cellulose (Puri et al. 2020; Zulkifli et al. 2018). Cow manure's high cellulose content can produce vast quantities of biogas (Effendy and Syarif 2018). Bagasse, which contains 40.59% cellulose, 15.91% hemicellulose, and 17.50% lignin, can also be used as a substrate for biogas production (Hidayati, Pujiati, and Rahayu 2016). Biological treatments are capable to degrade bagasse with a high cellulose concentration. Biological pretreatment is an emerging technology that has the potential to boost biogas yield (Zhao et al. 2020). In this process, cellulolytic microorganisms such as bacteria and fungi can be utilized. *Trichoderma* sp, *Rhizopus* sp, and *Aspergillus* sp are examples of cellulolytic mold (Copete-Pertuz et al. 2019; Pujiati, Ardhi, and Sulistyarsi 2013). Aspergillus species, such as *Aspergillus niger*, have been

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extensively applied as microorganisms in the biological pretreatment process for biogas production. Based on previous research, 20% concentration of *A. niger* can reduce the C/N ratio of bagasse by 56.64% (Pujiati and Widiyanto 2017). Pretreatment of orange peel waste with *A. niger* provided the highest biogas yield of 308.85 ml/g-VS with a methane concentration of 57%; this result is superior to the value before optimization (Su, Tan, and Xu 2016). This research will be conducted in order to enhance biogas production by using cellulolytic fungi such as *Aspergillus flavus*. *A. flavus* is a saprophytic, soil-dwelling fungus(Li and Zhu 2021; Wang et al. 2022; Xue et al. 2022). *A. flavus* isolated from the soil of the teak forest in Kare, Madiun utilized as a bagasse pretreatment agent at the specified concentration and fermentation period. The role of *A. flavus* as a microorganism in the biogas production pretreatment process has never been investigated. Additionally, a number of sugar manufacturing hubs in Madiun generate bagasse waste.

This research is necessary in an effort to convert the waste due to the abundance of bagasse waste. Based on the backgorunds, it is feasible to conduct research on the production of biogas from cow dung and bagasse substrates that have been biologically pretreated with *A. flavus.* Specifically, the aims of this study were to determine the effect of concentrations of *A. flavus* on the pretreatment process of bagasse substrate to optimize biogas production and to determine the effect of *A. flavus* fermentation time in the pretreatment process of bagasse substrate for optimization of biogas production.

Methods

Research on the potential of *A. flavus* in biogas production by adding bagasse substrate was carried out at the Biology Laboratory of the University of PGRI Madiun and the Institut Teknologi Sepuluh November, Surabaya. The C/N test was conducted at the Sugar Research Center of PTPN X Kediri. This research was carried out for approximately 3 months. The experimental research design was utilized to investigate the potential for biogas production utilizing the fungus Aspergillus flavus. The fungus was isolated using PDA media supplemented with CMC as the first step in the study technique. The factorial Completely Randomized Design was used to structure this investigation (CRD). Biogas pressure, biogas volume, and the C/N ratio will be measured in this study. Measurements are completed within the timeframe specified.

Materials and equipment

Autoclaves, analytical balances, spatulas, hot plates, petri dishes, stirring rods, electric stoves, refrigerators, Bunsen, lighters, digital scales, aluminum foil, plastic wrapping, infusion bottles, bed linen bottles, cotton pads, gauze, drip pipettes, inoculation needles, glass bottles, test tubes

The following ingredients were utilized in this investigation: Potato Dextrose Agar (PDA), Nutrient Agar (NA), agar powder, Carboxy methyl cellulose (CMC), distillated water, chloramphenicol, Griseofulvin, soil from the Madiun Kare teak forest, bagasse powder, Aspergillus flavus mold, cow dung, and water were utilized in this investigation.

Pre-treatment process

The substrate of bagasse was pretreated in two ways: physically and biologically. Physical pretreatment was washed and dried in the sun until the color changed, suggesting a reduction in water content, then cut ±2 cm, ground using a grinding machine, and sieved (Kodri, Argo, and Yulianingsih 2013). The humidity is then measured. 1 kilogram of bagasse substrate powder is mixed with 2 liters of water. It was placed in 12 bottles, each containing 60 g of substrate, and sterilized for 15 minutes at 121 ° c temperature in an autoclave. Biological pretreatment with cellulosic molds was performed by introducing Aspergillus flavus at concentrations of 0%, 40%, and 60% with fermentation times of 48 h, 96 h, 144 h, and 192 h. The stock culture was made by pouring 3 tubes of mold spores from 4 days old slanted agar into 700 ml of sterile distilled water.

Biogas production

Bagasse that had been pretreated using Aspergillus flavus and cow dung was put into a 1500 ml volume reactor. The calculation of the ratio of the substrate used is water: substrate (2:1) with a total volume of 750 ml. The ratio is 500ml of water to 250ml of substrate. The substrate used is calculated with a ratio of cow dung to bagasse of 75%: 25% (Siswanto and Susanto 2018)

Biogas pressure

The biogas pressure is measured by glancing at the number or value displayed by the U manometer. The magnitude of the pressure measurement provided by the U manometer represents the pressure of the produced biogas. The pressure is calculated using Boyle's law with the following formula (Putra et al. 2017):

$$\mathbf{P = \rho.g.h + atmospheric\ pressure}$$

Where:

P = Absolute Pressure (N/m^2)

ρ = Fluid density = 1000 kg/m^3

g = Gravitational = 9,81 m/s^2

h = water level (m)

1 atm = 101325 N/m^2

1 N/m^2 = 9,869 x 10^{-6}

Biogas volume

The biogas sample volume is determined by measuring the change in water on the U manometer (assuming: the biogas produced is the same as the change in water driven by gas in the hose). The following formula is used to compute the volume of biogas(Daniyan et al. 2019; Widyasmara, Pratiwiningrum, and Yusiati 2012):

$$\mathbf{V = \pi \times r^2 \times t}$$

Where:

V = Biogas Volume
Π = 3,14
r^2 = circle's radius
t = cylinder height

C/N ratio

The C and N concentrations were measured at the start of the investigation on bagasse substrate that had been fermented with inoculum. The Kedjhal technique was used for measurements (Pujiati, Ratna, and Dewi 2020; Rahman et al. 2020)

Data examination

The experimental data on biogas volume, methane and carbon dioxide content emerged from the treatment of changes in concentration and incubation period. To determine the effect of therapy, the data was analysed using SPPS (Statistical Product and Service Solutions) with the Analysis of Variance (ANAVA) test at a significance level of 5%. If the effect of the therapy is not obtained, the test will be repeated using Duncan's test at the 5% level to establish significance.

Results

Table 1. C/N Ratio data

No	Treatment	carbon (%)	Nitrogen (%)	C/N
1.	T_1F_1	7,60	0,06	190
2.	T_1F_2	8,16	0,09	91
3.	T_1F_3	7,18	0,06	120
4.	T_1F_4	6,07	0,04	152
5.	T_2F_1	7,82	0,05	156
6.	T_2F_2	6,96	0,04	174
7.	T_2F_3	6,30	0,04	158
8.	T_2F_4	5,93	0,02	147
9.	T_3F_1	7,65	0,05	153
10.	T_3F_2	7,47	0,05	149
11.	T_3F_3	6,30	0,05	126
12.	T_3F_4	5,13	0,05	103
13.	T_4F_1	7,26	0,04	182
14.	T_4F_2	7,23	0,05	145
15.	T_4F_3	5,63	0,05	113
16.	T_4F_4	5,26	0,03	175

Note: T_1 = 48 h Fermentation, T_2 = 96 h Fermentation, T_3 = 144 h of fermentation, T_4 = 192 h Fermentation; F_1 = 0% mould concentration, F_2 = 20% mould concentration, F_3 = 40% mould concentration, F_4 = 60% mould concentration

Biogas Pressure

The biogas pressure is measured by examining the number displayed by the U manometer, which is checked every day. The magnitude of the pressure shown by the U manometer has revealed the magnitude of the resulting pressure. During the fermentation process, the biogas pressure rises and then falls. The following table shows the overall biogas pressure:

Table 2. Total Biogas Pressure (atm)

Fermentation Time	Concentration			
	F1	F2	F3	F4
T1	1,00133	1,00172	1,00230	1,00172
T2	1,00152	1,00172	1,00181	1,00191
T3	1,00172	1,00191	1,00172	1,00201
T4	1,00123	1,00162	1,00191	1,00191

Note: T_1 = 48 h Fermentation, T_2 = 96 h Fermentation, T_3 = 144 h of fermentation, T_4 = 192 h Fermentation; F_1 = 0% mould concentration, F_2 = 20% mould concentration, F_3 = 40% mould concentration, F_4 = 60% mould concentration

Volume of Biogas

Biogas volume is the amount of gas produced by digesting every day. The volume of gas is calculated by glancing at the number displayed by the U manometer, which is monitored every day. The volume formula was then used to calculate the result. The following table shows the total amount of biogas:

Table 3. Total Biogas Volume (cm^3)

Fermentation Time	Concentration			
	F1	F2	F3	F4
T1	0,4	0,51	0,68	0,51
T2	0,45	0,51	0,54	0,57
T3	0,51	0,57	0,51	0,59
T4	0,37	0,48	0,57	0,57

Note: T_1 = 48 h Fermentation, T_2 = 96 h Fermentation, T_3 = 144 h of fermentation, T_4 = 192 h Fermentation; F_1 = 0% mould concentration, F_2 = 20% mould concentration, F_3 = 40% mould concentration, F_4 = 60%mould concentration

Discussions

C/N ratio

C/N ratio is measured as data from pretreatment process**.** The C/N ratio reflects the nutrient levels of a digestion substrate: a high C/N ratio results in a low protein solubilization rate, whereas a high C/N ratio indicates inadequate nitrogen to maintain cell biomass, rapid nitrogen degradation by microbes, and an elevated chance of ammonia inhibition(Gonçalves Neto et al. 2021). The C/N ratio has a significant impact on the biogas production. The carbon-to-nitrogen ratio of organic matter is essential for the survival of microorganisms. Organic material with a low C/N ratio decomposes faster than organic material with a high C/N ratio because enough N is released during decomposition to meet the N requirements of the decomposing bacteria (Yuan et al. 2016).

The analysis of the C/N ratio of bagasse substrate fermented with *A. flavus* revealed the lowest value on the T_1F_2 substrate after 48 h of fermentation at a concentration of 20%. While the C/N ratio on the T_2F_4 substrate remains quite high after 96 h of fermentation at a concentration of 60%, According to the graph, the C/N ratio of sugarcane bagasse fermented with mould did not decrease. Raw Bagasse had a C/N ratio of 160.92% (Ismaya et al. 2012). According to Table 1, the greatest C/N ratio of the substrate fermented for 96 h was 297%, and the inoculum concentration was 60%. The Increase of C/N ratio is caused by a high content of C elements and a low amount of N elements (Pujiati et al. 2021). Microbes require element C in bagasse for energy and the synthesis of methane. Bagasse has the potential to be utilised as a substrate for biogas generation based on this, but the C/N ratio is far from the ideal required for biogas production. The number of C/N ratio, which didn't change after treatment, could be caused by a number of environmental factors, such as the level of humidity and the size of the substrate sieves.

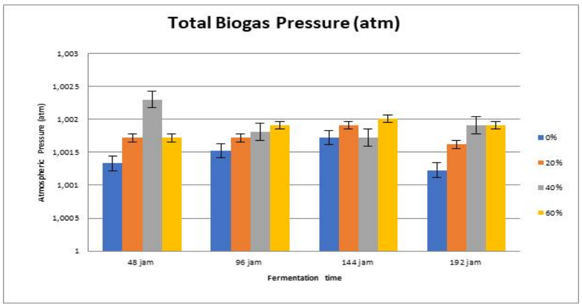

Figure 1. Graph of total biogas pressure

Biogas Pressure

The increase in the number displayed by the U manometer, which is linked to the digester faucet and measured daily, is used to determine the biogas pressure. According to the U manometer, the increase in number indicates the degree of the pressure generated by the biogas production process. Table 1 shows that the 48 hour fermentation time treatment with a concentration of 40% (T_1F_3) has the highest total biogas pressure (1.00230 atm), while the 144 hour fermentation time treatment with a concentration of 0% (T_4F_1) has the lowest total biogas pressure (1.00123 atm).

The overall pressure of the biogas produced is affected by differences in fermentation duration and concentration. The total biogas pressure graph in Figure 1 shows the highest pressure at 48 h of sugarcane bagasse fermentation time with 40% inoculum administration, with a pressure of 1.00230 atm, and the lowest pressure at 144 h of fermentation time with a concentration of 0%, with a pressure of 1.00123 atm. The resulting pressure grows with each fermentation time with different concentrations but lowers in the final fermentation period, which is 192 h of fermentation. This condition caused by a lack of nourishment, which no longer allows mold to grow, causing many molds to die.

The graph (Fig. 2) also shows that the pressure is always lowest at the beginning of fermentation. This means that at 24 h, the mold is still reacting to the substrate and the surrounding environment, so cell division hasn't happened yet because some enzymes may not have been made. Based on the Anova test results, it was discovered that the fermentation time and mold concentration had a significance value of 0.05, so H0 was rejected and H1 was approved, indicating that there is an effect of fermentation time and concentration on biogas pressure. The resulting pressure is affected by the period of fermentation and the amount of concentration. The quantity of microorganisms delivered affects the fermentation process; the fermentation process can be optimized by giving the appropriate amount of inoculum (Pujiati, Kiswardianta, and Wahyuni 2014).

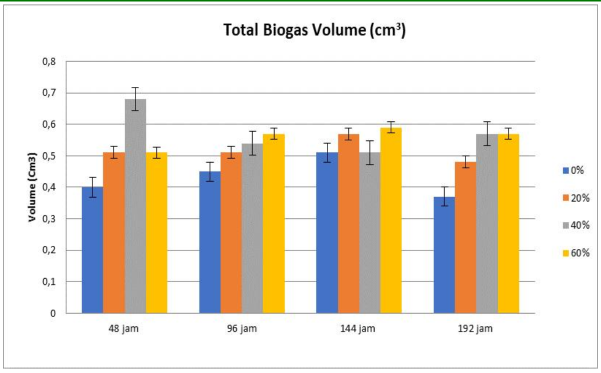

Figure 2. Graph of Total Biogas Volume

Biogas volume

The volume of biogas is calculated by measuring the pressure or changes in water on the U manometer and assuming that the volume produced is equal to the change in water caused by gas in the hose (Widyasmara, Pratiwiningrum, and Yusiati 2012).

Table 2 shows that the 48-hour fermentation time treatment with a concentration of 40% (T_1F_3) has the maximum total biogas volume of 0.68 cm3, while the 144-hour fermentation time treatment with a concentration of 0% (T_4F_1) has the lowest total biogas pressure of 0.37 cm3. The overall volume of biogas produced is affected by differences in fermentation time and concentration Figure 2 demonstrates that overall biogas production has grown. The sample with the greatest overall biogas volume was T_1F_3 (48 h of fermentation, 40% concentration), which measured 0.68 cm^3 total. The sample with the lowest volume was T_4F_1 (fermentation time 192 h, concentration 0%), which measured 0.37 cm^3. The data represents a 25-day accumulation of gas pressure measurements.

According to the research, the concentration of *A. flavus* and the time of fermentation affect the levels of C/N ratio, biogas pressure, and biogas volume. The greatest pressure of 1.00230 atm with a C/N ratio of 120% was recorded in sample T_1F_3 (treatment within 48 h of fermentation with a 40% concentration of *A. flavus* mould). The lowest was 1.00123 atm with a C/N ratio of 182% in the sample T_4F_1 (Treatment at 192 h of fermentation with 0% mould concentration).

Sample T_1F_3 (treatment within 48 h of fermentation with a 40% concentration of *A. flavus* mould) produced the most biogas, with a volume of 0.68 cm3 and a C/N ratio of 120%. While the T_3K_1 sample (treatment at 192 h of fermentation with 0% mould concentration) had the lowest C/N ratio (182%), it was 0.37 cm3. This fits with the volume and pressure of the biogas that was made, especially after 48 h of fermentation and a concentration of 40% (T_1F_3). This shows that the time of fermentation affects the drop in the C/N ratio but not the amount of concentration supplied.

Based on the research, it can be concluded that the addition of organic waste, including bagasse and the pre-treatment process with *A. flavus*, can increase biogas production by around 40%. The long-term goal of this research is to find a mold formula that can increase biogas production, especially biogas production that uses more organic material.

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