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Methane Production from Coffee Pulp by Microorganism of Rumen Fluid and Cow Dung in Co-digestion

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Coffee pulp is abundantly available in Indonesia every year, about 1.65×10^6 t of coffee pulp were produced during the 2011 - 2015. However, they have been poorly utilized or dumped in the environment. Meanwhile, utilizing coffee pulp for production of methane (CH_4) is one of the most demanding technologies for production of environmentally friendly energy. This study aims to investigate the methane production from coffee pulp by some variables; microorganism existed in cow dung, rumen-fluid, and cow dung/rumen fluid mixture in anaerobic batch reactor with 3.6 L working volume at mesophilic temperature (30 - 35 °C). Some parameters such as Total Solid (TS), Volatile Solid (VS), Volatile Fatty Acids (VFAs), Chemical Oxygen Demand (COD) conversion and CH_4 generated as a function of digestion time were analysed and compared with each variable and another substrate: rice straw. The result showed that the rate of TS, VS conversion of almost all digesters was low during 30 d of digestion. Consequently, a low of CH_4 generated was detected, except in cow dung digester which had a higher concentration of CH_4 after 20 d of digestion. The highest COD value decreasing was from cow dung reactor of 78.05 % that was converted to VFAs. The VFAs value was represented by acetic acid as the main substrate for generating CH_4. The digestion rate was limited by bacteria growth because of the presence of toxic compounds in coffee pulp such as caffeine, tannin, and free phenol. Nevertheless, the conversion of TS, VS, COD and methane production still continued, though with a slower rate. At the final digestion time, the optimum accumulation of CH_4 was generated from cow dung digester of 85.1 $Ndm^3/kg_{COD\ removal}$, but it was approximately ten times as small as optimum accumulation of CH_4 from rice straw. This shows that different substrate source can affect the amount of generated biogas.

1. Introduction

The utilization of agro-industrial wastes as the substrate of biogas production has been an alternative source of energy that is developed in almost worldwide. In addition, it is one of the ways to reduce the quantity of an environmental pollutant which is interesting topic in recent days, especially in relation with the concept of renewable energy development.

Biogas is the name of a gas mixture of methane, carbon dioxide, hydrogen, nitrogen, hydrogen sulfide, etc. produced by bacterial conversion of biomass, in which methane is the most desirable gas because of its high heating value. Biomass can give particularly favorable energy balance when considering the energy yield from the biomass [output] to the provided primary energy [input]. With the output/input ratio of 28.8 MJ/MJ, biomass appeared to be a very efficient source of biogas among all the energy production technologies through biological and thermochemical routes (Deublein and Steinhauser, 2010). In this case, the one of potential biomass and also wastes of Indonesian agro-industry for methane production is coffee pulp.

Coffee is a leading agro-industry commodity in Indonesia that generates significant amount of waste from its processing, ranging from 30 - 50 % of total weight of coffee produced, depending on the type of processing: wet or dry (Oliveira and Franca, 2016). Coffee husks and Coffee pulp are the major solid waste that can be produced during those processing.

Coffee processing in Indonesia was generally done by wet processing where the generated coffee pulp was

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about 1.65 Mt during 2011 - 2015 season with average amount of 686 × 10^6 kg of coffee produced each year (Indonesian Directorate General of Estate Crops, 2015). That abundant coffee pulp can cause an environmental problem if it is not utilized immediately because it is the major pollutant agent of the river near coffee processing area. If it is just dumped in the environment, the presence of uncontrolled emission of CH_4 and N_2O was inevitable (Corro et al., 2013). To prevent that problem, utilization of coffee pulp for biogas production under anaerobic digestion presents as the solution. However, its application still is a challenge because coffee pulp has high contents of caffeine, tannin and free phenol (Corro et al., 2013) which are known as anti-nutrition and toxic agent for biological processes (Pandey et al., 2000). On the other hands, coffee pulp has high bacterial nutrients such as cellulose, hemicellulose and protein (Table 2), which is good for bacterial growth.
In the previous research, it was stated that the toxic material in coffee pulp can be minimized by microbial degradation (Rojas et al., 2003). Furthermore, Corro et al. (2013) reported that firstly degrade toxic material in coffee pulp required so many bacterial concentrations. For those reasons, this study aims to investigate the possibility of improving cumulative methane production by using co-digestion of coffee pulp-cow dung, -rumen fluid, and -cow dung/rumen fluid mixture in anaerobic batch reactor at mesophilic temperature (30 - 35 °C).

1.1 Cow dung

The application of cow dung for generating biogas has been popular and easy. Cow dung contains a substantial number of bacteria but low amount of cellulose, lignocellulose, lignin and other organic compounds which are important substrates for supporting bacterial growth and methane production. Biomass addition from food and non-food based portion of crops such as corn, sugarcane, leave, rice straw etc. is always done to handle that weakness. Corro et al. (2013) tried to generate methane from coffee pulp/cow dung mixture in co-digestion. Furthermore, He compared the amount of methane produced from the coffee pulp/cow dung mixture with the sole coffee pulp and sole cow dung in digestion. Due to that mixture strategy, its methane yield proved higher than that of sole coffee pulp and sole cow dung in digestion.

1.2 Cow's rumen fluid

The amount of waste rumen contents (solid and liquid phase) from the nearest slaughterhouse by the author's institute is very abundant, about 5,200 L/d from 260 cows (average number of the slaughtered cow everyday) or about 1,560,000 L/month. By utilizing it for bioprocess, it is expected that it can reduce the load wastewater processing plants due to slaughterhouse effluents.
Cow's rumen fluid consists of a complex anaerobic microbial population. There are two quadrillion of bacteria, one million protozoa, fungi, and archaea reside in cow's rumen (Baba et al., 2013). They were mostly a cellulolytic microorganism with high enzyme activity for degradation cellulosic material (Table 1). These made the rumen have a very short solid residence time, around 25 - 30 h (Yue et al., 2013). For those advantages, some researchers used cow's rumen fluid to produce methane from bagasse and maize bran (Kivaisi and Eliapenda, 1995), maize straw (Jin et al., 2014), rice straw (Widjaja et al., 2016), and paper waste (Baba et al., 2013) with significant yield improvement over of 30 % to 70 % of the theoretical methane yield. Unlike coffee pulp which has toxic contents as inhibitor of methane production, these types of biomass generally had a problem on lignin degradation process that bound cellulose and hemicellulose. The use of cow's rumen fluid for methane production from coffee pulp is still rarely done, notably for mixture of cow dung and rumen fluid. This will be explained in the next chapter.

Table 1: Major microorganism reside in rumen (Takenaka, 2008)

Function	Kind of Bacteria	Bacterial Morphology
Degrading cellulose	*Fibrobacter succinogens*	Gram -, bacilli/ rods
	Ruminococcus albus	Gram +, coccus/cocci
	Ruminococcus flavefaciens	Gram +, coccus/cocci
Hemicellulose and pectin eater	*Prevotella ruminocola*	Gram -, bacilli/rods
	Butyrivibrio fibrisolvens	Gram +, bacilli/rods
Starch Fermenter	*Ruminobacter amylophilus*	Gram -, rods
	Streptococcus bovis	Gram +, rods

2. Methods

2.1 Substrate

Robusta coffee pulp (the skin and mesocarp of the coffee berry) was obtained from West Tanjung Jabung Regency, Jambi Province. It was directly collected from the de-pulping machine during wet processing of coffee berry and dried for 7 (seen) hours from 08:00 to 15.00 for 3 d under solar radiation to reduce its moisture content. This treatment reduced weight of coffee pulp about 50 % of fresh coffee pulp. Then, it was transported to the

laboratory in plastic sacks. Moreover, the size of dried coffee pulp was reduced to 35 mesh using disc mill machine. This was need for microbes to carry out its digestion and provide large surface area for adsorbing the substrate that would increase microbial activity and hence increased biogas production. Dried coffee pulp was not treated using chemical and biological pre-treatment to remove or reduce its certain chemical content. The chemical composition of the coffee pulp in present study is shown in Table 2.

Table 2: Composition of the dried coffee pulp

Components	% dry weight basis
Cellulose	58.36
Hemicellulose	21.8
Lignin	5.05
Pectin	2.16
Protein	0.81
Tannin	3.11
Caffeine	0.91
Polyphenol (free phenol)	3.78

2.2 Collecting cow dung and rumen fluid

Rumen fluid was derived from slaughterhouse in Pegirian, Surabaya, Indonesia near the author's institute. About 5 Litter of rumen fluid was collected from rumen contents that were filtered using muslin to seperate from its coarse. Meanwhile, the fresh cow dung was obtained from nearby slaughterhouse in Pegirian. Then, it was immediately placed in sterile vessel and stored in incubator shaker at 37 °C at 140 rpm.

2.3 Preparation of the Inoculum

Inoculums were prepared with adding 1.05 gram of the coffee pulp to each variable; cow dung, rumen fluid, and cow dung/rumen fluid mixture (1:1 ratio) of 15 % of working volume into 1 L erlenmeyer respectively. Then, some of chemicals such as 4 g/L NH_4Cl. 0.06 g/L KH_2PO4, 0.025 g/L $CaCl_2$, 0.005 g/L $NiCl_2$, 0.005 g/L $MnCl_2$, 0.005 g/L $CoCl_2$, 2 g/L CH_3COONa, 0.1 g/L yeast extract, 0.025 g/L $MgCl_2$, and 0.03 g/L Fe-EDTA were added as microbial nutrition into that erlenmeyer respectively. Subsequently, the top of erlenmeyers was covered by rubber stopper, incubated and stirred in incubator shaker for 12 h with 137 rpm and 37 °C.

2.4 Biogas (methane) production

In the present study, laboratory scale of anaerobic reactor/digester was made up of polypropylene with a total volume of 6 L and working volume of 3.6 L. It was equipped with manometer, control valve, thermometer and slurry sampling point (Figure 1). The digester was filled with coffee pulp and water (1:2 ratio) and the inoculum variable. There were 3 digesters: Digester A was contained the coffee pulp and cow dung (CP + CD); Digester B was contained the coffee pulp and rumen fluid (CP + RF); Digester C was contained the coffee pulp and cow dung/rumen fluid mixture (CP + CD + RF). pH value was checked every day and kept on the optimum pH (6.8 – 7.2). If pH value was decreasing, 1 M NaOH was added to achieve that optimum pH. Methane production was kept for observation at mesophilic temperature (30 - 40 °C) for 30 d of digestion process.

2.5 Analysis

Total Solid (TS) and Volatile Solid (VS) were estimated as described by Costa (1998). Chemical oxygen demand (COD) was measured by Standard Method 20th Edition - Examination of Water & Waste Water, Methods 5220-D-Closed Reflux Colorimetric Methods with KHP (Potassium Hydrogen Phthalate) as a standard solvent and then read by spectrophotometer with wave length of 620 nm. VFAs concentration including acetic, propionate, and butyrate acid was measured by Gas Chromatography (GC) HP-6890 equipped with Porapak Column (Length: 30 m; diameter: 530 μm, thickness: 40 μm), FID detector as front detector at 275 °C and TCD detector as back detector at 250 °C. Initial temperature of injector was 275 °C, with pressure of 17.21 psi and Helium as a carrier gas. Methane concentration in ppm unit was analyzed using HP 19095P-QO_4 (Length: 30 m; diameter: 530 μm, thickness: 40 μm) that bounded polystyrene-divinilbenzene (DVB) column. It is excellent to determine methane and CO_2. The column and injection temperature was 270 °C and 170 °C respectively. N_2 was used as carrier gas at flow rate of 30 mL/min. Cumulative methane production was calculated and shown in Ndm^3/kg_{COD} removal.

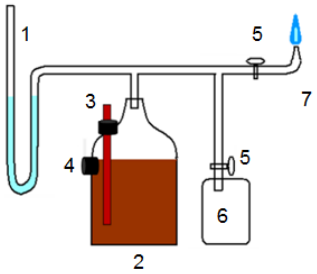

Figure 1: The anaerobic digester set-up, (1): Manometer; (2): Digester; (3): Thermometer; (4): Slurry sampling point; (5): Control valve; (6): Collection bag; (7): Burner tip

3. Results and discussion

Attempts to increase the methane yield production from a biomass can be done in several ways are including the use of a consortium of microorganisms, keeping the pH value at optimum conditions, set a suitable temperature digester, an appropriate organic loading rate (OLR) and hydraulic retention time (HRT), C:N ratio, particle size of the substrate, pretreatment and innovations in the reactor design (Yadvika et al., 2004). While in this study, from those ways, some factors have been chosen and varied, while some remaining factors was set equal or negligible. One of the overlooked was the value of C:N ratio (roughing of the substrate used), OLR and HRT. While the setting factors were optimum pH (6.8 to 7.3) (Hu et al., 2004), particle size of substrate (35 mesh), temperature in the digester (mesophilic) and reactor design: batch system. The varied factor was consortium of microorganisms that the results of its investigation are presented in next chapter.

3.1 Total solid (TS) and volatile solid (VS)

Dilution of slurry in the digester can be seen from the number of total solids (TS) and volatile solid (VS), which states the ability of microorganisms to degrade organic material in the process of hydrolysis. Analysis of TS and VS was done every 3 d for 30 d during the process of anaerobic digestion by taking the liquid in the digester. Table 3 and Figure 2 show the TS and VS value of all the digester. The fastest TS conversion rate occurred in the digester CP + CD + RF, but the value diminution overtaken by the TS value of digester CP + CD of 51.12 % on 30-d digestion time. TS impairment is caused by hydrolysis activity of microorganisms forming intermediate products in the form of volatile fatty acids (VFAs) to be converted into biogas by methanogens through methanogenesis step (Vishwanath et al. 1992).

Table 3: Initial and final value of Total Solid and Volatile Solid determined for all digester for 30 d

Digester	Total Solid (TS) (wt%)		% conversion	Volatile Solid (VS) (wt%)		% conversion
	Initial	Final		Initial	Final	
CP+CD	29.55	14.46	51.07	27.10	13.62	49.74
CP+RF	32.82	22.78	30.59	29.76	12.62	57.59
CP+CD+RF	34.66	19.24	44.49	26.68	15.35	42.47

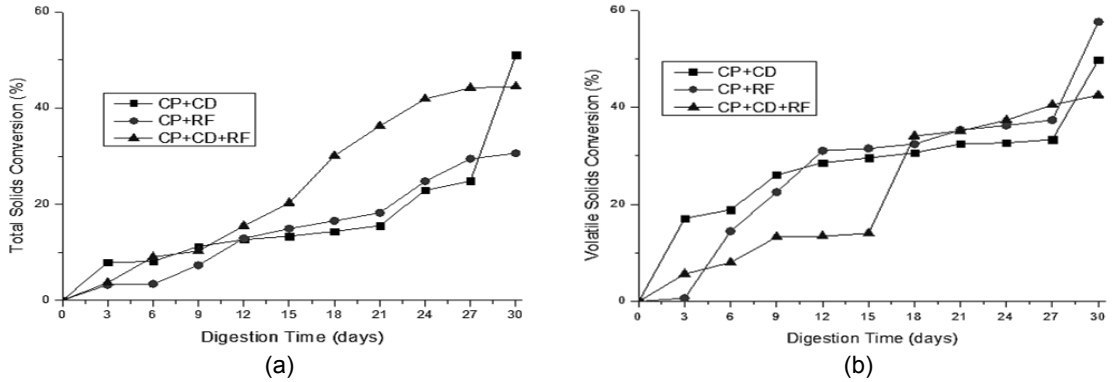

Figure 2: (a) Total solid conversion; (b) Volatile solid conversion of three digesters as a function of digestion time

From Figure 2, the conversion rate of TS in digester CP + CD + RF was faster because of its high quantity of microorganism reside in the digester. Thus, its hydrolysis process can be faster. But, its conversion rate of VS was slower than the others because the acid-producing bacteria might have long adaptation time before it converts soluble organic compounds in VS to acids, acetate, etc. The digester CP + CD and CP + RF had slow conversion rate of TS. This was caused by their microorganisms, which required more time to adapt the stressfulness of new environment where there are toxic compounds in them, such as caffeine, tannin, and free phenols (polyphenols) (Corro et al., 2013).

3.2 Kinetics of volatile fatty acids (VFAs)

In the biogas production process, a substrate is always converted to be intermediate product before being converted into bio-methane (Eq(1)-(5)). These products are acetic acid, hydrogen, carbon dioxide, and other lower weight simple volatile organic acids such as propionic and butyric acid which were in turn converted to acetic acid (Yadvika et al., 2004). The acetic acid were converted into a mixture methane and carbon dioxide by the methanogenic bacteria. A high concentration of VFA would not guarantee to get efficient biogas process, because the accumulation of VFA will make the coffee substrates lack of nitrogen (Battista et al., 2015). That is not favorable for bacterial life. Figure 3 shows the total acetic acid evolution during 30 d of digestion time. Total acetic acid was determined according to the acetic its self and potential acetic from propionate and butyrate that was converted base on reaction Eq(1), (3).

$$CH_3CH_2COOH \text{ (propionate)} + 2H_2O \longrightarrow CH_3COOH \text{ (acetate)} + CO_2 + 3H_2 \quad (1)$$

$$CH_3CH_2COOH \text{ (propionate)} + \tfrac{1}{2} H_2O \longrightarrow 7/4\ CH_4 + 5/4\ CO_2 \quad (2)$$

$$2CH_3(CH_2)_2COOH \text{ (butyrate)} + 2H_2O \longrightarrow 4CH_3COOH \text{ (acetate)} + CH_4 \quad (3)$$

$$CH_3COOH \text{ (acetate)} \longrightarrow CH_4 + CO_2 \quad (4)$$

$$4\ H_2 + CO_2 \longrightarrow CH_4 + 2H_2O \quad (5)$$

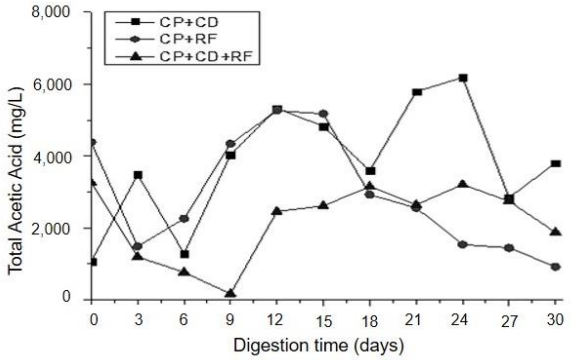

Figure 3: Evolution of VFA concentration of acetic, propionate and butyric acid

From Figure 3, it is known that total acetic acid in digester CP + RF and CP + CD + RF initially decreased, in which the largest decline occurred in digester CP + CD + RF (coffee pulp-cow dung/rumen fluid mixture), which fell from 3,256 mg/L to 167 mg/L within 9 d followed by an increase and decrease until the end of the experiment. A decline of Total Acetic acid concentration was due to the conversion of acetic acid to biogas (CH_4, CO_2, H_2, etc.). Although the depletion of Total Acetic Acid was significant in digester CP + RF and CP + CD + RF, the generated methane was not significant. This was probably caused by a lot of methane-forming bacteria which was inhibited or died by the toxic materials in coffee pulp before all the substrates were changed to acetic acid.

3.3 Chemical oxygen demand (COD) and cumulative methane production

The biogas formation can be indicated by decreasing of COD level, which led to amount of biogas as a function of digestion time because microorganisms need time to degrade organic compounds to be biogas (Widjaja et al., 2016). The highest COD value decreasing was from cow dung reactor of 78.05 % which has cumulative methane production of 85.1 $Ndm^3/kg_{COD\ removal}$ (Table 4). It was approximately ten times as small as optimum accumulation of CH_4 from rice straw-rumen fluid digester on another author's research, where it generated of 811.1 $Ndm^3/kg_{COD\ removal}$. This shows that different substrate source can affect the amount of generated biogas,

especially for coffee pulp substrate that has microbial inhibition component.

Table 4: COD and cumulative methane production values of three digesters for 30 d

Digester	COD (mg/L)		COD Removal (%)	Methane gas ($Ndm^3/kg_{COD\ removal}$)	
	Initial	Final		Coffee pulp	Rice straw #
CP+CD	42,205.81	9,265.00	78.05	85.1	557.5
CP+RF	23,081.59	16,565.15	28.23	11.3	811.1
CP+CD+RF	22,533.48	11,510.34	48.92	60	386.4

4. Conclusions

Methane production using co-digestion of coffee pulp-cow dung, -rumen fluid, and -cow dung/rumen fluid mixture in anaerobic batch digester at mesophilic temperature (30 - 35 °C) was conducted which the optimum accumulation of CH_4 was from cow dung digester of 85.1 $Ndm^3/kg_{COD\ removal}$, approximately ten times as small as the optimum accumulation of CH_4 from rice straw. Using co-digestion strategy to the coffee pulp without pretreatment could not give a significant improvement of methane production. The digestion rate was limited by bacteria growth because of the presence of toxic compounds in coffee pulp. Pretreatment process to reduce the toxic compounds of coffee pulp is highly recommended to achieve a high amount of methane yield.

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