The effects of microorganism on coffee pulp pretreatment as a source of biogas production

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Abstract. Coffee pulp waste composition consist of cellulose, hemicellulose, lignin, pectin and caffeine, tannin, and polyphenol as inhibitor substance. The high cellulose compound in coffee pulp can be used for alternative raw materials in the manufacture of biogas. This study aims to define the composition of the mixture of microorganisms of *Pseudomonas putida, Trichoderma harzianum*, and *Aspergillus niger* that are best to use in coffee pulp pretreatment to degrade inhibitor substance. The best result of pretreatment will be applied to biogas production. The first step is to do a pretreatment of the coffee pulp with variable *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger* with a ratio of 1:1:1, 1:2:1, 1:1:2, 1:2:2, 2:1:1, 2:1:2, 2:1:1 (v:v:v), then variables that are most excellent in degrading inhibitor substance are selected. The second step, is doing anaerobic fermentation for 20 days at mesophilic temperature (30-40^{o}C) on a reactor working volume of 4.5 L. In the making of biogas, a varied starter as much as 10% of the total are put into the reactor in the form of a mixture of cow dung : rumen fluid with a ratio of 1:0, 0:1, 1:1, 1:2, 2:1 (w/v). The parameters measured include the decreasing of the inhibitor substance, Chemical Oxygen Demand (COD), biogas (CH_4 and CO_2) and calorific value of combustion (Heating value). This study results a composition of ingredients within the pretreatment process which includes a mixture of microorganisms with a ratio of *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger* is 1:2:1 (v:v:v). For biogas, a mixture of cow dung and rumen fluid can produce higher methane gas is the ratio of cow dung : rumen fluid is 1:2 (w/v) with the concentration of methane (CH_4) formed at 1.825%. At the concentration of methane is 1.825%, the heating value obtained is 76.032 kcal/kg with volume biogas 0.0032 m^3/ kg converted of COD.

1. INTRODUCTION

One of the many industrial waste obtained from processing coffee beans include coffee pulp. Its composition consists of 57.9% cellulose, 21.63% hemicelluloce, 5.21% lignin, 2.28% pectin and inhibitor substance such as 0.86% caffeine, 4.81% tannins, 3.48% polyphenol. Coffee pulp is high in cellulose, which as a raw material, can be generated into an alternative source for the production of biogas.. However, some substances within coffee pulp contains inhibitor, such as caffeine, tannins, and polyphenols which are able obstruct the production of biogas [1].

Degradation of the toxic material contained in coffee pulp such as caffeine, tannins and polyphenols, can be executed by the use of microorganisms [1]. Enzymes in microorganisms is involved within the process of bacterial inhibitor degradation, by breaking down the inhibitor. The process of degradation of inhibitor substance in coffee pulp, helps the microorganism to turn the cellulose in coffee pulp into biogas.

Ethanol and sodium hydroxide are the chemical used in the pretreatment process. Within the pretreatment, lignin is broken down to increase the cellulose in the coffee pulp [2]. The drawback of chemical pretreatment is the expensive ingredients used and the toxicity of the raw material itself.

Whereas in biological pretreatment, non-toxic parts can be produced as the microorganisms grow on the inhibitor substances. In short, biological pretreatment is more environmentally friendly than the chemical pretreatment because harmful materials such as chemicals that will be bad for the environment are not being used.

In degrading caffeine, microorganisms such as *Klebsiella, Serratia*, and *Pseudomonas* found the highest percentage of caffeine degradation in *Klebsiella* with 100% degradation percentage in 10 hours [3]. *Pseudomonas putida* has of 67.2% percentage of caffeine degradation within 48 hours.

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Several microorganisms can degrade Tannins, however fungi species have a specialty in degrading tannins such as Chaetomium, *Fusarium, Rhizoctonia, Clyndrocarpon, and Trichoderma harzianum* [4] and microorganisms that can degrade polyphenols such as *Aspergillus, Fusarium, Coprinus, Penicillium* and the highest degradation rate is *Aspergillus niger* of 8 mg/L/h.

It can be analyzed in previous research that *Pseudomonas putida*, *Trichoderma harzianum*, and *Aspergillus niger* have special ability to degrade each inhibitor substance. So the three selected microorganisms to degrade the inhibitor substances as a variable in degrading inhibitor substance and then applied in the manufacture of biogas.

2. EXPERIMENT DETAILS

2.1Sampling and coffee pulp treatment

Coffee pulp samples were obtained from PTPN XII plantations, Malang, East Java, Indonesia. The coffee pulp was prepared by separating leaves, rocks, and other materials and leaving only the coffee pulp.

The water content of the coffee pulp is decreased through the process of drying. Then the dried coffee pulp is reduced in size about ± 100 mesh.

2.2 Preparation of medium and microorganism

2.2.1 Medium for Bacteria

4 Grams of nutrient broth, 6.25 grams of agar with 1.5 grams of glucose into 500 mL of boiling water are mixed to create the liquid medium. The autoclave then sterilizes at 121^{o}C, and media was transferred into test tube and cooled until 28^{o}C. Local strain of Pseudomonas putida was moved to agar medium. An incubator was used to inoculate the bacteria and media at 30^{o}C.

2.2.2 Medium for Fungi

19.5 Grams of PDA powder and 6.25 grams of agar into 500 mL of boiling water were mixed as the liquid medium. The autoclave then sterilizes at 121^{o}C, and media was transferred into test tube and cooled until 28^{o}C. Local strain of *Trichoderma harzianum* and *Aspergillus niger* were then moved to agar medium. Fungi and media ware inoculated inside incubator at 30^{o}C.

2.3 Pretreatment Coffee Pulp

Mixing the coffee pulp with water at 1:4 ratio results in substrate. Substrate was prepared by mixing 42.5 grams of coffee pulp and 170 mL of water in Erlenmeyer (Fig 1). Then, a mixture of microorganisms as much as 15% of the amount of substrate around 30 mL was moved from agar medium to substrate. Substrate in the reactor were inoculated inside incubator shaker at 30^{o}C and 100 rpm. An aerobic system is maintained through the substrate and microorganisms in bioreactor. There were 7 bioreactors with different variables (ratio microorganisms *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger* are 1:1:1, 1:2:1, 1:1:2, 1:2:2, 2:1:1, 2:2:1, and 2:1:2 (v:v:v)).

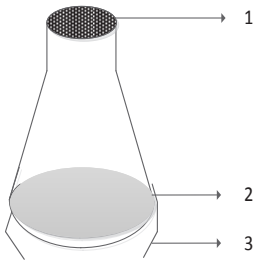

Fig 1. Bioreactor for Pretreatment of Coffee Pulp
1. Cover of Erlenmeyer
2. Substrate and mixture of microorganisms
3. Clamp of shaker

For 7 days, pretreatment of coffee pulp is being executed. Every 2 days, samples are taken from the bioreactors to be analyzed. The analysis include the decrease of inhibitor substance and increase of microorganisms in bioreactors. From the process pretreatment, the best comparison of microorganisms added with the indicated amount of decrease of inhibitor substance the highest is obtained. The best variable will be applied to biogas manufacture.

2.4 Manufacture of Biogas

2.4.1 Preparation of Starter

Cow dung and rumen fluid are used as starter in this experiment. There were 5 biogas reactors with different starter variable. Starter added to the ratio of cow dung: rumen fluid is 1:0, 0:1, 1:1, 1:2, 2:1 (w/v). Based on a study about the manufacturing of biogas by Abdillah Jaka et al in 2013, starter mixed with 10% of the best result of pretreatment coffee pulp and fermented for 5 days. Then, starter is fed into a biogas reactor that containing 90% of total coffee pulp.

2.4.2 Anaerob Fermentation

A separation between substrate and the filtrate is done and the substrate is then dried with an oven for 24 hours and with the temperature of 105^{o}C before mixed with starter. The volume of biogas reactor used is 6 L with a substrate volume of 4.5 L and starter volume of 0.5 L. For 20 days fermentation of biogas is carried out to produce methane (CH_4). The operating state of biogas fermentation is mesophilic temperature (30-40^{o}C) and in anaerobic system (Fig 2).

Every 4 days, samples substrate and gas from fermentation taken from biogas reactors and to analyzed pH, Chemical Oxygen Demand (COD),

biogas (CH_4 and CO_2) and caloric value of combustion (Heating Value).

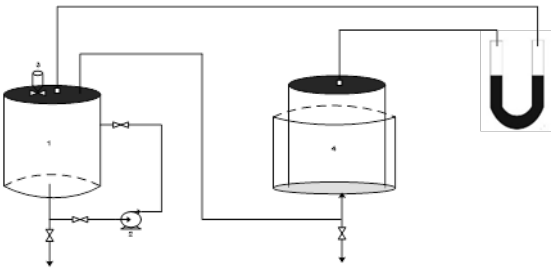

Fig 2. Biogas Reactor

1. Feed Tank
2. Pump
3. Inlet Feed
4. Gas Holder
5. Manometer

2.5 Total Cell Counts

During the degradation of inhibitor substances, total cells counts were determined. The counting method was counting chamber. 1 ml of slurry was diluted by 9 ml aquadest. Dilution was put in haemacytometer. Cell counts were observed and counted under microscope. Cell counts were done everytime for a week. The result was plotted in graphic a form, compared with time and decrase of inhibitor substaces.

2.6 Analysis of Biogas

Biogas sample consist of methane (CH_4) and carbon dioxide (CO_2) from biogas reactor then analyzed at Energy Laboratory, Sepuluh Nopember Institute of Technology, Surabaya, by gas chromatography (GC) method and nitrogen as gas carrier at 200^{o}C.

3. RESULT AND DISCUSSION

3.1 Composition of Coffee Pulp

Table 1. Composition of Coffee Pulp at PTPN XII Malang

Composition Of Coffee Pulp	Percent Composition (%)
Cellulose	57.9
Hemicellulose	21.63
Lignin	5.21
Pectin	2.28
Tannin	4.81
Caffeine	0.86
Polyphenol (Total Phenol)	3.48

That composition is the result of dry coffee pulp analysis before pretreatment. Based on Van Dam of 2003, composition of coffee pulp is different from each other. It is because of the different in source, type, and maturity of raw materials.

3.2 Coffee Pulp Pretreatment

The addition of microorganisms such as *Pseudomonas putida*, *Trichoderma harzianum*, and *Aspergillus niger* spill accelerate the process degradation of inhibitor substance in coffee pulp. The addition of microorganisms was supposed to decrease inhibitor substance such as caffeine, tannins, and polyphenol that was contained in the coffee pulp. The results of the analysis related to the inhibitor substance in seventh bioreactors were shown in Fig 3 to Fig 9.

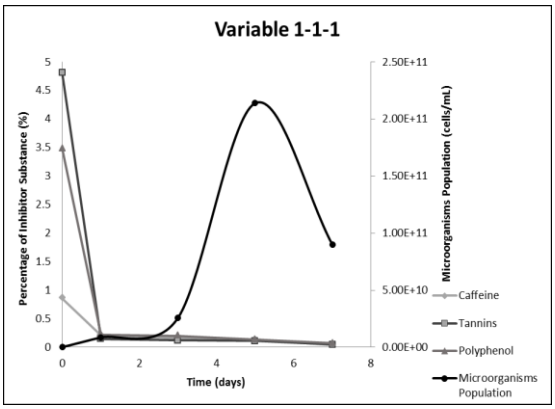

Fig 3. The correlation between the percentage of inhibitor substances, the increase in microorganisms population and time on the ratio of *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger* of 1:1:1

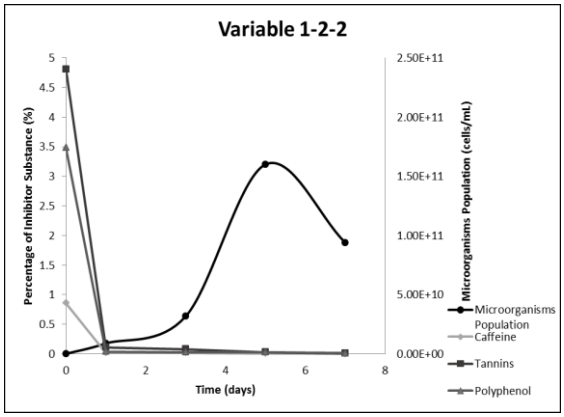

Fig 4. The correlation between the percentage of inhibitor substances, the increase in microorganisms population and time on theratio of *Pseudomonas putida :Trichoderma harzianum : Aspergillus niger* of 1:2:2

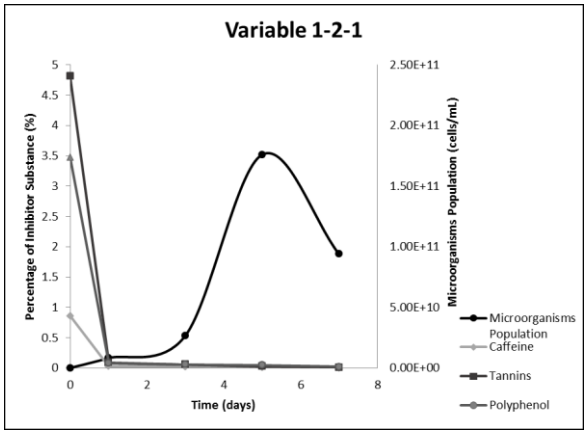

Fig 5. The correlation between the percentage of inhibitor substances, the increase in microorganisms population and time on the ratio of *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger* of 1:2:1

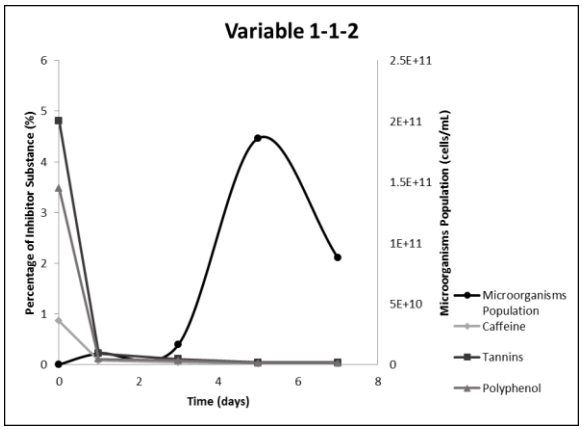

Fig 6. The correlation between the percentage of inhibitor substances, the increase in microorganisms population and time on the ratio of *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger* of 1:1:2

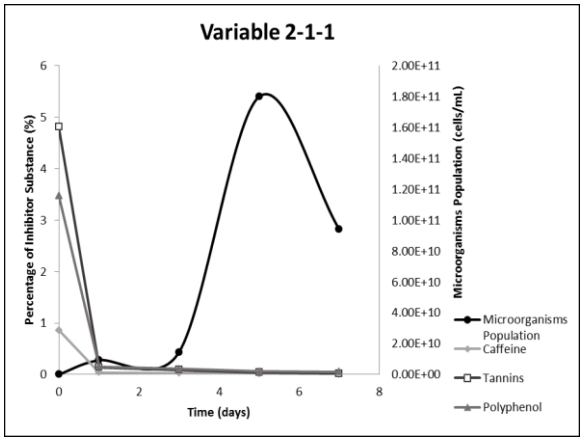

Fig 7. The correlation between the percentage of inhibitor substances, the increase in microorganisms population and time on the ratio of *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger* of 2:1:1

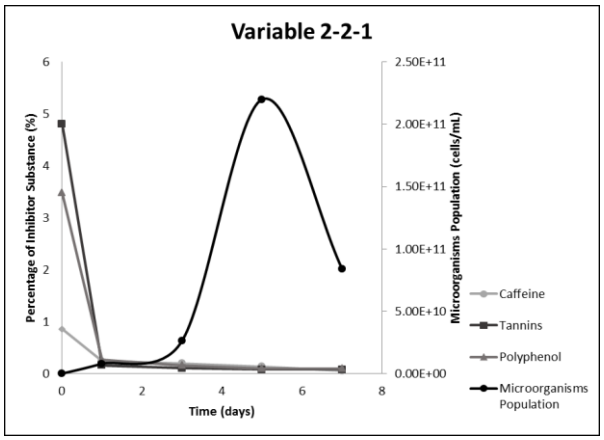

Fig 8. The correlation between the percentage of inhibitor substances, the increase in microorganisms population and time on the ratio of *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger* of 2:2:1

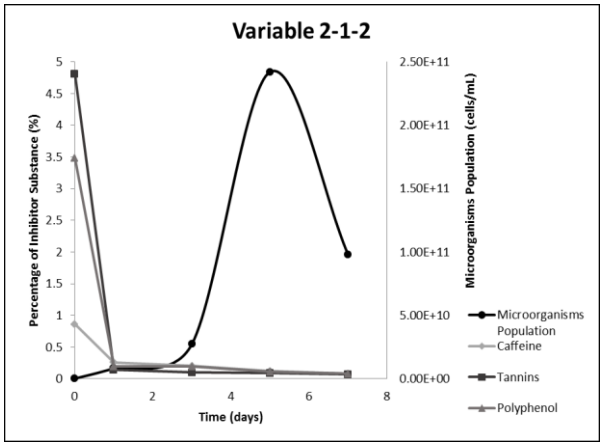

Fig 9. The correlation between the percentage of inhibitor substances, the increase in microorganisms population and time on the ratio of *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger* of 2:1:2

Concentration of inhibitor substance decreased day by day for all bioreactors. It shown that microorganism are growing by being able to adapt on the substrate so that the microorganism would degrade the inhibitor substance. Caffeine compounds are degraded into xanthine and tannins and phenol are degraded into citric acid.

In bioreactor 1, with addition of *Pseudomonas putida, Trichoderma harzianum*, and *Aspergillus niger* with ratio 1:1:1, at the end of process caffeine was decreased from 0.86% become 0.04%, tannins was decreased from 4.81% become 0.05%, and polyphenol was decreased from 3.48% become 0.07%.

In bioreactor 2, with addition of *Pseudomonas putida, Trichoderma harzianum*, and *Aspergillus niger* with ratio 1:2:2, at the end of process caffeine was decreased from 0.86% become 0.005%, tannins was decreased from 4.81% become 0.01%, and polyphenol was decreased from 3.48% become 0.0011%.

In bioreactor 3, with addition of *Pseudomonas putida, Trichoderma harzianum*, and *Aspergillus niger* with ratio 1:2:1, at the end of process caffeine was decreased from 0.86% become 0.001%, tannins was decreased from 4.81% become 0.01%, and polyphenol was decreased from 3.48% become 0.02%.

In bioreactor 4, with addition of *Pseudomonas putida, Trichoderma harzianum*, and *Aspergillus niger* with ratio 1:1:2, at the end of process caffeine was decreased from 0.86% become 0.02%, tannins was decreased from 4.81% become 0.04%, and polyphenol was decreased from 3.48% become 0.03%.

In bioreactor 5, with addition of *Pseudomonas putida, Trichoderma harzianum*, and *Aspergillus niger* with ratio 2:1:1, at the end of process caffeine was decreased from 0.86% become 0.01%, tannins was decreased from 4.81% become 0.02%, and polyphenol was decreased from 3.48% become 0.05%.

In bioreactor 6, with addition of *Pseudomonas putida, Trichoderma harzianum*, and *Aspergillus niger* with ratio 2:2:1, at the end of process caffeine was decreased from 0.86% become 0.06%, tannins was decreased from 4.81% become 0.08%, and polyphenol was decreased from 3.48% become 0.1%.

In bioreactor 7, with addition of *Pseudomonas putida, Trichoderma harzianum*, and *Aspergillus niger* with ratio 2:1:2, at the end of process caffeine was decreased from 0.86% become 0.08%, tannins was decreased from 4.81% become 0.07%, and polyphenol was decreased from 3.48% become 0.08%.

Decrease of caffeine from various variables can be seen in Fig 10. It shown that addition of microorganisms with a ratio 1:2:1 has the highest caffeine decreased of 99.88%.

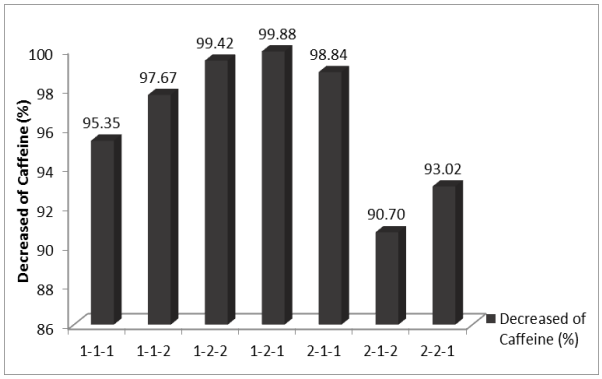

Fig 10. The correlation of Decreased Caffeine (%) on Various Ratio of *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger*

Decrease of tannins from various variables can be seen in Fig 11. It shown that addition of microorganisms with a ratio 1:2:2 and 1:2:1 has the highest tannins decreased of 99.79%. Tej K. Bhat (1998) [5] explained that Trichoderma harzianum has ability to degrade tannins to gallic acid. At that ratio, Trichoderma harzianum has more ratio than Pseudomonas putida and Aspergillus niger, so Trichoderma harzianum is more effective in degrading tannins.

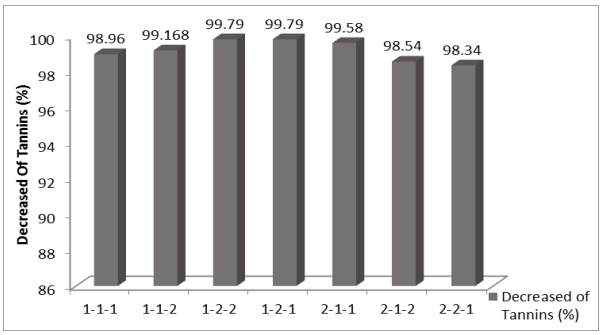

Fig 11. The correlation of Decreased Tannins (%) on Various Ratio of *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger*

While decrease of polyphenol from various variables can be seen in Fig 12. It shown that addition of microorganisms with ratio 1:2:2 has the highest polyphenol decreased of 99.68%.

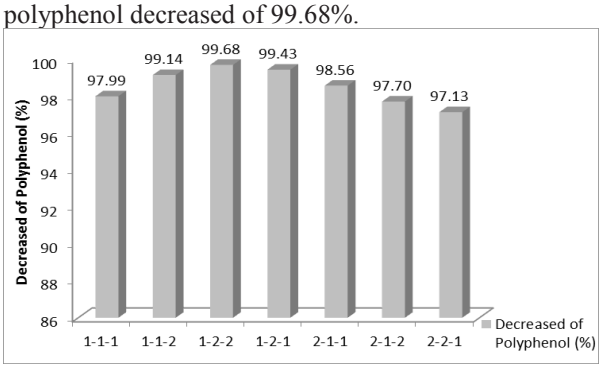

Fig 12. The correlation of Decreased Polyphenol (%) on Various Ratio of *Pseudomonas putida: Trichoderma harzianum : Aspergillus niger*

In addition to being seen in decreasing the amount of inhibitor substances also seen the cellulose produces at the end of the process. It is because cellulose plays an important role in the formation of biogas. Cellulose can be converted into methane gas with help of microorganisms. The amount of cellulose in various variables can be seen in the Fig 13.

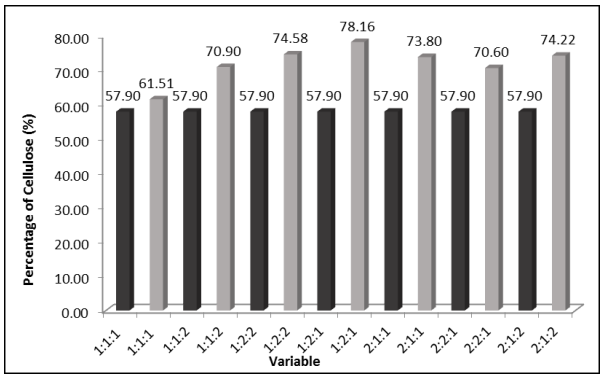

Fig 13. Percentage cellulose on Various Variables on Days 0 and Days 7

Fig 13 showed the increased of cellulose in various variables at the end process. The highest amount of cellulose in the addition of microorganisms with a ratio of 1:2:1 from 57.90% to 78.16%. So the ratio addition

of microorganisms Pseudomonas putida : Trichoderma harzianum : Aspergillus niger is 1:2:1 was selected and will be used in biogas manufacture.

3.3 Manufacture of Biogas

In the manufacture of biogas, variable used is the ratio of the addition of cow dung and rumen fluid as starter with the ratio of cow dung : rumen fluid is 1:0, 0:1, 1:1, 1:2, and 2:1.

The results of the analysis related to the increase methane gas concentrations in the five biogas reactors to the fermentation time were shown in Fig 14.

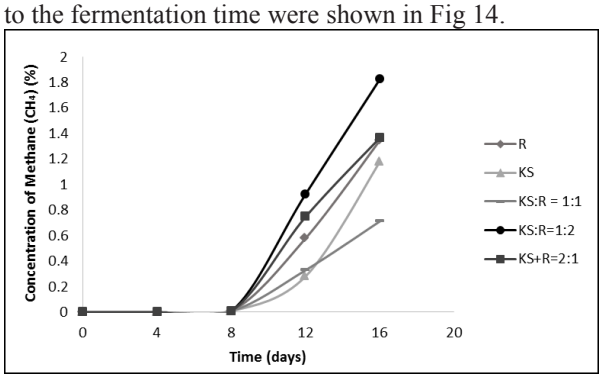

Fig 14. The Correlation Between Increase in Concentration of CH_4 (%) on Time

Fig 14 shows the correlation between methane gas concentration and fermentation time. Starter such as cow dung and rumen fluid use substrate as carbon source to do their metabolism and to reproduce. Fig 14 showed that in biogas reactor, methane gas increased slowly. On days 0 to days 8, methane gas has not formed. It is because microorganisms in cow dung and rumen fluid still adapt to the environment which there are toxic substances such as caffeine, tannins, and polyphenol from coffee pulp [1]. Furthermore, methane gas that has not been formed in the biogas reactor is due to microorganisms still at the hydrolysis and asidogenesis stage [5].

The results of biogas formation for 20 days on variable cow dung : rumen fluid is 1:2 with methane gas concentration of 1.825%. It is because the main microorganisms (Ruminococcus sp) in rumen fluid more and more specifically in degrading cellulose and organic material to methane (CH4) than microorganisms in cow dung [6]

According to previous research (Corro, 2013)[1], biogas that uses coffee pulp as substrate formed methane gas for 1 month fermentation time with methane gas formed very small. Benefit of pretreatment biologically on coffee pulp obtained results 1.825% within 16 days of fermentation. This indicate an improvement that has been made by previous research using coffee pulp without pretreatment to produce biogas.

The accumulated volume of biogas from day 0 to day 20 is shown in Fig 15. Methane gas volume has increased slowly.

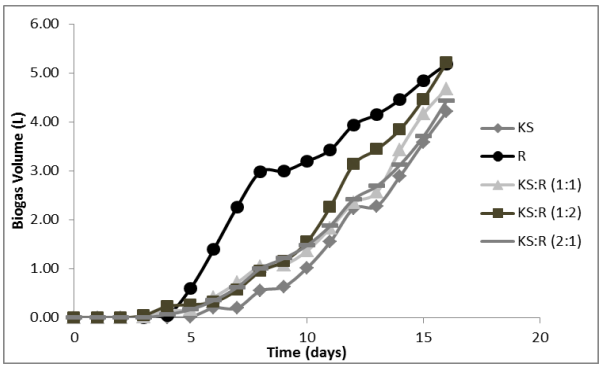

Fig 15. The Correlation of Biogas Accumulated Volume to Time (days) at Various Ratio of Addition Cow Dung : Rumen Fluid

On the days 0 to days 4 can be seen in all variables not yet formed biogas. After day 4, all variables begin to produce biogas. For the result of volume of biogas at the end of process is at ratio cow dung : rumen fluid is 1:0 of 4.61 L/days, at ratio 0:1 of 5.68 L/days, at ratio 1:1 of 5.12 L/days, at ratio 1:2 of 5.71 L/days, and at ratio 2:1 of 4.86 L/days. From the data obtained the highest volume of biogas accumulation on variable cow dung : rumen fluid at ratio 1:2. Thus, it can be calculated methane gas (CH_4) resulting from 0.0032 m^3/kg converted COD.

Tabel 2. Heating value on various variables in biogas reactor

Waktu (Hari)	KS			R			KS : R (1:1)		
	LHV (BTU/ft^3)	Densitas (lb/ft^3)	LHV (kcal/kg)	LHV (BTU/ft^3)	Densitas (lb/ft^3)	LHV (kcal/kg)	LHV (BTU/ft^3)	Densitas (lb/ft^3)	LHV (kcal/kg)
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	2.293	0.123	10.378	5.069	0.122	23.032	2.715	0.123	12.319
16	10.795	0.122	49.247	12.247	0.122	55.933	6.297	0.122	28.633

Waktu (Hari)	KS : R (1:2)			KS : R (2:1)		
	LHV (BTU/ft^3)	Densitas (lb/ft^3)	LHV (kcal/kg)	LHV (BTU/ft^3)	Densitas (lb/ft^3)	LHV (kcal/kg)
4	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
12	8.253	0.122	37.588	6.608	0.122	30.061
16	16.595	0.121	76.032	8.496	0.122	38.808

In measuring the value of heating value in biogas, composition gas greatly affect the heating value, especially methane gas. If composition of methane gas is high then the heating value obtained will be higher. The highest heating value of biogas in reactor is with variable cow dung : rumen fluid is 1:2 with heating value of 76.032 kcal/kg on end the process.

4 CONCLUSION

From the research that has been conducted, it could be concluded as follows :

1. Biological pretreatment by using microorganisms allows to degrade the inhibitor substance in coffee pulp with the ratio of addition of *Pseudomonas putida : Trichoderma harzianum : Aspergillus niger* of 1:2:1 is best composition for degrading the component. In the variable of 1:2:1, the amount of inhibitor substances decreased by 99.8% for

caffeine, 99.7% for tannins, and 99.4% for polyphenol.

2. Biogas with highest methane concentration was produced by a mixed variable between cow dung and rumen fluid (1:2) of 1.825% with 20 days of fermentation time. The highest heating value was obtained when the highest methane composition was 1.825% in variable cow dung: rumen fluid (1:2) of 76.032 kcal/kg.

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