Anaerobic Digestion at 45℃ for Sludge Treatment: A Trade-off between Performances and Capability in Producing Class a Biosolids

Nuruol Syuhadaa Mohd, Baoqiang Li, Amber Hameed, Safia Ahmed, and Rumana Riffat

***Abstract*—Anaerobic digestion at mesophilic and thermophilic temperatures have been widely studied and evaluated for the purpose of sludge stabilization. However, limited extensive research has been conducted on anaerobic digestion in the intermediate zone of 45℃, mainly due to the notion that limited microbial activity occurs within this zone. The objectives of this research were to evaluate the performance and the capability of anaerobic digestion at 45℃ in producing class A biosolids, in comparison to a mesophilic and thermophilic anaerobic digestion system operated at 35℃ and 55℃, respectively. 45℃ anaerobic digestion systems were not able to achieve comparable methane yield and high quality effluent as mesophilic system, though the systems produced biogas with 66.08±2.83% methane. No ammonia inhibition was observed and the digesters were able to achieve volatile solids (VS) reduction of 47.79±1.86%. Moreover, the pathogen counts were less than 1,000 MPN/g dry solids, thus, producing Class A biosolids. However, the 45℃ systems suffered from high acetate accumulation, but sufficient buffering capacity was observed. Correspondingly, the dominant methanogen existed in 45℃ system was thermo-tolerant acetate-utilizing methanogen of *Methanosarcinaceae* species.**

***Index Terms*—45℃ anaerobic digestion, acetate accumulation, class a biosolids.**

I. Introduction

A large number of studies have been done to evaluate the performance of mesophilic and thermophilic anaerobic digesters [1]-[6]. However, very few studies have extensively investigated the possibility of operating anaerobic digesters in the intermediate zone, between mesophilic and thermophilic range [7]-[11]. This is due to the assumption that limited activity of microorganisms occurs within the intermediate zone of 40℃ to 50℃. The assumption is that, within this zone, neither mesophilic nor thermophilic microorganisms would flourish, as the microorganisms would try to cope with the changing environment, hence cause limited digestion activity.

Manuscript received June 2, 2015; revised August 5, 2015. This study was supported by a grant from USAID under the Pakistan-US Science and Technology Cooperation Program.

N. S. Mohd was with George Washington University, Washington, DC 20052 USA. She is now with the Department of Civil Engineering, University of Malaya, Kuala Lumpur, 50603, Malaysia (e-mail: nuruol.syuhadaa@gmail.com).

B. Li and R. Riffat are with the Civil and Environmental Engineering Department, George Washington University, Washington, DC, 20052, USA (e-mail: baoqiang0606@gwu.edu, riffat@gwu.edu).

A. Hameed and S. Ahmed are with the Department of Microbiology, Quaid-i-Azam University, Islamabad, 45320, Pakistan (e-mail: amberh@yahoo.com, safiamrl@yahoo.com).

Apparently, this has triggered questions regarding its validity as Gao *et al*. observed that at 45℃, their anaerobic digestion system was still producing gas and even significantly similar to the mesophilic system at 37℃ [8]. This finding was similar to observations by Peces *et al.* [10]. They found that there was no great variation between anaerobic digestion systems operating at 37℃ and 45℃ in terms of methane production, volatile solids (VS) content and pH, except for volatile fatty acids (VFA) concentration that increased significantly with increasing temperature. However, none of these studies have done any further investigation in finding the reasons behind the accumulation of VFAs observed in their digestion system as well as the capability of the system in producing class A biosolids.

Considering the above findings, it implies that microbial activities are still occurring within the intermediate zone. To determine whether they are mesophilic activities, or thermophilic activities, or possibly activities involving both types of microorganisms, further investigations need to be conducted. Therefore, the current research was initiated to investigate the performance of anaerobic digestion within the intermediate zone, specifically at 45℃. In addition to that, the investigation on the capability of anaerobic digestion at 45℃ in producing class A biosolids will also be conducted.

In the U.S, land application of biosolids or processed sludge is regulated by the U.S.EPA under The Standards for the Use or Disposal of Sewage Sludge (Title 40 of the Code of Federal Regulations (CFR), Part 503) [12]. The regulation classifies biosolids according to the assumed pathogen content resulting from the sludge treatment process. Class A biosolids are considered to be pathogen-free and will have no restrictions on crop choice or human access to land application sites used for disposal. Class B biosolids may have detectable amount of pathogens but have been reduced to levels that do not pose a threat to public health and the environment, and certain restrictions must be met when it is used for land application. Additionally, according to the regulation, class a biosolids can only be produced through treatment processes involving high temperatures such as thermophilic anaerobic digestion [12]. Hence, if the 45℃ anaerobic digestion system can successfully produce the same quality of biosolids, this process will be more economical in comparison to the conventional thermophilic anaerobic digestion process.

II. Materials and Methods

A. Anaerobic Digestion System

In this study, two single-stage 45℃ anaerobic digestion systems were operated in parallel to each other at which the second system, 45℃ (2) AD served as a duplicate to the first system 45℃ (1) AD. 45℃ (1) AD system was operated for 25 months while 45℃ (2) AD system was operated for 8 months. In addition to that, two control systems, which were conventional mesophilic and thermophilic systems were also operated. The mesophilic 35℃ Control AD was run for 24 months while the thermophilic 55℃ Control AD was run for 4 months, after 2 years of operation at 45℃. The operational volumes of all 45℃ ADs and 55℃ Control AD were 15 L each, while the operational volume of 35℃ Control AD was 13 L (Table I). The TS content of influent were all 6.5% except for 35℃ Control AD which was 5.0% (Table I). The average organic loading rate (OLR) was 5.22 kg VS/m^3.day for both 45℃ and 55℃ Control AD systems (Table I). As for 35℃ Control AD, the OLR was 4.07 kg VS/m^3.day (Table I).

The OLR for 45℃ and 55℃ systems were generally higher, considering that it was observed previously that anaerobic digestion systems operating at higher temperature were able to tolerate higher organic loading [3], [4], [6]. The SRT of all digesters were the same at 10 days. The SRT of 10 days was selected as it was regarded as the optimum time needed by the methanogens to complete the digestion process.

B. Experimental Apparatus

The digesters were made of high density polyethylene (HDPE) 25 L brewery tanks of Hobby Beverage Equipment Company (Temecula, California). The heating system for the digesters was controlled by a thermostat connected to a temperature sensor inserted into the digester. Each digester was covered with aluminum foil and temperature adjustable heating tape was placed on top of the foil. The aluminum foil was used to ensure even heat distribution to the digesters and to provide protection from the heating tape so that physical failure of the polyethylene would not occur. The temperature of two of the digesters was maintained at 45℃, and the other two control digesters were operated at 35℃ and 55℃. Gas mixing was applied to each digester by circulating the headspace gas to the bottom of digesters, by using a peristaltic pump. Because all digesters were kept completely mixed throughout the study, and feeding and wasting were done in equal amounts, solids retention time (SRT) of each reactor was equal to hydraulic retention time (HRT). Each digester was equipped with a gas collection flask and wet tip gas meter to measure volume of gas production. Fig. 1 is a schematic diagram of the anaerobic digestion systems.

C. Operation

The anaerobic digester system was initiated by inoculating the empty digester with seed sludge from well operating mesophilic anaerobic digesters in Alexandria Wastewater Treatment Plant, AlexRenew (operated by city of Alexandria, VA). This is to benefit from the seed sludge that usually contains abundant amounts of useful microorganisms such as methane formers and acid forming bacteria. After approximately 3 days of seed feeding, the digester system was then fed with raw municipal sludge obtained from Blue Plains Advanced Wastewater Treatment Plant (operated by DC Water and Sewer Authority, DC Water). During this initial phase, no effluents are taken out as the microorganisms need to have ample time to establish themselves within the new environment.

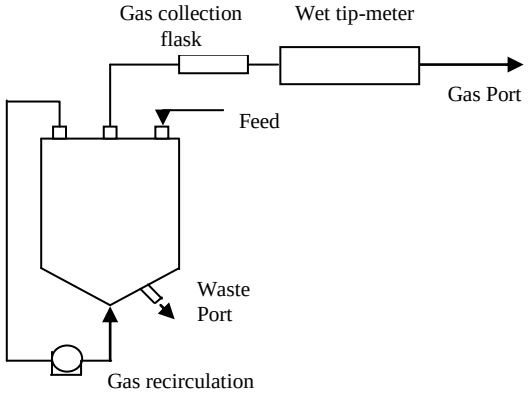

Fig. 1. Schematic diagram of anaerobic digestion systems.

After approximately two weeks, duration that was considered sufficient for microorganisms' adaptation, the feeding and effluent withdrawing schedules were started regularly. The digesters were fed once a day and an equal amount of digested sludge or effluent was withdrawn directly before feeding. After six cycles of SRT, which is after 2 months, the analytical tests were initiated for the digester effluent as well as for the influent.

The raw feed sludge for the system was collected from Blue Plains Advanced Wastewater Treatment Plant. The raw sludge was the effluent after the thickening process and was collected once every two weeks and stored at 4℃ prior to its use as feed. The average total solids (TS) content of the raw sludge was approximately 13%. Prior to the daily feeding routine, the raw sludge was diluted with tap water to make a feed of 6.5% total solids for 45℃ and 55℃ digesters and 5.0% total solids for 35℃ digester. The feed sludge was then acclimated to 45℃, 35℃ or 55℃ by incubating them at the desired temperature for approximately 2 hours. The other characteristics of the feed sludge, such as pH, chemical oxygen demand (COD), ammonia, alkalinity and VFA varied widely throughout the study and the values for feed sludge were provided in Table I.

D. Experimental Methods

The digested sludge was analyzed for several parameters. The pH was measured daily, while the alkalinity, total solids (TS), volatile solids (VS), soluble COD (sCOD), total COD (tCOD), total ammonia nitrogen (TAN) and volatile fatty acids (VFA) were analyzed weekly. Alkalinity, TS, VS, COD and TAN tests were conducted in accordance to Standard Methods 2320 B, 2540 B, 2540 E, 5220 D and 4500-NH_3 B & C, respectively [13]. The composition of VFA was analyzed using a Shimadzu Gas Chromatograph Model GC-2010 (Kyoto, Japan) with flame ionization detector (FID) and equipped with a Restek Stabilwaxfi-DA capillary column. The column temperature was 145℃, and the injector and flame ionization detector temperature was 250℃. The calibration curves were obtained using five aqueous solutions of organic acids: acetic, propionic, butyric, valeric and caproic, in the concentration range of 25 to 1000 μL/L. The digested sludge was also analyzed for pathogens once per month. Pathogen count was evaluated using Most Probable Number (MPN) Method as described in EPA Method 1680

[14]. Additionally, in order to investigate the microorganism colonies within the digesters, the digested sludge was also sent to MRDNA Laboratories (Texas) for methanogen illumine assay. Methanogen illumine assay was conducted by the Metagenomes technique, utilizing MiSeq 2*250bp sequencing.

TABLE I: INFLUENT AND EFFLUENT CHARACTERISTICS OF 45°C AD, 35°C CONTROL AD AND 55°C CONTROL AD SYSTEMS

	Feed	45°C AD	35°C Control AD	55°C Control AD
		Influent Characteristics		
Digester Volume (L)	N/A	15	13	15
SRT (days)	N/A	10	10	10
Flow (L/day)	N/A	1.5	1.3	1.5
TS (%)	N/A	6.5	5.0	6.5
VS (%)	N/A	5.2	4.1	5.2
OLR (g VS/L.day)	N/A	5.22	4.07	5.22
		Effluent Characteristics		
Daily CH_4 Production (L/day)	N/A	21.86 ± 5.49	22.58 ± 6.09	6.03 ± 2.98
Ultimate CH_4 Production (L)	N/A	1.414 ± 0.222	1.478 ± 0.108	0.329 ± 0.140
Specific CH_4Yield (m^3 CH_4/kg VS removed)	N/A	0.564 ± 0.141	0.910 ± 0.246	0.249 ± 0.097
CH_4 Content (%)	N/A	66.28 ± 4.61	69.16 ± 5.00	60.97 ± 2.42
TAN (mg/L)	407 ± 107	1,740 ± 200	1,385 ± 228	2,392 ± 221
Free NH_3 (mg/L)	1.1 ± 0.5	82.0 ± 12.8	28.6 ± 8.0	239.0 ± 37.9
Acetate (mg/l)	1,398 ± 574	1,007 ± 160	94 ± 27	2,510 ± 209
Propionate (mg/L)	727 ± 125	506 ± 145	16 ± 7	1,008 ± 153
Butyrate (mg/L)	325 ± 97	163 ± 58	28 ± 7	272 ± 42
Valerate (mg/L)	165 ± 67	88 ± 8	N/A	155 ± 29
Total VFA (mg/L)	2,491 ± 350	1,814 ± 396	126 ± 24	3,944 ± 15
pH	6.08 ± 0.29	7.41 ± 0.15	7.30 ± 0.18	7.42 ± 0.24
Alkalinity (mg/L)	3,380 ± 888	10,131 ± 1623	8,037 ± 1059	9,893 ± 755
VFA-to-Alkalinity (ratio)	N/A	0.16 ± 0.10	0.01 ± 0.01	0.38 ± 0.02
TS Reduction (%)	N/A	36.17 ± 6.45	37.12 ± 6.72	24.87 ± 8.64
VS Reduction (%)	N/A	47.80 ± 5.38	46.85 ± 5.91	30.20 ± 7.46
sCOD Concentration (mg/L)	11,101 ± 2675	7,237 ± 2617	2,083 ± 597	10,064 ± 2,225
sCOD Reduction (%)	N/A	34.80 ± 24.37	81.24 ± 5.38	9.19 ± 5.02
tCOD Concentration (mg/L)	72,222 ± 22,988	46,586 ± 12,080	32,098 ± 5,735	64,054 ± 16,527
tCOD Reduction (%)	N/A	36.25 ± 14.63	55.63 ± 8.18	11.31 ± 8.28
Pathogen Count (MPN/g dry solids)	7.16×10^6	2.52×10^2	2.55×10^2	2.09×10^2

TABLE II: METHANOGEN ANALYSIS OF 45°C AD, 35°C CONTROL AD AND 55°C CONTROL AD SYSTEMS

	Count			Percentage (%)		
Genus	45°C AD	35°C Control AD	55°C Control AD	45°C AD	35°C Control AD	55°C Control AD
Methanosphaera	114	17	32	0.05	0.01	0.02
Methanosarcina	202,769	169,981	131,737	80.99	73.3	83.9
Methanobrevibacter	1,098	320	3,179	0.44	0.14	2.02
Methanoculleus	59	174	241	0.02	0.08	0.15
Methanobacterium	44,865	60,679	15,632	17.92	26.17	9.96
Methanobacteriaceae	498	135	614	0.2	0.06	0.39
Methanosaeta	84	21	189	0.03	0.01	0.12
Methanothermobacter	871	563	5,260	0.35	0.24	3.35
Total Methanogens Count	250,358	231,890	156,884	100%	100%	100%

The total biogas production was monitored daily using a Wet Tip gas meter (Nashville, Tennessee). Gas composition was analyzed using SRI 8610C Gas Chromatograph (Menlo Park, California) equipped with a thermal conductivity detector (TCD) and Helium was used as the carrier gas.

III. Results and Discussions

Daily and ultimate methane production of 45℃ AD, 35℃ Control AD and 55℃ Control AD systems for a specific period of operation were presented in Table I. It was observed that methane productions of 45℃ AD was comparable, but not significantly similar to 35℃ Control AD (Table I). Whereas for 55℃ Control AD system, the methane production was the lowest among all (Table I). Apparently, this observation showed that methane was produced at all temperatures, with thermophilic system demonstrating the lowest methane production. The absence of any clear and sharp decline at 45℃ implies that there may not be a transition zone between mesophilic and thermophilic zones that inhibit the methane forming activity. Instead, as mesophilic methane formers exist in mesophilic zone, and thermophilic methane formers exist in thermophilic zone, there might be an overlap between these two zones that still allow methane formation by both types of microorganisms. Additionally, though methane production of 45℃ system was not significantly similar to mesophilic system, it could be considered on par with that of the 35℃ Control digester, as it was able to produce comparably equal amount of methane throughout the process. As for 55℃ Control AD system, the limited amount of methane produced was attributed to its high TAN and free ammonia content that subsequently contributed to high VFA levels that are known to be inhibitory to methanogens.

The decreasing trend over the increase of temperature was also observed in specific methane yield. Apparently, for every 10℃ increase in temperature, the specific methane yield was decreased by about half (Table I). Among all, only 35℃ Control AD system yielded methane within the recommended range of 0.6 to 1.6 m^3 CH_4/kg VS removed suggested by Gerardi [15], while 45℃ AD system yielded methane slightly below the lower end of the recommended value (Table I). High methane yield in 35℃ system was mainly due to the organic loading rate used, as well as the temperature being the optimum for mesophilic methane formers. For 45℃ system, the lower yield may be associated to its operation within the intermediate zone of mesophilic and thermophilic, therefore not providing an optimum growth environment for either type of methane formers, and subsequently affecting the amount of methane produced. While for 55℃ system, despite its operation within the optimum range of thermophilic methanogens, the methane production was minimal, and this might be attributed to the accumulation of VFAs as a result of ammonia stress observed within the 55℃ digester system.

As for the composition of methane in headspace gas, examination of Table I revealed that methane composition in 45℃ AD systems were lower than 35℃ Control AD but higher compared to 55℃ Control AD systems Similarly, the significantly low methane content in 55℃ AD systems was most likely attributed to ammonia inhibition exhibited by the system.

Throughout the operation period, TAN concentrations of 45℃ AD system, with an average of 1,740±200 mg/L, were higher than 35℃ Control AD at 1,385±228 mg/L but lower than 55℃ Control AD at 2,392±221 mg/L (Table I). In addition to that, in the 45℃ system, TAN concentrations were slightly higher than the lower limit of the inhibitory region of 1,500 and 3,000 mg/L [16]. Due to the substantial VFA accumulation observed within the systems, high TAN concentrations may have exerted a detrimental effect on the methanogens, particularly on the acetate-utilizing methane formers. However, significant inhibition was not observed, as the 45℃ digesters have been operated for more than 2 years with sufficient VS destruction and methane production, as well as stable pH distribution. In comparison to 35℃ mesophilic system, the average TAN concentrations in both 45℃ AD and 55℃ Control AD systems were higher. This was attributed to two factors. First, it was possibly due the fact that at higher temperature, the proteinaceous materials were hydrolyzed into ammonia more readily than at lower temperature. Second, it was possibly because the mesophilic population contained less versatile protein degraders in comparison to the thermophilic population, hence, leaving more protein undegraded, thus explaining the lower TAN concentrations at lower temperature [17].

A similar trend was also observed in free ammonia concentrations. The concentration in 45℃ AD systems was considerably higher than 35℃ Control AD but distinctively lower than 55℃ Control AD (Table I). Moreover, the levels in 45℃ and 35℃ system was lower than the inhibitory level of 100 to 150 mg/L free ammonia reported by Braun *et al*. [18]. Apparently, high TAN observed at higher temperature digesters, coupled with high pH, has contributed to high free ammonia content, which is known to be inhibitory for anaerobic fermentation and toxic to the methanogens. However, the inhibition was only evidenced in 55℃ system due to its minimal amount of methane production and high accumulation of VFAs in the digester.

Other than ammonia, the inhibition was also assessed based on the accumulation of VFAs. It was clearly indicated in Table I that all type of VFAs were at the highest in 55℃ Control AD, followed by 45℃ AD system and very minimal in 35℃ Control AD system.

Noticeably, as acetate accounted for a significant majority of the total VFA concentration, it was thus the most sensitive VFA indicator of digester operation. In 35℃ Control AD system, the large reduction of acetate concentration was in stoichiometric agreement with its high specific methane yield and reduction of soluble COD achieved through the mesophilic process. This was due to the fact that 35℃ is an optimum temperature for the acetate-utilizing methanogens. Furthermore, at this temperature, the digestion of proteinaceous materials did not create a large amount of ammonia that can be inhibitory to the methanogens, therefore explaining the high reduction of acetate within the stress-free system.

On the other hand, 45℃ digester system produced high acetate accumulation. The final concentrations of these parameters were significantly higher compared to those in 35℃ system. They were four reasons that possibly

contributed to this situation. First, at higher temperature, more proteinaceous materials were degraded and created ammonia-stress condition which had a negative influence on acetate-utilizing methanogens in comparison to the other microbes (i.e. acetate-producing acidogens). As a result, acetate was produced faster than they could be utilized, hence allowing them to accumulate within the system. Second, the abundance of acetate in the effluent suggested that instead of functioning as a fully methanogenic digester, it was serving more as an acid-phase digester. In other words, the methanogenesis process had not reached a complete steady state yet. If more time were to be given to the methanogens, more appreciable acetate reduction would be expected. Thirdly, at 45℃, instead of acting as a transition zone that inhibits microbial activity, there might be an overlap between these two zones that allow the methane to be formed despite the accumulation of VFA. The said methane formers might be the hydrogen-utilizing thermophilic methanogens instead of acetate-utilizing mesophilic methanogens. Presumably, the colonies of hydrogen-utilizing thermophilic methanogens which was at its optimum at higher temperature, were more abundant in number, hence leaving the other methanogens' substrate, acetate, not fully utilized. Lastly, it might also be caused by the way the digesters were started up. 45℃ and 55℃ digesters were started up by increasing the temperatures slowly from the mesophilic operating temperature to the thermophilic operating temperature. This technique was likely to lead to the development of a population that was different from a population obtained through a true thermophilic digester. The population was most probably a thermo-tolerant acetate-utilizing mesophilic methanogens, a mesophile that can survive at high temperature but whose optimum growth rate occurs at mesophilic temperatures [19]. Thus, the operating temperature of 45℃ might not be the optimum temperature for the microbes, hence affecting their methanogenic activity. As the temperature was further increased, the methanogenesis became less effective, therefore explaining the accumulation of acetate and VFA within the system.

The first, third and fourth assumptions were further supported by the findings of even higher acetate accumulation in 55℃ Control AD. Apparently, the higher the temperature, the higher the rate of the proteinaceous materials breakdown. In addition to that, as 55℃ is the optimum temperature for hydrogen-utilizing thermophilic methanogens, the population was presumably more exuberant, and the population of true acetate-utilizing mesophilic methanogens or thermo-tolerant acetate-utilizing mesophilic methanogens were gradually diminishing, due to the high ammonia content and high temperature, hence, leaving more acetate unutilized.

As for the presence of propionate, though was commonly believed to also inhibit methanogenesis, the concentrations were well below the tolerable range of 800 mg/L to 3,000 mg/L [20]. While the presence of butyrate and valerate were remained minimal in all digesters.

Other than methane production, ammonia content and VFA accumulation, buffering capacity is also a crucial indicator of the well-being of anaerobic digesters. The average pH of 45℃ AD and 55℃ Control AD were generally higher, at 7.41±0.15 and 7.42±0.24, than that of 35℃ Control AD at 7.30±0.18 (Table I). The pH increase in 45℃ and 55℃ AD in comparison to the 35℃ mesophilic system was most likely due to a reduced solubility of carbon dioxide at higher temperature. In overall, pH values of all digesters were stable throughout the operation period and were within the optimum pH value of 6.6 – 7.6 [21]. Thus, demonstrated that, in spite of high VFA accumulation observed in the systems, it had not reduced the pH to the levels that would cause digester failure.

Correspondingly, the alkalinity levels of 45℃ AD and 55℃ Control AD were also higher than that of 35℃ Control AD (Table I). With regard to alkalinity, McCarty suggested that an anaerobic digestion system requires an alkalinity of at least 1,500 mg/L in the presence of biogas containing about 30 percent carbon dioxide [22]. Within our 45℃ and 55℃ systems, the alkalinity was observed to be as high as 10,000 mg/L and apparently, high alkalinity was favorable as it would provide effective buffering against a pH drop due to the accumulation of VFAs. Higher alkalinity usually indicates a greater amount of protein conversion resulting in high ammonia content as well as salts. In a digester, these salts produce natural buffers, which normally remain fairly constant at about 3,000 to 4,000 mg/L [23]. Additionally, other than ammonia and salts, the increase in alkalinity was also attributed to its high concentration of VFA observed in 45℃ and 55℃ systems. As VFA concentrations began to increase, they were neutralized by the bicarbonate alkalinity, thus, forming volatile acid alkalinity [21]. Therefore, instead of only having bicarbonate alkalinity, which is normally observed in mesophilic systems, the 45℃ and 55℃ systems now had total alkalinity composed of bicarbonate alkalinity and volatile acid alkalinity.

As for VFA-to-alkalinity ratio, while the value of 45℃ AD and 35℃ AD systems were well below the suggested value of 0.4, the value of 55℃ system at 0.38±0.02 were marginally close to the inhibition value of 0.4 (Table I) [24]. Clearly, the higher ratios in 45℃ AD and 55℃ AD systems were primarily a result of the higher VFA concentration in the systems. Overall, all of the ratios remained below the maximum allowable limit, thus explaining their ability to buffer against the changes in pH caused by the accumulation of VFA in the system. In general, these findings were in agreement with Angelidaki *et al.* who demonstrated that small pH changes were common in highly buffered systems with high ammonia content, even though the systems were severely stressed by VFA accumulation [25].

The performance of the digesters was also assessed based on their ability in reducing TS and VS content. In 45℃ system, TS and VS reductions were significantly identical to that achieved in mesophilic control system and both were higher in comparison to 55℃ Control AD system (Table I). In addition to that, both 45℃ AD and 35℃ Control AD digesters were also able to exceed the required average VS destruction efficiency of 45% for Class A biosolids criterion for vector attraction reduction [12]. As for 55℃ Control AD, the VS destruction efficiency was less than the required regulated efficiency. Greater TS and VS reductions in 35℃ and 45℃ systems implied an increase in dewatering capabilities of the digested effluent due to its reduced viscosity and a better separation of solids. In addition to that, it also suggested an increase in sludge reduction as well as

sufficient sludge stabilization. While in 55℃ systems, less solids removal indicated an inhibited activity which caused incomplete solids stabilization within the system. Furthermore, similar TS and VS destructions of 45℃ system to mesophilic system also demonstrated that 45℃ system was capable of removing enough solids despite the indication of inhibition observed within the system.

In terms of COD, the effluent quality of 45℃ AD was inferior in comparison to that of the 35℃ Control AD system, but significantly superior to the 55℃ Control AD system (Table I). A similar trend was also observed in sCOD and tCOD removal efficiencies of all digesters. Reductions in removal efficiencies were observed when the temperature increased from 35℃ to 45℃ and 55℃. These reductions signified that the degradation of soluble organic matter and complex organic solids were highly affected by temperature. In other words, the group of microorganisms involved in this process worked at its maximum capabilities at 35℃ and the efficiencies decreased as the temperature increased. In addition to that, it might also suggest that different microbial colonies might have different affinity towards the substrate. Apparently, the mesophilic colonies in 35℃ digester had more affinity towards the substrate compared to the thermophilic colonies found in higher temperature digesters. The slow degradation of soluble organic matter represented as sCOD at high temperature was further supported by the accumulation of VFAs found in higher temperature digesters. The trend of increasing sCOD concentrations with the increase of temperature was consistent with trends in VFA concentrations found in higher temperature digesters. This was due to the fact that sCOD is usually composed of up to 90% of VFAs [26]. A similar increasing trend of tCOD concentrations over the increase of temperature was also observed in all systems.

Another important factor to consider, if the sludge is to be used as a fertilizer or soil amendment, is the content of pathogenic bacteria. From Table I and Fig. 2, it was observed that 45℃ AD and 55℃ Control AD systems had successfully achieved 3-log reduction of fecal coliforms, while 35℃ Control AD systems did not. The concentration of fecal coliforms in the digested sludge from 45℃ AD and 55℃ AD systems had never exceeded 1,000 MPN/g total solids, thus, met the U.S. federal fecal coliform requirements for Class A biosolids [12]. The higher deactivation of pathogens in 45℃ and 55℃ systems were probably a result of the combined effects of high temperature, VFA, TAN, free ammonia and long retention time of 10 days.

Additionally, as shown in Table II, the three dominant genus of methanogens that flourished in all systems were *Methanosarcina*, *Methanobacterium* and *Methanothermobacter*. Among all, *Methanosarcina* was the primary methanogen which represented approximately 73.3% to 83.9% portion of all methanogens present. 45℃ AD was observed with the most *Methanosarcina* count, while 55℃ Control AD was observed with the least. This was because of the fact that *Methanosarcina* was the only methanogen among those three dominant methanogens that could grow in both mesophilic and thermophilic conditions [15]. The reverse trend was observed with the *Methanobacterium*, as *Methanobacterium* has optimal growth between approximately 35℃ to 45℃ [15]. Any temperature condition above 45℃ will retard the growth of *Methanobacterium*. The *Methanobacterium* was at the highest at 26.17% in 35℃ Control AD, followed by 17.92% in 45℃ AD, and dropped to 9.96% in the 55℃ Control AD. The highest amount of *Methanothermobacter* flourished in the 55℃ AD at 3.35%, followed by 0.35% in 45℃ AD and 0.24% in 35℃ AD, respectively. *Methanothermobacter* has optimal growth between 55℃ to 75℃ [15].

As methane-forming bacteria are a group of highly temperature sensitive bacteria, fluctuations in digester temperature should be as small as possible. It was even more sensitive in the thermophilic digester (<1℃) than the mesophilic digester (<2-3℃), thus more difficult to maintain the optimum condition for methanogen growth in the thermophilic digester. In addition to that, there are several microbiological characteristics associated with thermophilic anaerobes that may adversely affect the digester performance. First, low bacterial growth or yield of anaerobes is evidenced in the thermophilic digester. Second, the endogenous death rate of these bacteria in thermophilic digestion system is significantly high. Last, there is a lack of diversity of anaerobes in the thermophilic digester [15].

To sum up, the high amount of *Methanosarcina* observed in the 45℃ AD showed that there was no strict inhibition of *Methanosarcina* growth within intermediate temperature zone. Other than inhibition effect, *Methanosarcina* seems to flourish within a wide temperature range of 35℃ to55℃. Also, relatively less difference was found in the dominant methanogen group comparison between 35℃, 45℃ and 55℃ AD. This was possibly due in part to the start-up method of both 45℃ and 55℃ Control AD, which was conducted with seed sludge from 35℃ AD.

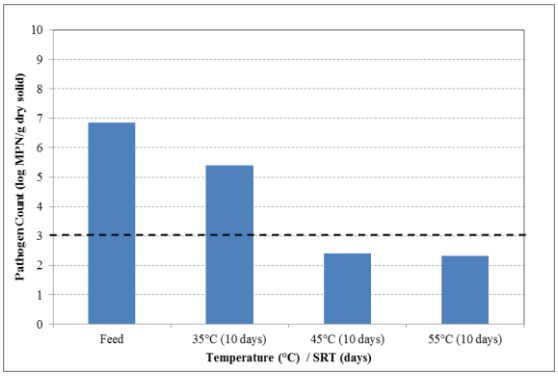

Fig. 2. Pathogen count of 45℃ AD, 35℃ control AD and 55℃ control AD systems.

IV. Conclusions

45℃ anaerobic digestion systems were operated for more than 2 years with significant solids destruction, comparable methane production, no ammonia-stress condition as well as stable pH distribution. However, the systems suffered from high VFA accumulation though the high VFA content was not detrimental, as evidenced by its adequate buffering capacity and abundant production of methane. In addition to that, the systems had successfully achieved the regulated pathogen destruction of less than 1,000 MPN/g dry solids, thus producing Class A biosolids. The two dominant genus of

methanogens that flourished in 45℃ anaerobic digestion system were *Methanosarcina* and *Methanobacterium* with *Methanosarcina* being the primary methanogen.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of the staff of Blue Plains Advanced Wastewater Treatment Plant in Washington, DC, for providing the sludge for the digesters.

REFERENCES

[1] D. M. D. Gabb, J. M. Hake, and S. Ghosh, "Influence of staging, mean cell residence time and thermophilic temperature on the thermophilic anaerobic digestion process," *Water Environment Research*, vol. 78, no. 5, pp. 497-509, 2006.
[2] J. C. Kabouris, U. Tezel, S. G. Pavlostathis, M. Engelmann, J. A. Dulaney, A. C. Todd, and R. A. Gillette, "Mesophilic and thermophilic anaerobic digestion of municipal sludge and fat, oil and grease," *Water Environment Research*, vol. 81, no. 5, pp. 476-485, 2009.
[3] M. Kim, Y. H. Ahn, and R. E. Speece, "Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic," *Water Research*, vol. 36, no. 17, pp. 4369-4385, 2002.
[4] R. A. Labatut, L. T. Angenent, and N. R. Scott, "Conventional mesophilic vs. thermophilic anaerobic digestion: A trade-off between performance and stability?" *Water Research*, vol. 53, pp. 249-258, 2014.
[5] K. Maneerat, N. Boonyarit, T. Somkiet, and P. Chantaraporn, "Comparative mesophilic and thermophilic anaerobic digestion of palm oil mill effluent using upflow anaerobic sludge blanket," *Water Environment Research*, vol. 84, no. 7, pp. 577-587, 2012.
[6] J. Zabranska, J. Stepova, R. Wachtl, P. Jenicek, and M. Dohanyos, "The activity of anaerobic biomass in thermophilic and mesophilic digesters at different loading rates," *Water Science & Technology*, vol. 42, no. 9, pp. 49-56, 2000.
[7] F. Abouelenien, Y. Kitamura, N. Nishio, and Y. Nakashimada, "Dry anaerobic ammonia-methane production from chicken manure," *Applied Microbiology and Biotechnology*, vol. 82, no. 4, pp. 757-764, 2009.
[8] W. J. Gao, K. T. Leung, W. S. Qin, and B. Q. Liao, "Effects of temperature and temperature shock on the performance and microbial community structure of a submerged anaerobic membrane bioreactor," *Bioresource Technology*, vol. 102, no. 19, pp. 8733-8740, 2011.
[9] J. K. Kim, B. R. Oh, Y. N. Chun, and S. W. Kim, "Effects of temperature and hydraulic retention time on anaerobic digestion of food waste," *Journal of Bioscience and Bioengineering*, vol. 102, no. 4, pp. 328-332, 2006.
[10] M. Peces, S. Astals, and J. Mata-Alvarez, "Response of a sewage sludge mesophilic anaerobic digester to short and long-term termophilic temperature fluctuations," *Chemical Engineering Journal*, vol. 233, pp. 109-116, 2013.
[11] J. S. Zhang, K. W. Sun, M. C. Wu, and L. Zhang, "Influence of temperature on performance of anaerobic digestion of municipal solid waste," *Journal of Environmental Sciences*, vol. 18, no. 4, pp. 810-815, 2006.
[12] A Plain English Guide to the EPA Part 503 Biosolids Rule, EPA/832/R-93/003, U.S. Environmental Protection Agency, Washington, DC, 1994.
[13] *Standard Methods for the Examination of Water and Wastewater*, 21st ed., American Water Works Association, Washington, DC, 2005.
[14] EPA Method 1680: Fecal coliforms in sewage sludge (biosolids) by multiple-tube fermentation using Lauryl Tryptose Broth (LTB) and EC medium, U.S. Environmental Protection Agency, Washington, DC, 2006.
[15] M. H. Gerardi, *The Microbiology of Anaerobic Digesters*, New Jersey: John Wiley, 2003.
[16] P. L. McCarty, "Anaerobic waste treatment fundamentals – Part three – Toxic materials and their control," *Public Works*, vol. 95, no. 11, pp. 91-94, 1964b.
[17] C. Gallert and J. Winter, "Mesophilic and thermophilic anaerobic digestion of source-sorted organic waste: Effect of ammonia on glucose degradation and methane production," *Applied Microbiology and Biotechnology*, vol. 48, no. 3, pp. 405-410, 1997.
[18] R. Braun, P. Huber, and J. Meyrath, "Ammonia toxicity in liquid piggery manure digestion," *Biotechnology Letter*, vol. 3, no. 4, pp. 159-164, 1981.
[19] Y. Han and R. R. Dague, "Laboratory studies on the temperature-phased anaerobic digestion of domestic primary sludge," *Water Environment Research*, vol. 69, no. 6, pp. 1139-1143, 1997.
[20] R. E. Speece, *Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industries*, Nashville, TN: Archae Press, 2008.
[21] P. L. McCarty, "Anaerobic waste treatment fundamentals – Part two – Environmental requirements and control," *Public Works*, vol. 95, no. 9, pp. 123-126, 1964a.
[22] P. L. McCarty, "Anaerobic waste treatment fundamentals – Part four – Process design," *Public Works*, vol. 95, pp. 95-99, 1964c.
[23] P. L. McCarty and R. E. McKinney, "Volatile acid toxicity in anaerobic digestion," *Journal of Water Pollution Control Federation*, vol. 33, no. 3, pp. 223-232, 1961.
[24] Q. Zhao and G. Kugel, "Thermophilic/Mesophilic digestion of sewage sludge and organic wastes," *Journal of Environmental Science and Health*, vol. 31, no. 9, pp. 2211-2231, 1996.
[25] I. Angelidaki, L. Ellegaard, and B. K. Ahring, "A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: Focusing on ammonia inhibition," *Biotechnology and Bioengineering*, vol. 42, pp. 159-166, 1993.
[26] D. Bolzonella, F. Fatone, P. Pavan, and F. Cecchi, "Anaerobic fermentation of organic municipal solid wastes for the production of soluble organic compounds," *Industrial and Engineering Chemistry Research*, vol. 44, pp. 3412-3418, 2005.

Nuruol S. Mohd graduated with the bachelor of engineering (environmental) from University of Malaya, Malaysia, the master of science in environmental engineering from Drexel University, USA and the Ph.D. of the same field from George Washington University. USA. She is now working at University of Malaya, Malaysia as a senior lecturer. Her area of expertise is wastewater treatment specifically in biological processes.