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Specific Methanogenic Activity from Anaerobic Biomass Originated from Full-Scale Anaerobic Digester Treating Domestic Sewage Sludge

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Abstract: Methanogens are anaerobic microorganisms that are able to process organic matter to methane via two pathways; aceticlastic and hydrogenotrophic. This microorganism is easily found in digested sludge from anaerobic digester. In practice, digested sludge is commonly used as inoculum for a new anaerobic digester. The quality of the inoculum is measured by performing specific methanogenic activity test. The objective of this study is to examine the specific aceticlastic methanogenic activity from active anaerobic biomass, in which acetate was a substrate. The anaerobic biomass was taken from an active local anaerobic digester treating domestic mixed sewage sludge, working at ambient mesophilic range. The VS destruction of the anaerobic digester dictates the anaerobic biomass characteristics, particularly VS (%) subsequently interfering the value of specific aceticlastic methanogenic activity. Digested sludge having biomass with higher VS (%) showed good aceticlastic methanogenic activity and are recommended to be used as inoculum for anaerobic biodegradability studies.

Key words: Methanogens, digested sludge, inoculums, methanogenic activity, sewage sludge

INTRODUCTION

The anaerobic digestion of organic matter produces methane. Methane is mostly produced from acetate ($C_2H_3O_2^-$) or hydrogen (H_2) and carbon dioxide (CO_2) or formate (HCO_2^-) (Demirel and Scherer, 2008). Aceticlastic and hydrogenotrophic methanogens involved in the terminal processes of methanogenesis were obtained from complex organic matter. Aceticlastic methanogens are responsible to convert acetate to methane while hydrogenotrophic methanogens transform hydrogen to methane (Demirel and Scherer, 2008). About 70-72 % of methane produced in the digester originates from the decarboxylation of acetate (Gujer and Zehnder, 1983; Hussain and Dubey, 2013). Therefore, acetate is mainly considered as precursor for methane production.

Normally, new anaerobic digesters use the digested sludge taken from an active anaerobic digester as inoculum for start-up (Budiyono *et al*., 2009; Li *et al*., 2014; Zhao and Viraraghavan, 2014). The Specific Methanogenic Activity (SMA) measures the potential of inoculum in producing methane, indirectly showing the efficiency of the inoculum (Hussain and Dubey, 2013; Muxi *et al*., 1992; Regueiro *et al*., 2012). SMA measures the methanogenic activity and closely relates to the metabolic functions of the microbial community and they do not directly quantify microorganism. SMA is defined as the substrate-dependent methane production rate per unit mass of biomass (Lu *et al*., 2008) and is written as gCOD-CH_4/g VSd (Sorensen and Ahring, 1993) or gCOD-CH_4/g VSSd (Regueiro *et al*., 2012). The poorly digested sludge usually have SMA value of 0.04 mg COD-CH_4/mg VSSd. On the other hand, SMA values of 0.01-0.04g COD-CH_4/g VSSd are for sludge taken from conventional anaerobic digester (Hussain and Dubey, 2013). The above SMA values were derived from unknown feed unfortunately.

SMA tests can be done using different substrate, including acetate/acetic acid mixture of volatile fatty acid and H_2/CO_2 (Regueiro *et al*., 2012; Morris *et al*., 2014; Angelidaki and Ahring, 1993). When the acetate/acetic acid is used as feed then the best terminology to describe this process would be Specific Aceticlastic Methanogenic Activity (SAMA). Whereas, SMA refers to the test where the feed is a mixture of volatile fatty acids. Hydrogenotrophic methanogenic activity reflects the activity measurements using H_2/CO_2 as feed (Regueiro *et al*., 2012; Morris *et al*., 2014; Raposo *et al*., 2006). For sludge to be considered as inoculum, the specific activity on acetate (or SAMA) should be a minimum of 0.1 gCH_4-COD/g VSSd (Angelidaki *et al*., 2009). The SAMA and SMA value for anaerobic sludge

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taken from a full scale mesophilic anaerobic digester (CSTR, 36°C) of a sewage treatment plant were 0.15±0.01 g COD/gVSSd and 0.20±0.04 g COD/gVSSd, respectively (Regueiro *et al*., 2012). From another study, the digester's sludge taken from a municipal wastewater treatment plant produced SAMA between 28 and 35 m^2 CH_4/gVSSd and lower SMA ranges from 5-6 m^2 CH_4/gVSSd (Raposo *et al*., 2006). Using a typical conversion of 350 m^2 CH_4 equals to 1 g COD (Mottet *et al*., 2010) then the SAMA and SMA values are 0.08-0.1 g CH_4-COD/gVSSd and 0.014-0.017 CH_4 COD/gVSSd, respectively. The objective of this study is to evaluate the feasibility of specific methanogenic activity test for monitoring the performance of aceticlastic groups involved in the terminal process of anaerobic digestion in non-granular biomass.

MATERIALS AND METHODS

Sample collection: Anaerobic biomass or anaerobically Digested Sludge (AnDS) was taken from a full-scale anaerobic digester working at ambient mesophilic range which treats domestic sewage sludge in Malaysia, on 1/6/2015 and 27/7/2015. The biomass was stored at 4°C in the laboratory until use.

Analytical methods: Samples of AnDS were analysed for Total Solids (TS), Volatile Solids (VS) and alkalinity. TS and VS were measured following the method described in the Standard Method procedure 2540G. Triplicate samples were prepared. About 30 m^2 of fresh AnDS was used for each measurement. The AnDS was placed at 105°C for 24 h to obtain the VS value. While, VS is the loss of weight caused by the ignition of a sample (dried at 105°C previously) at 550°C for 2 h in a furnace (Gianico *et al*., 2013). VS (%) represents the biomass content in the AnDS as described by AMPTS as:

$$VS(\%) = \frac{M_{105} - M_{550}}{M_{wet}} \quad (1)$$

Where:

M_{105} = Weight of dried residue and dish after evaporate at 105°C (g)

M_{550} = Weight of dried residue and dish after ignition at 550°C (g)

M_{wet} = Weight of fresh sample and dish (g)

Samples for alkalinity measurements were centrifuged at 6000 rpm for 3 min and the supernatant was used for further analysis (Bjornsson *et al*., 2001). About 30 m^2 of supernatant was used for alkalinity measurement and triplicate samples were prepared. The alkalinity measurement follows the Standard Method procedure 2320B. The initial pH of the 30 m^2 supernatant was recorded and 0.1 N Hydrochloric acid (HCl) was added until the pH reached 5.75 and 4.30 for measuring the partial alkalinity and total alkalinity, respectively.

The Intermediate Alkalinity (IA) is determined by calculating the difference between Total Alkalinity (TA) and Partial Alkalinity (PA). The Intermediate Alkalinity (IA) is Related to Volatile Fatty Acid (VFA) presence (Ripley *et al*., 1983):

$$IA = TA - PA \quad (2)$$

Where:

PA = Partial alkalinity

TA = Total alkalinity

The Specific Aceticlastic Methanogenic Activity (SAMA): Aceticlastic activity was measured by mixing acetate ($CH_3COONa.3H_2O$) with anaerobic medium to form substrate solution (later called as substrate). The anaerobic medium for methanogenic activities was prepared using K_2HPO_4, NaH_2PO_4, NH_4Cl, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, $FeCl_3.6H_2O$, $CoCl_2.6H_2O$, $MnCl_2.4H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, HBO_3, $(NH_4)6Mo_7O_2.4H_2O$, Na_2SeO_3, $NiCl_2.6H_2O$, EDTA, HCl (36%), Resazurin and Yeast (Angelidaki and Sanders, 2004).

The AnDS was used an inoculum. The AnDS was fed with acetate ($CH_3COONa.3H_2O$) overnight to activate the microorganisms within it before the SMA test was conducted. The mass of acetate to be added to 200 m^2 of AnDS was calculated based on the total COD concentration of substrate and the VS concentration of AnDS. About liter of AnDS was activated. Equations 3 and 4 were used to determine the acetate ($CH_3COONa.3H_2O$) for AnDS activation:

$$\frac{V_{subs}}{V_{inoc}} = \frac{b \times 0.5}{d} \quad (3)$$

Where:

b = VS concentration of inoculum (g L^{-1})

d = Total COD of substrate (g L^{-1})

0.5 = Due to inoculum to substrate ratio (L/S^{-1}) equals 2

$$Acttate_{in\ 200\ mL\ of\ inoculum} = \frac{0.4}{1 + \frac{b \times 0.5}{d}} \quad (4)$$

Where:

0.4 = Total volume for the mixture of substrate and inoculum (kg)

Acetate = g

Each reactor should be prepared once, at one time as shown in Fig. 1. After filling the reactor bottle (either sample or blank), the reactor bottle was flushed using

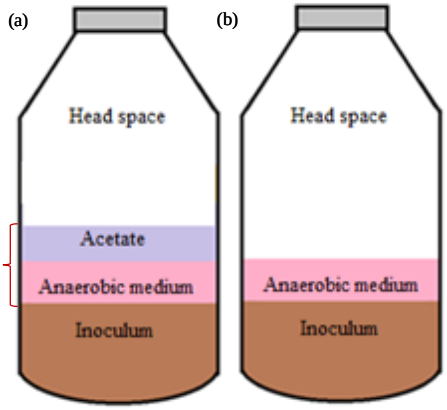

Fig. 1: SAMA batch reactors: a) sample and b) blank

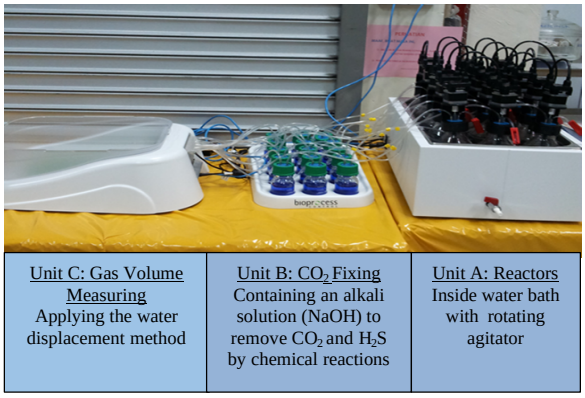

Fig. 2: Automatic Methane Potential Test System (AMPTS II)

pure N_2 gas for 2 min to ensure anaerobic conditions in the headspace of the reactors. Then, the reactor bottles were incubated at 37°C. During the experiment the mixture in the reactor bottles was mixed continuously at 160 rpm. The expected CH_4 content of the biogas varies between 50-70% (Bioprocess, 2014). However, for this study, the expected CH_4 content of produced biogas is set as 60%. The methane was measured using volumetric measuring principles, therefore the over-estimation of methane was taken into consideration during the development of AMPTS II (Stromberg *et al*., 2014). Each batch reactor was placed in Unit A-AMPTS II (Fig. 2) for automated monitoring up to 7 days. Triplicate samples and duplicate blanks were prepared each for measuring the aceticlastic activity. The net methane production from the sample is the value after subtracting the methane production from the blank. The SAMA test was carried out on the third day after sample collection because TS measurements take 2 days to complete. The total mass of the mixture (substrate and inoculum) filled into the reactor bottles for SMA tests was fixed to 400 g. The mass of substrate and inoculum added to the reactor bottle was calculated using total COD concentration as shown in Eq. 5-8. For this experiment, the inoculum to substrate ratio (I/S) is selected as 2:1 (or I/S = 2.0):

$$M_{inoc} + M_{subs} = 400_g \quad (5)$$

$$\frac{M_{inoc} \times COD_{inoc}}{M_{subs} \times COD_{subs}} = 2 \quad (6)$$

Firstly, Eq. 5 and 6 were rearrange and rewritten to calculate the mass of inoculum as shown in Eq. 7:

$$M_{inoc} = \frac{400 \times 2 \times COD_{subs}}{COD_{inoc} + (2 \times COD_{subs})}$$

Then, by rearranging Eq. 5-8 the mass of substrate was determined:

$$M_{subs} = 400_g - M_{inoc}$$

RESULTS AND DISCUSSION

Anaerobic process status of mesophilic anaerobic digester: The pH and alkalinity of AnDS were measured to monitor the mesophilic anaerobic digestion process. The value of intermediate to total alkalinity (IA/PA) ratio was higher than 0.3 indicating the presence of instability of anaerobic digestion process (Ripley *et al*., 1983). The anaerobic digestion process took place at the pH ranged from 6.0-8.3 (Angelidaki and Sanders, 2004). The IA/PA ratios for AnDS were 0.14 and 0.19 on 1/6/15 and 27/7/2015, respectively, indicating that the anaerobic process of the local full scale anaerobic digester is in stable condition during the sampling day. This was confirmed by the pH value of AnDS which ranged from 7.1-7.2.

The destruction of organic matter is the main objective of an anaerobic digester and VS is commonly used to represent the organic matter. The performance of the anaerobic digester could be estimated based on the percentage of Volatile Solid (VS) destruction. The VS destruction efficiency was calculated by using the difference between Vs_{subs} (measured from the mixed domestic sewage sludge which goes directly into the Mesophilic Anaerobic Digester, data not presented) and VS_{inoc} (from AnDS) divided by the Vs_{subs} (Zhao and Viraraghavan, 2004). The VS destruction efficiencies for the mesophilic anaerobic digestion (MAD) which is also the sampling point of AnDS were 38 and 29% on 1/6/2015 and 27/7/2015, respectively. This values are lower from the typical VS destruction percentage which ranged from

Table 1: Solid and alkalinity concentration of AnDS

	Sampling date	
Parameters	1/6/2015	27/7/2015
pH	7.60000	7.1000
VS/TS	0.64000	0.65000
Partial alkalinity (Mg/L as $CaCO_3$)	1222.22±41.94	1455.56±19.25
Total alkalinity (mg/L as $CaCO_3$)	1388.89±34.69	1727.78±25.46

1222.22±41.94 = Average±Std. Dev.; triplicate samples were prepared for all measurement; Coefficient of Variation (CV) for each data is<%

Table 2: Specific Acetolastic Methanogenic Activity (SAMA in gCH_4-COD/gVSd)

Sampling date	Sample	Blanks
1/6/2015		
Average SAMA	0.128	0.04700
Std	0.002	0.00100
CV (%)	1.560	2.13000
Net SAMA		0.08100
27/7/2015		
Average SAMA	0.067	0.04600
Std	0.002	0.00200
CV (%)	2.990	4.35000
Net SAMA		0.02100

Triplicate samples and duplicate blanks were prepared; CV = Coefficient of Variation, <5.0%

40-60% when the digester feed with the mixed sludge (primary and secondary sewage sludge (Parkin and Owen, 1986). Water consumption is a possible reason for the lower VS destruction. Anaerobic digester performance is difficult to predict and control due to the fact that the substrate, operating condition (organic loading rates, retained or suspended bacterial culture) and anaerobic digester microorganisms interrelated among them (Williams *et al*., 2013).

Anaerobic biomass characteristics: The solid concentrations (TS and VS) and biomass concentration (VS in %) for samples taken on 27/7/2015 was lower than what was observed from samples taken on 1/6/2015. However, the organic content of the AnDS is almost similar as indicated by the VS/TS value as shown in Table 1. The AnDS characteristics are shown in Table 1.

Specific Aceticlastic Methanogenesis Activity (SAMA): Table 2 shows the average Specific Aceticlastic Methanogenic Activity (SAMA) of triplicate samples and duplicate blanks measured in this study. From the methane gas production of the blank tests in the SAMA tests, it can be seen that the AnDS is stabilized, indicated by the low average SAMA value of 0.047 and 0.046 for samples taken on 1/6/2015 and 27/7/2015, respectively.

The net SAMA value is showing the potential of AnDS (anaerobic biomass) in producing the methane when fed by the acetate. It is obvious that the AnDS taken on 27/7/2015 was inefficient in producing methane as indicated by low nett SAMA value (0.021). While, the AnDS taken on 1/6/2015, is showing greater capability (almost four times higher) in producing methane. This result is expected because the biomass content (VS in %) for both samples differs significantly. VS (%) from AnDS of 1/6/2015 is about four times higher than VS (%) observed in AnDS of 27/7/2015. As shown in Table 2, the Coefficient of Variation (CV) values for sample and blank reactors containing AnDS of 27/7/2015 were higher than what were observed for AnDS of 1/6/2015. The significant difference of the CV as observed for sampling date of 27/7/2015 is possibly due to the non-uniform distribution of anaerobic biomass of each reactors especially when the VS (%) of the biomass is about 0.1%. Similar observations were seen in the Biomethane Potential (BMP) tests which were carried out concurrently during the measurements of SAMA for each sampling. The results showed that the CV of blank reactor filled with AnDS of 27/7/2015 is greater than 15%. This value is about twofold from the value observed from the blank reactor filled with AnDS of 1/6/2015.

CONCLUSION

The hypothesis of this study is the biomass concentration is related to the VS destruction of the anaerobic digester. This is possible because new cells develop during organic reduction and organic reduction performance is shown by the VS destruction. This also means that an anaerobic digester with higher VS destruction also has abundant and stabilized biomass. This was successfully observed in this study. The methane production via aceticlastic pathway was also observed from the anaerobic biomass having lower VS (%). However, better result was observed from anaerobic biomass with higher VS (%). Therefore, anaerobic biomass from anaerobic digester with higher VS (%) is recommended as suitable inoculum for BMP tests. On the other hand, anaerobic biomass with lower VS (%) should not be selected because it contained less methanogens and less capable in producing higher methane.

Despite the importance of methanogens in methane production is well understood, the routine monitoring of methanogens from the local full scale anaerobic digestion treating domestic sewage sludge is never known. This is due to the diverse methanogenic communities that dominate the different full-scale digesters. It is suggested that for future work, the activity study is not limited to SAMA and combine with molecular tools to further enhance the understanding of these microorganisms' complex environments prior to inoculum selection. This helps in interpreting the obtained data.

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