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Assessment of Specific Methanogenic Activity from Cow Dung

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Abstract: The specific methanogenic activity (SMA) is a test to measure the producing potential of an anaerobic bacteria until it's allowing a relevant organic loading rates to be applied for a selected substrate. Commonly, acetate is used as substrate for the SMA test. Anaerobic bacteria were mostly taken from an anaerobic digester and cow dung was also implemented as a source of an anaerobic bacteria. However, the results of SMA of cow dung was less reported. Therefore, this study is initiated to determine the potential of methane production from the cow dung by using the SMA test. Prior the SMA test, the cow dung was characterized for solids where the results showed that the cow dung is having 12.00 g L^{-1} for total solid and 10.50 g L^{-1} for volatile solid. The SMA test was conducted at mesophilic condition by using an automatic methane potential system test (AMPTS II) and the SMA of the cow dung was found as 0.04 in unit g COD-CH_4 $g^{-1}VS^{-1}d^{-1}$. The significance of this research is to determine the anaerobic bacteria potential of cow dung for use in the anaerobic digestion process, which offers numerous advantages for manufacturing, particularly in industrial applications such as methane production (fuel).

Keywords: SMA, cow dung, anaerobic, solids

1. Introduction

Earth is facing an ongoing environmental threat such as global warming due to the emission of greenhouse gases [1]. Million tons of waste is generated from various sources (municipal, industrial, and agricultural sources) and most of it is disposed at landfill [2]. The accumulated waste at landfill release greenhouse gases, such as methane into the atmosphere and causes global warming [3]. To mitigate the global warming issue and provide better alternative to reduce the waste, anaerobic digestion is chosen as a promising method by composting the waste and digest the organic matter and produce clean biogas as the final product [4]. Anaerobic digestion can be performed in either batch test or continuous test. Nandi et al. [5] conducted anaerobic digestion using continuous test while Rajput & Sheikh [6], Dhamodharan et al. [7], and Zhua & Jha [8] using batch test. Anaerobic digestion involves four stages such as hydrolysis, acidogenesis, acetogenesis and methanogenesis [9].

The biogas produce from the anaerobic digestion can be considered as a renewable energy that can replace the conventional energy sources such as fossil fuels and oil [10]. Anaerobic digestion is a biochemical process involving bacteria that will breakdown the organic waste and convert the waste into biogas comprises of methane [11]. Methane is recognized as a renewable energy [12]. Other than methane, anaerobic digestion also can produce a nutrient-rich digestate that can be used as fertilizer of for soil conditioner properties [1]. The production of methane from anaerobic digestion is influenced by several factor such as the inoculum used, the inoculum to substrate ratio (I/S), the method of BMP used, the buffering system, the operating temperature, and the period of the digestion process [13].

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Anaerobic bacteria used to convert organic matter to methane originate from the inoculum used for the anaerobic digestion process [10]. During methanogenesis process, the methanogenic bacteria consumed the products from the acetogenesis process and converted them into methane (CH_4) and carbon dioxide (CO_2) [14], [15]. The frequent inoculum employ in anaerobic digestion are cow manure, pig manure and cattle manure [16]. According to Aragaw et al. [16], anaerobic digestion that used cow dung as inoculum improves the digestion process stability and methane production due to the great buffering capacity of the inoculum. Nandi et al. [5], Rajput & Sheikh [6], Dhamodharan et al. [7], and Zhua & Jha [8] used cow dung for anaerobic digestion of various organic waste. The selection of inoculum to be used in anaerobic digestion is a vital step and this selection of inoculum can be determined by conducting specific methanogenic activity test (SMA) [10].

In addition, fresh inoculum is often recommended to conduct anaerobic digestion [17], [18]. Fresh inoculum, on the other hand, is not always readily available [18]. For example, the inoculum (digestate) for full-scale applications may need to be shipped from the full-scale anaerobic digestion facility to the laboratory [18]. For lab-scale applications, the inoculum may need to be gathered over a period of time to amass the volume of inoculum required to conduct the experiment, or it may need to be held until the laboratory infrastructure is available [18]. This raises questions about the impact of varied transportation and storage circumstances on inoculum activity, and consequently on the inoculum's efficacy when utilised in the experiment [18].

SMA test is conducted to evaluate the microbial activity of the bacteria present in the inoculum as well as their capability in producing methane [19]. The SMA test can act as an operating parameter to test the performance and stability of the anaerobic digestion system [20]. In addition, the SMA testing is also utilized to evaluate the adaptation of the mixture to anaerobic treatment and even evaluate the rate of methanogenic process and also the methanogenic capacity of a reactor through quantification of the active biomass [21]. Through the results obtain from the SMA testing, the potential microbial activity in the inoculum can be estimated [10]. The loading capacity of the anaerobic digestion system can be determined through SMA testing as well, therefore relevant organic loading rate can be applied in the anaerobic digestion system [22]. SMA testing was often performed at the start of the anaerobic digestion process to assess inoculum activity development [20].

SMA testing was carried out by mixing a known amount of substrate with a determined amount of inoculum [20]. In SMA testing, the substrate applied is different that the substrate applied for anaerobic digestion where for SMA testing the common substrate used are acetate, cellulose and propionate [22]. Acetate is the most popular substrate applied in SMA testing and it becomes the energy source for the anaerobic bacteria [20]. Acetate is a favorable substrate to the methanogenic bacteria as more than 70% of methane originate from the conversion process of acetic acid in the anaerobic digestion process [23]. As a result, the ability of anaerobic digester sludge to convert acetate to methane is a critical determinant in process capability [20]. The SMA is measured by putting a certain amount of biomass and a certain amount of substrate (acetate) in concentration that can maximize the biogas production and then the methane released is measured [10]. The methane results were expressed as mlCH4 g/VS day or as chemical oxygen demand equivalent to methane mg COD g VS-1 day-1 [24].

Anaerobic digestion is a process that requires delicate control and design of the system [20]. Thus, SMA testing is important to be applied before the biomethane potential testing (BMP) to ensure no inhibition or insufficient loading rate/ microbial activity occur in the reactor that can minimize the biogas production [10]. Unfortunately, in Malaysia, the microbial activity in SMA testing is less addressed [23]. Thus, the aim for this study is to investigate the SMA by using cow dung as the source of the microorganisms. In this study, the cow dung used is characterized in solids through total solids (TS) and volatile solids (VS) testing.

2. Methodology

2.1 Cow Dung Sampling

Cow dung was chosen as the inoculum used for the SMA testing [20]. 1.0 kg of cow dung was collected from a cow barn located in Batu Pahat as shown in Fig. 1. The collected cow dung was kept in a plastic container and stored temporarily in the container during the journey back to UTHM's laboratory. The cow dung was stored in a cold room at 4°C before testing [25]. Experimental results demonstrated that, regardless of storage temperature, methanogenic activity decreased over time with storage [18]. At 4 °C, however, the rate of decline in methanogenic activity was two to five times slower than at 22 and 37 °C, respectively [18].

2.2 Specific Methanogenic Activity

The SMA testing aim was to measure the capability of inoculum in producing the methane and expressing the potential of the inoculum to be used in anaerobic digestion [20]. The SMA testing were carried out at day 3 after the cow dung collection, because the cow dung was characterized first in terms of TS and VS measurements [26]. Normally, the SMA result was reported in unit gCOD-CH_4/g VS or gCOD-CH_4/g VSS [27].

The most utilized substrate for the SMA testing is acetate [20]. The SMA testing with acetate, measures the acetoclastic methanogenic activity and the methane produced from the hydrogenoclastic methanogenic was ignored [22].

It is when the acetic is used as substrate of the test, so it would give the good terminology to conduct this process of the Specific Aceticlastic Methanogenic Activity (SAMA) [26].

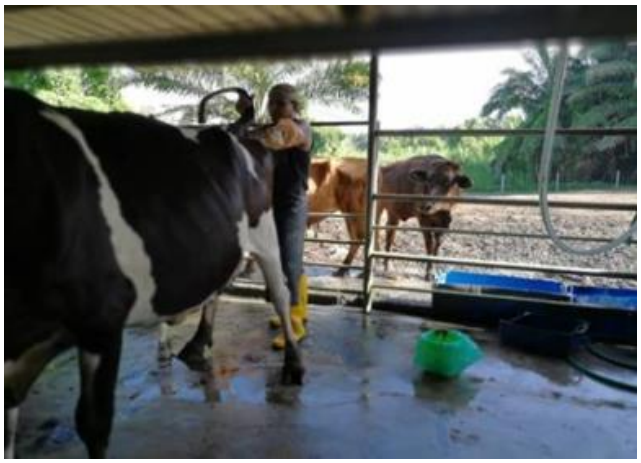

Fig. 1 - The cow barn (Al Zafe, Batu Pahat)

Because methane is the most important product in anaerobic digestion, the SMA may be more representative than the other activities. It is demonstrated by Ary et al. [28], by using sodium acetate and acids (butyric, propionic, and Valeric) as substrates, the results shows that acetate was the preferable choice for SMA. Obviously, there are hydrophilic methanogenic bacteria that receive H_2 from the acidification stage but given that only 26% of organic matter is transformed into acids and lipids to make hydrogen, acetoclastic methane bacteria deserve our attention [28]. The acetate content was also between 6 and 6.6 g L^{-1} and is the best concentration for obtaining a representative SMA curve [28].

The SMA testing involves the combination of macronutrients, micronutrients, fosfat A and fosfat B. The macronutrients and micronutrients used were prepared by using $FeCl_3$ $6H_2O$, $CoCl_2$ $6H_2O$, $MnCl_2$ $4H_2O$, $CuCl_2$ $2H_2O$, $ZnCl_2$, H_3BO_3, $(NH_4)6$ Mo_7O_2 $4H_2O$, Na_2SeO_3, $NiCl_2$ $6H_2O$, EDTA, HCl, resazurin, yeast extract, NH_4Cl, $CaCl_2$ $2H_2O$, and $MgSO_4$ $7H_2O$. While for fosfat A and fosfat B was prepared by using K_2HPO_4 and NaH_2PO_4. The macronutrients and micronutrients was used to aid in stimulating the microorganisms to produce high methane [23].

SMA blank solution and SMA substrate solution were prepared by using the macronutrient, micronutrient, fosfat A and fosfat B as shown in table 1. The blank and substrate solution were prepared for 1.5 L at both. The SMA blank solution and SMA substrate solution were stored in a cool room (refrigerator) at UTHM's laboratory, and the solution were used when the solution temperature is at the room temperature. The different between SMA blank solution and SMA substrate solution is the addition of $NaAc.3H_2O$ (Sodium acetate trihydrate) of 6.0 g as a source of acetate.

Table 1 - Chemical composition for preparing the SMA blank solution and SMA substrate solution

Chemical composition	Quantity	Purpose
Fosfat A	45.75 ml	SMA Blank Solution
Fosfat B	29.25 ml	
Macronutrients	9.00 ml	
Micronutrients	0.90 ml	
Fosfat A	45.75 ml	SMA Substrate Solution
Fosfat B	29.25 ml	
Macronutrients	9.00 ml	
Micronutrients	0.90 ml	
$NaAc.3H_2O$ (Sodium acetate trihydrate)	6.00 g	

The activated of cow dung were used for the SMA test. The activated of cow dung is prepared by adding 17.3 g of acetate in 1L of inoculum (cow dung). The mixture is left overnight for the activation process. The amount of acetate for activation process is determined by considering the TS and VS of cow dung, the total COD of SMA substrate, and the working volume of reactor of 400 mL. Table 2 shows the SMA setup at both for the blank and also the sample reactor. Fig. 2 illustrate the SMA Blank and the SMA Sample reactor.

Table 2 - Setups for SMA blank and SMA sample reactor

	SMA Blank reactor	SMA Sample reactor
SMA blank solution (mL)	330	
SMA substrate solution (mL)		330
Activated cow dung (in ml)	70	70
Number of prepared reactors	2	3

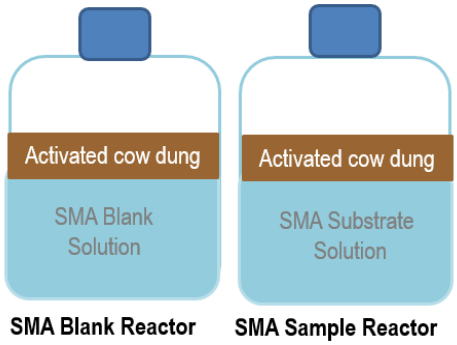

Fig. 2 - SMA blank and SMA sample reactor

The AMPTS II was used for SMA measurement [22]. The SMA blank and SMA sample reactors were placed in the thermostatic for automatic monitoring until the methane production is insignificant. Temperature was kept constant at mesophilic temperature of 37 ± 1°C in the thermostatic water bath, and the reactors bottle were mixed at the 160 rpm continuously [26]. Fig. 3 showed the SMA setup for this study using AMPTS II with only five reactors were used. This system consists of three main components: the sample incubation system, the CO_2 absorption tray and the measurement tool for the amount of gases. To measure the volume of the biogas generated, methane flows from the wash bottles (CO_2 absorption tray) into the gas volume measuring system [29]. The SMA was determined according to equation below:

$$SMA = \frac{g\ COD\ CH^4}{g\ VS\ day} \quad (1)$$

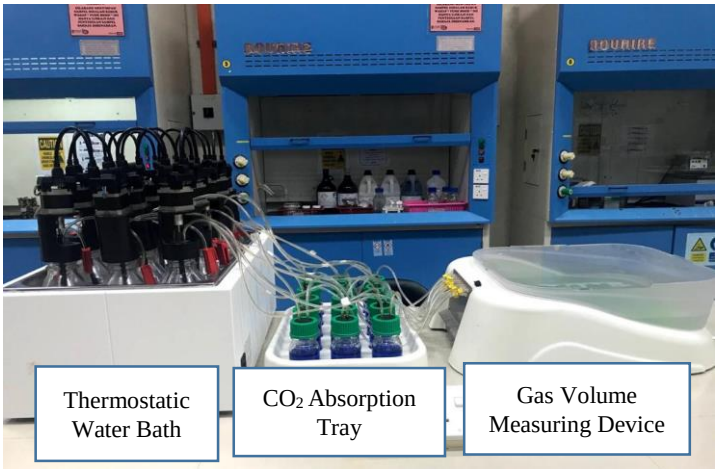

Fig. 3 - AMPTS II (only 5 reactors used for SMA testing)

2.3 Characteristics Measurement in Solids of Cow Dung

The total solids (TS) and volatile solids (VS) were conducted according to the Standard Method 2540G by APHA (2005) as described in [30]. Before TS testing all the crucible used were dried in a drying oven for 1 hour. After that, the lid and cup of the crucible were measured alone without sample and the value was recorded. 30 g of cow dung (sample)

was poured into the crucible and triplicate sample was prepared. Then the crucible containing sample was weighed and the value was jotted down. Then all the crucible containing sample will be put into the drying oven and left to dry for 24 hours at temperature of 105°C. After the overnight drying process, the crucibles were left to cool in a desiccator (approximately 30 minutes) as shown in Fig. 4 and then finally the final dry weight of crucible were measured.

Fig. 4 - Desiccator containing crucibles to be cooled

Moving on to the measurement of VS after TS testing was completed, the same crucibles was used to be dried in a furnace for 2 hours at temperature of 550°C. Then the crucibles were left to cool in the desiccator and the final weight of the crucibles were weighed and recorded. The TS and VS value was calculated according to the equations below:

$$\%\ \text{total solids} = \frac{(A-D)}{(C-B)} \times 100\% \tag{2}$$

$$\%\ \text{volatile solids} = \frac{(A-D)}{(C-B)} \times 100\% \tag{3}$$

$$\text{mg total solids L}^{-1} = \frac{(A-D)\times 1000}{\text{sample volume, mL}} \tag{4}$$

$$\text{mg volatile solids L}^{-1} = \frac{(A-D)\times 1000}{\text{sample volume, mL}} \tag{5}$$

where, A = weight of dried residue + dish (mg), B = weight of dish, C = weight of wet sample + dish (mg), and D = weight of residue + dish after ignition (mg).

3. Results and Discussions

3.1 Characteristics of Cow Dung

The digestion efficiency of cow dung was studied based on the results observed from the monitoring process of VS, TS, VS/TS and production of biogas with its methane content [1]. Table 3 shows the solids concentration of cow dung observed from this study.

The results obtained for this study is relatively similar with what observed in [31]. Jha et al. [31] recorded a TS value of 11.28 g L^{-1} and a VS value of 10.02 g L^{-1} for cow dung. But Sánchez-Hernández et al. [32] observed a higher value of TS (25.40 g L^{-1}) and VS (15.30 g L^{-1}) respectively. This differenced was possible due to difference feedlot prepared based on different country or region [32]. Based on the data tabulated in Table 3 below, the theory of the high percentage of VS/TS were correctly true. This is because most of the researchers that implement the specific methanogenic activity by using the cow dung with the substrate of acetate would possible to get the result as same as others [31].

The value of VS/TS ratio for this study exceed 50% which is 88%. The inoculum source have significant effects on the solid concentration and usually cow dung has high value of VS/TS ratio [31, 32]. Jha et al. [31] and Sánchez-Hernández et al. [32] observed a VS/TS value of 88.82% and 60.23%. Both of the VS/TS value exceed 50%. This is because the manure of animals may serve as major feedstock for the manufacture of biogas and it also means with the high percent of proportion VS/TS indicates that a substantial fraction of manure in biodegradable process [31].

Table 3 - Solids concentration of cow dung, (N=3)

Total solid (g L^{-1})	Volatile solid (g L^{-1})	VS/TS (%)
12.00 ± 0.94	10.5 ± 0.24	88 ± 3.48

3.2 Specific Methanogenic Activity (SMA)

The cow dung for this study recorded an SMA value of 0.04 g COD CH_4 g^{-1} VS^{-1} d^{-1}. In addition, the ideal anaerobic digested were reported to have a SMA value ranging from 0.01 – 0.04 g COD CH_4 g^{-1} VS^{-1} d^{-1} [20]. This is because the anaerobic bacteria of cow dung were usually used for the anaerobic digestion process, due to their traits of having high potential of microbial activity thus producing favorable value of SMA [20]. The SMA value obtained may influenced by many factors such as type of substrate, the feature of the sludge, environmental conditions, and the experimental procedures [20]. Moreover, a storage of inoculum at 4°C also helps to reduce the loss of microbial activity [18].

4. Conclusion

For this study, the cow dung tested for the SMA activity proved to be having an active microbial activity that can promotes high methane production. The SMA value obtain for this study is in the ideal range of a good microbial activity which is 0.04 g COD CH_4 g^{-1} VS^{-1} d^{-1}. The VS/TS ratio for this study also exceeded 50% indicating the cow dung used has a major effect in the producing high biogas. Based on the results obtained it is safe to say that cow dung is suitable to be used as inoculum for anaerobic digestion process.

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