Journal of Engineering and Applied Sciences 11 (10): 2155-2160, 2016
ISSN: 1816-949X

Influence of Inoculum to Substrate Ratio on the Biochemical Methane Potential of Domestic Sewage Sludge in Batch Tests

Roslinda Seswoya and Ahmad Tarmizi Abdul Karim
Micropollutant Research Centre (MPRC),
Faculty of Civil and Environmental Engineering,
Universiti Tun Hussein Onn Malaysia (UTHM), 86000 Parit Raja, Johor, Malaysia

Abstract: Anaerobic digestion is adapted worldwide to treat sewage sludge, mostly under mesophilic condition and producing methane. The feasibility of anaerobic digestion of aged domestic sewage sludge which was stored for 3 weeks was evaluated under mesophilic condition. Completely mixed batch reactors were used to compare the methane production from aged domestic sewage sludge at inoculum to substrate of 2.0 and 1.5. The tests were carried out at 37°C and run against a control of inoculum without substrate. Modified Gompertz Model was employed to reveal the principle kinetics of methane yield from the anaerobic digestion. The results showed that the methane yield was higher at higher I/S ratio had average value of 1336.6 mL CH_4/g VS added. However, the higher cumulative methane production was observed at lower ratio of I/S and had nett average value of 794.23 mL CH_4. Model simulation indicated Modified Gompertz model best fitted the laboratory data, showing the value of kinetic equation parameters between model and actual differ slightly.

Key words: Anaerobic, mesophilic, domestic, modified Gompertz model, batch

INTRODUCTION

Biogas, a by-product of anaerobic digestion of organic materials contained huge amount of methane, a gas which is seen as a future renewable energy. The organic material as input to anaerobic digesters can varies, such as municipal solid waste, waste oils, animal fat, agricultural waste, manure and sewage sludge. However, sewage sludge is known to produce higher methane yield (Appels *et al*., 2011). Anaerobic digestion is the most applied technology on sewage sludge (Miron *et al*., 2000), precisely under mesophilic condition (Skiadas *et al*., 2005)

The research on anaerobic biodegradability of municipal sewage sludge is done worldwide (Skiadas *et al*., 2005; Astals *et al* ., 2013; Bolzonella *et al*., 2012) including municipal sewage sludge containing low organic content (Forster-Carneiro *et al*., 2010). Biochemical Methane Potential (BMP) assay was a standard protocol to estimate the methane generation and the biodegradability of sewage sludge in anaerobic digestion. The BMP assay was carried out using the glass serum bottle or glass reactor where the reactor has been shaken manually and biogas measurement as well (Li *et al*., 2014; Zhen *et al*., 2015). It is difficult to compare the BMP results from other researchers due to the different experimental set-up and modification to suit each researchers requirements (Stromberg *et al*., 2014). Recently, BMP assays were conducted using the Automatic Methane Potential Test System (AMPTS II) which successfully minimise human errors (Stromberg *et al*., 2014; Rajagopal *et al*., 2013). A study on biomethane potential of Malaysian domestic sewage sludge has been carried out by (Chua *et al*., 2013) particularly on co-digestion of sewage sludge and food waste. However, the anaerobic reactor was not incubated at any temperature and was placed at shaded area which the average temperature is 28.5°C. The sewage sludge of 0.75 kg (total initial mass) produced biogas 622.5 ml/kg^{-1} mass of total solid.

Inoculum to Substrate (I/S) ratio is generally presented in VS basis (Astals *et al*., 2013; Feng *et al*., 2013). Different types of inoculum and I/S ratio are affecting the lag phase of anaerobic digestion of municipal sewage sludge. Seung (Lim and Fox, 2013) used anaerobic granular sludge as inoculum and I/S of 1:1, 1:3 and 1:8 for BMP assays using thickened municipal sewage sludge as substrate. The elapse time for cumulative methane yield (or biomethane potential) was about 15 day at any I/S ratio. The highest cumulative

---

Corresponding Author: Roslinda Seswoya, Micropollutant Research Centre (MPRC),
Faculty of Civil and Environmental Engineering,Universiti Tun Hussein Onn Malaysia (UTHM),
86000 Parit Raja, Johor, Malaysia

methane yield was observed for I/S ratio of 1:3. However, the lag phase was not observed in the study carried out by Astals *et al*. (2013). With I/S ratio of 2:1 which was used when assaying the Biochemical Methane Potential (BMP) of municipal sewage sludge and the inoculum originated from the sewage sludge anaerobic digester (lab scale).

Anaerobic biomass in the inoculum is the key factor for the successful anaerobic digestion of organic matter. Unfortunately, there is no specific value of I/S for start-up the batch reactor. For start-up the batch reactor, plenty of microorganisms (in inoculum) should be available to start the reaction. Therefore, it is necessary to determine the optimum I/S for the anaerobic digestion of domestic sewage sludge.

The objectives of this study are to evaluate the biochemical methane potential of domestic sewage sludge (aged) under mesophilic condition and to determine the suitable I/S ratio for the methane production of domestic sewage sludge under mesophilic condition.

MATERIALS AND METHODS

Substrate: A domestic sewage sludge that had been stored for 21 days in a refrigerator at 4^0C was used as the substrate. This sludge is a mixture of primary and secondary sludge and was taken from the inlet point of a full-scale anaerobic digester, working at ambient mesophilic range. It had a pH of 5.5, VS of 16.00 g/L^{-1} VS/TS ratio of 0.76. and VS (%) of 0.44.

Inoculum: Anaerobically digested sludge from the similar digester was used as the inoculum. It had a pH of 7.0, VS of 12.50 g L^{-1}, VS/TS ratio of 0.61 and VS (%) of 0.34.

Reactors: The reactors were the Duran bottle with a 500 mL working volume. The tubing lid is equipped with the motor for mixing. The tubing in the reactor lid is to allow venting of the biogas (CH_4, CO_2 and H_2S) to the alkali solution to eliminate CO_2 and H_2S and leave CH_4 to be recorded by water displacement. AMPTS-II, the commercial batch reactor was used in this study as shown in Fig. 1 and complied to the aforementioned description. During the experiment the mixture in the reactor bottles was mixed continuously at 160 rpm. Luostarinen *et al.* (2009) also applied 160 rpm for mixing of the batch reactor.

BMP assays: The anaerobic biodegradability and methane production potential of the substrate was determined over the following range of Inoculum to Substrate (I/S) ratio: 2.0 and 1.5. The mass of substrate and inoculum filled into the reactor bottle was determined using Eq. 1 to 4 based on VS (%) as described by AMPTS II however, if the value of I/S is changed to 1.5, then 2 in Eq. 2 and 3 was substituted by 1.5.

Fig. 1: Automatic Methane Potential Test System (AMPTS II)

$$M_{inoc} + M_{subs} = 400_g \tag{1}$$

$$\frac{M_{inoc} \times VS_{inoc}}{M_{subs} \times VS_{subs}} = 2 \tag{2}$$

$$M_{inoc} = \frac{400 \times 2 \times VS_{subs}}{VS_{inoc} + 2 \times VS_{subs}} \tag{3}$$

$$M_{subs} = 400_g - M_{inoc} \tag{4}$$

The following amounts of substrate (M_{subs}): 111.75 and 136.30 g of domestic sewage sludge were added to 288.25 g and 263.70 g of inoculum (M_{inoc}) in order to obtained I/S ratios of 2.0 and 1.5, respectively. The reactor was prepared once at a time. For the sample reactor, the substrate and inoculum was firstly mixed. Before mixing, the substrate and inoculum must be shaken to homogenize the solid concentration followed by pouring into the sample reactor as well as blank reactor (only inoculum). The mass of inoculum were always similar for sample and blank reactor. Pure nitrogen was flushed for 2 min to create the anaerobic condition in the reactor after the initial pH was recorded. The reactor bottles were incubated at 37°C. The methane generation was monitored until insignificant methane production was observed.

Triplicate reactors for sample and duplicate blank reactors were prepared as shown in Fig. 2. All batch anaerobic digestion tests had no additional nutrients or minerals. The initial pH for the mixture in sample and blank reactors are between 7.0-7.5. The anaerobic digestion

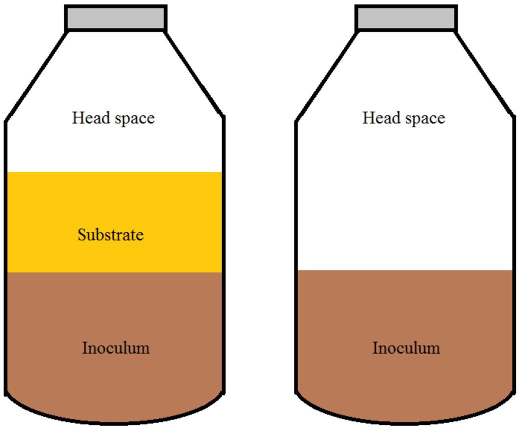

Fig. 2: BMP assays reactor: a) Sample; b) Blank

process takes place at the pH ranged from 6.0-8.3 (Angelidaki and Sanders, 2004). The methane production (potential) was expressed specifically per mass volatile solid sadded (L CH_4/ kg-VS_{added}) (Luostarinen *et al*., 2009). According to the BMP is defined as:

$$BMP = \frac{VS-(VB\times\frac{M_{VS,IS}}{M_{VS,IB}}}{M_{VS,SS}} \quad (5)$$

Where:

VS = Accumulated methane volume from sample bottle(s) (mL)

VB = Accumulated methane volume from blank bottle(s) (mL)

$M_{VS,\,SS}$ = Volatile solids amount of substrate in sample bottle (s) (g)

$M_{VS,\,IS}$ = Volatile solids amount of inoculum in sample bottle (s) (g)

$M_{VS,\,IB}$ = Volatile solids amount of inoculum in blank bottle (s) (g)

Analytical methods: Samples of domestic sewage sludge and anaerobically digested sludge were analysed for TS (g/L^{-1}), VS (g/L^{-1}) and VS (%). TS and VS were measured following the method described in the Standard Method procedure 2540G (Gianico *et al*., 2013). The 30 mL of fresh sample (domestic sewage sludge and anaerobically digested sludge) was used for the measurement of TS and VS. TS (or dry matter) is the material remaining after water evaporation from a sample placed at 105°C for 24 h. The VS (or organic matter) correspond to the loss of weight caused by the ignition of a sample (previously dried at 105°C) at 550°C for 2 h in a muffle furnace (Jeong *et al*., 2007). The VS (%) was calculated using equation described by Bioprocess Control as:

$$VS(\%) = \frac{MD-MB}{MW} \quad (6)$$

Where:

MD = Weight of dried residue and dish (mg)

MB = Weight of residue and dish after ignition (mg)

MW = Weight of dish and sample (mg)

Batch kinetic modelling: The modified Gompertz (Zhen *et al*., 2015) was used to describe the kinetics of methane production from sewage sludge. This kinetic modelling was applied by (Li *et al*., 2014) for the batch reactors study. The mathematical modified Gompertz model is described in Eq. 7.

$$M = M_0 \exp\left\{-\exp\left[\frac{R_m e}{M_0}(\lambda - t) = +1\right]\right\} \quad (7)$$

Where:

M = Cumulative methane yield (mL/g VS $_{added}$)

Mo = Ultimate methane yield (mL/g VS $_{added}$)

Rm = Maximum methane production rate (mL/g VS_{added}/day)

t = Digestion time (day)

e = 2.718

λ = The lag phase time (day)

RESULTS AND DISCUSSION

Methae production: Figure 3 shows the evolution of methane accumulated production during the 26 days of assay, dotted graph showing I/S of 2.0 and I/S of 1.5 was shown by line graph. The accumulated methane for blanks for each ratio were similar throughout the monitoring period. However, the methane production for I/S of 2.0 was not uniform even after 20 day monitoring. The BMP stopped at day 26 due to insignificant of methane production from each reactor. No changes of methane production for blank of I/S = 2.0 and I/S = 1.5 were observed starting at day 16 and 22, respectively. While, the insignificant methane production from sample reactors started at day 18 and 21 for I/S = 2.0 and I/S = 1.5, respectively. Other researchers using sewage sludge for BMP tests observed the plateau condition after 15-20 days (Astals *et al*., 2013; Lim and Fox, 2013). The huge differences of accumulated methane between blanks and sample for each I/S ratio showed that the inoculum is stable. However, this could not be confirmed because specific methanogenesis activity was not measured. The pH of the mixture of sample and blank reactor at the end of the BMP assays are in the acceptable value for anaerobic process takes place. The net cumulative methane for test at I/S = 1.5 was greater than what was measured by BMP test at I/S = 2.0 as shown in Table 1.

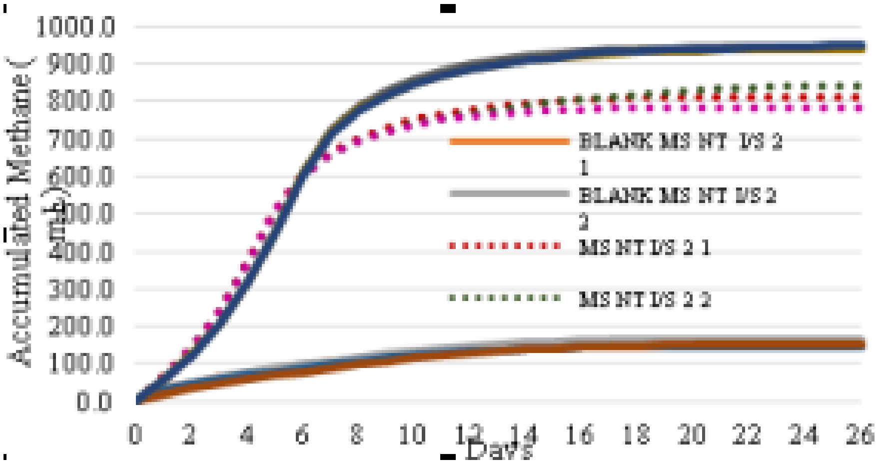

Fig. 3: Cumulative methane production of aged domestic sewage sludge

Table 1: Net cumulative methane production of 26 days assay

I/S = 2.0		I/S = 1.5	
Sample ID	Methane (mL)	Sample ID	Methane(mL)
Blank 1	275.20	Blank 1	268.20
Blank 2	286.80	Blank 2	269.40
Average = 281.00		Average = 268.80	
STD = 8.20		STD = 0.85	
CV (%) = 2.92		CV (%) = 0.32	
Sample 1	901.20	Sample 1	1054.80
Sample 2	927.50	Sample 2	1044.70
Sample 3	929.30	Sample 3	1046.90
Average = 919.33		Average = 1048.80	
STD = 15.73		STD = 5.31	
CV (%) = 1.71		CV (%) = 0.51	
∑ Methane (mL) = 638.33		∑ Methane (mL) = 780.00	

CV (%) = (STD/Average) X 100%

Table 2: Biomethane Potential

Digestion time	I/S = 2.0	I/S = 1.5
Day	BMP (mL CH_4/g VS)	BMP (mL CH_4/g VS)
0	0.0	0.0
1	68.1	53.6
2	196.5	139.5
3	371.3	261.5
4	601.7	419.2
5	862.7	624.6
6	1038.7	871.6
7	1133.2	1040.5
8	1186.8	1132.4
9	1225.0	1184.4
10	1250.1	1217.1
11	1264.6	1241.6
12	1275.4	1263.9
13	1285.0	1280.5
14	1292.9	1288.0
15	1299.8	1294.9
16	1307.2	1301.8
17	1313.2	1306.5
18	1318.5	1309.7
19	1323.0	1312.7
20	1327.2	1315.2
21	1331.4	1317.5
22	1334.3	1319.3
23	1336.6	1321.3
24	1336.6	1322.4
25	1336.6	1323.4
26	1336.6	1323.7

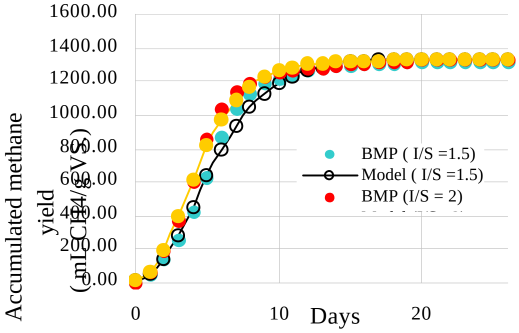

Fig. 4: Laboratory data and modified Gompertz plots of cumulative methane yield

Table 3: Kinetic equation parameters of methane production

Parapeters	I/S = 2(L)	I/S = 2(G)	I/S = 1.5(L)	I/S = 1.5(G)
Mo	1336.56	1327.02	1323.71	1337.51
Rm	245.74	220.99	226.20	181.38
l	0.33	1.21	0.17	1.50

L = lab; G = Gompertz

Methane production rate: By applying Eq. 5 and average data presented in Table 1, the biomethane potential were determined as shown in Table 2. The maximum biomethane potential for I/S of 2.0 and 1.5 were almost similar as shown in Table 2. However, the highest was observed for I/S = 2.0. This is suggesting that the bigger net cumulative methane production was not promising the higher biochemical methane potential.

Considering the steepest slope of each biomethane potential curve, the methane production rate was calculated. The maximum methane production rates were 10.24 mL CH_4/g VS.hr and 9.42 mL CH_4/g VS.hr for I/S = 2.0 and I/S = 1.5, respectively.

Kinetic modelling: Table 3 and Fig. 4 summarize the results of fitting the modified Gompertz model to digestion data. Lag phase time (l), reflects the acclimation of microbes to the specific substrate and environmental condition in the digestion. In this study, short lag phase times (<2.0 day) were observed for each digestion (I/S of 2.0 and 1.5) and this is supporting that initial viability was maintained for all conditions tested.

CONCLUSION

The methane yield at two different inoculum to substrate ratios (2.0 and 1.5,) showed only slight variation between them. Higher methane yield was observed at higher ratio and had average value of 1336.6 mL CH_4/g VS_{added}. Generally, the higher methane production rate would result in the higher methane yield. Despite of this, the accumulated methane production for I/S = 1.5 was higher than what was observed at I/S = 2.0, yet this does not assured higher methane yield. In assessing impact of I/S towards anaerobic degradability of domestic sewage sludge, I/S of 2.0 should be one of the variable. The kinetic equation parameters obtained from Modified Gompertz Model showed only slight difference. The capability of aged domestic sewage sludge in producing methane is unexpected. This suggested that it is worth paying attention on energy (CH_4) recovery from digestion of domestic sewage sludge. Furthermore, domestic sewage sludge is the by-product of sewage treatment plant which is impossible to be reduced.

ACKNOWLEDGEMENTS

The researcher wish to thank Universiti Tun Hussein Onn Malaysia for the funding of this research. We also wish to thank Unit Inovasi, Indah Water Konsortium for their cooperation.

NOMENCLATURE

BMP = Biochemical Methane Potential (t)
Digestion time (day)
(AMPTS II) = Automatic Methane Potential Test System (λ)
The lag phase time (day)
(I/S) = Inoculum to Substrate (e), (2.718)
M = Cumulative methane yield (mL/g VS_{added})
Mo = Ultimate methane yield (mL/g VS_{added})
Rm = Maximum methane production rate (mL/g Vs_{added}/day)
V_S = Volume from sample bottle (s) (mL)
V_B = (mL) (s) volume from blank Bottle
M = Cumulative methane yield (mL/g VS_{added})
MD = Weight of dried residue and dish (mg)
MB = Weight of residue and dish after ignition (mg)
MW = Weight of dish and sample (mg)

REFERENCES

Angelidaki, I. and W. Sanders, 2004. Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Bio Technol., 3: 117-129.

Appels, L., J. Lauwers, J. Degreve, L. Helsen and B. Lievens et al., 2011. Anaerobic digestion in global bio-energy production: Potential and research challenges. Renewable Sustainable Energy Rev., 15: 4295-4301.

Astals, S., E.M. Gutierrez, F.T. Arevalo, E. Aymerich and G.J.L. Heras et al., 2013. Anaerobic digestion of seven different sewage sludges: A biodegradability and modelling study. Water Res., 47: 6033-6043.

Bolzonella, D., C. Cavinato, F. Fatone, P. Pavan and F. Cecchi, 2012. High rate mesophilic, thermophilic and temperature phased anaerobic digestion of waste activated sludge: A pilot scale study. Waste Manage., 32: 1196-1201.

Chua, K.H., W.L. Cheah, C.F. Tan and Y.P. Leong, 2013. Harvesting biogas from wastewater sludge and food waste. Proceedings of the IOP Conference Series on Earth and Environmental Science, Vol. 16, March 5-6, 2013, IOP Publishing, Putrajaya, Malaysia, pp: 1-4.

Feng, L., Y. Li, C. Chen, X. Liu and X. Xiao et al., 2013. Biochemical Methane Potential (BMP) of vinegar residue and the influence of feed to inoculum ratios on biogas production. Bioresour., 8: 2487-2498.

Forster-Carneiro, T., V. Riau and M. Perez, 2010. Mesophilic anaerobic digestion of sewage sludge to obtain class B biosolids: Microbiological methods development. Biomass Bioenergy, 34: 1805-1812.

Gianico, A., C.M. Braguglia, R. Cesarini and G. Mininni, 2013. Reduced temperature hydrolysis at 134°C before thermophilic anaerobic digestion of waste activated sludge at increasing organic load. Bioresour. Technol., 143: 96-103.

Jeong, T.Y., G.C. Cha, S.S. Choi and C. Jeon, 2007. Evaluation of methane production by the thermal pretreatment of waste activated sludge in an anaerobic digester. J. Ind. Eng. Chem., 13: 856-863.

Li, J., S.M. Zicari, Z. Cui and R. Zhang, 2014. Processing anaerobic sludge for extended storage as anaerobic digester inoculum. Bioresour. Technol., 166: 201-210.

Lim, S.J. and P. Fox, 2013. Biochemical methane potential (BMP) test for thickened sludge using anaerobic granular sludge at different inoculum-substrate ratios. Biotechnol. Bioprocess Eng., 18: 306-312.

Luostarinen, S., S. Luste and M. Sillanpaa, 2009. Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant. Bioresour. Technol., 100: 79-85.

Miron, Y., G. Zeeman, V.J.B. Lier and G. Lettinga, 2000. The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems. Water Res., 34: 1705-1713.

Rajagopal, R., J.W. Lim, Y. Mao, C.L. Chen and J.Y. Wang, 2013. Anaerobic co-digestion of source segregated brown water (feces-without-urine) and food waste: For Singapore context. Sci. Total Environ., 443: 877-886.

Skiadas, I.V., H.N. Gavala, J. Lu and B.K. Ahring, 2005. Thermal pre-treatment of primary and secondary sludge at 70°C prior to anaerobic digestion. Water Sci. Technol., 52: 161-166.

Stromberg, S., M. Nistor and J. Liu, 2014. Towards eliminating systematic errors caused by the experimental conditions in Biochemical Methane Potential (BMP) tests. Waste Manage., 34: 1939-1948.

Zhen, G., X. Lu, T. Kobayashi, Y.Y. Li and K. Xu et al., 2015. Mesophilic anaerobic co-digestion of waste activated sludge and Egeria densa: Performance assessment and kinetic analysis. Appl. Energy, 148: 78-86.