Sains Malaysiana 50(7)(2021): 1827-1841
http://doi.org/10.17576/jsm-2021-5007-01

Temperature Phased Anaerobic Digestion at the Intermediate Zone of 45 °C: Performances, Stability and Pathogen Deactivation

(Pencernaan Anaerobik Fasa Suhu di Zon Pertengahan 45 °C: Prestasi, Kestabilan dan Pendeaktifan Patogen)

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ABSTRACT

Temperature phased anaerobic digestion (TPAD) systems with conventional sequences (first stage of 55 °C and second stage of 35 °C) have been widely studied. However, very limited studies were available on TPAD system with the first stage operated at the intermediate zone of 45 °C, mainly due to the notion that limited microbial activity occurs within this zone. The objective of this research was to evaluate the performance, stability and the capability of 45 °C TPAD in producing class A biosolids, in comparison to a conventional TPAD. Four combinations of TPAD systems were studied, 45 °C TPAD 2.5/10 (1st stage solids retention time (SRT) 2.5 days/2nd stage SRT 10 days), 45 °C TPAD 7.5/10, 55 °C TPAD 2.5/10 and 55 °C TPAD 7.5/10. Among all, 45 °C TPAD 7.5/10 was found to have the best performances, attributed to its high volatile solids (VS) destruction (58%), minimal acetate accumulation (127 mg/L), high methane yield (0.58 m^3 CH_4/kg VS removed), high COD destruction solid COD (sCOD; 74% and total COD (tCOD) 54%) and minimal free NH_3 content (67.5 mg/L). As for stability, stable pH distribution, high alkalinity content and low VFA to alkalinity ratio, indicated a well-buffered system. Additionally, the system had also able to produce class A biosolids. Therefore, proved that TPAD system operated at the intermediate zone of 45 °C can perform better than the conventional TPAD, hence, highlighting its economic advantage.

Keywords: 45 °C TPAD; 45 °C anaerobic digestion; class A biosolids; TPAD

ABSTRAK

Sistem pencernaan anaerobik fasa suhu (TPAD) dengan urutan konvensional (peringkat pertama 55 °C dan tahap kedua 35 °C) telah dikaji secara meluas. Walau bagaimanapun, terdapat kajian yang sangat terhad pada sistem TPAD dengan tahap pertama yang beroperasi di zon pertengahan 45 °C, disebabkan oleh anggapan bahawa aktiviti mikroorganisma adalah terhad di dalam zon ini. Objektif kajian ini adalah untuk menilai prestasi, kestabilan dan keupayaan TPAD 45 °C dalam menghasilkan biopepejal kelas A, berbanding dengan TPAD konvensional. Empat gabungan sistem TPAD dikaji, 45 °C TPAD 2.5/10 (tahap-1 SRT 2.5 hari/ tahap-2 SRT 10 hari), 45 °C TPAD 7.5/10, 55°C TPAD 2.5/10 dan 55°C TPAD 7.5/10. Antara semua sistem, 45 °C TPAD 7.5/10 didapati mempunyai prestasi terbaik, disebabkan oleh penghapusan VS yang tinggi (58%), pengumpulan asetat minimum (127 mg/L), hasil metana yang tinggi (0.58 m^3 CH_4/kg VS dikeluarkan), penghapusan COD yang tinggi (sCOD; 74% dan tCOD 54%) dan kandungan NH_3 yang minimum (67.5 mg/L). Bagi aspek kestabilan, pengedaran pH yang stabil, kandungan alkali yang tinggi dan nisbah VFA kepada kealkalian yang rendah, telah menunjukkan sistem penampanan yang baik. Di samping itu, sistem ini juga mampu menghasilkan biopepejal kelas A. Oleh itu, membuktikan bahawa sistem TPAD yang beroperasi di zon pertengahan 45 °C menunjukkan prestasi lebih baik daripada TPAD konvensional, dengan itu, menunjukkan kelebihan daripada segi ekonomi.

Kata kunci: 45 °C TPAD; 45 °C pencerna anaerobik; biopepejal kelas A; TPAD

INTRODUCTION

A broad array of anaerobic digestion systems has been studied extensively for the treatment of municipal wastewater. Majority of these systems operated at mesophilic temperatures of 30 to 40 °C. Though effective in reducing the organic content of wastes, the mesophilic systems can achieve only limited destruction of pathogens, hence restricting the final use of the biosolids generated after the digestion process (López et al. 2020; Mohd et al. 2015; Sassi et al. 2018). Due to this major drawback, sludge treatment facilities have shown widespread interest in a more efficient thermophilic anaerobic digestion. Thermophilic system has proved to be able to produce class A biosolids, but on the other hand, the technology brings together major disadvantages such as poor process stability, poor effluent quality as well as highly sensitive operation factors (Böske et al. 2015; De Vrieze et al. 2016; Huang et al. 2020).

Due to these disadvantages, both mesophilic and thermophilic anaerobic digestion systems are combined as one system to offer the advantages of both while eliminating the problems associated with these systems when operated independently (Leite et al. 2016; Srisowmeya et al. 2019). This two-stage combined anaerobic digestion system is known as temperature phased anaerobic digestion (TPAD). TPAD is a two-stage anaerobic digestion system, which consists of two digesters in series, operated at higher thermophilic temperature (typically 55 °C) in the first stage and lower mesophilic temperature (typically 35 °C) in the second stage. In the first thermophilic stage, the rate-limiting hydrolysis step of wastewater is accelerated by elevated temperatures, while in the second mesophilic stage, the syntrophic acetogens and methanogens are provided with permissive conditions where inhibitions are decreased (Aboudi et al. 2017; Hameed et al. 2019; Yuan et al. 2019).

Through TPAD system, the thermophilic step is able to produce class A biosolids through high temperature pathogen deactivation and at the same time enhance digester capacity through higher rate thermophilic kinetics (Akgul et al. 2017; Hagos et al. 2017). More rapid solids hydrolysis at higher temperature, coupled with staged reaction kinetics is believed to improve VS removal efficiencies in TPAD system (Fernández-Rodríguez et al. 2016; Qin et al. 2017). Additionally, TPAD system is also capable in handling increased solids loading and has a better capability to absorb shock loadings in comparison to a single-stage mesophilic or thermophilic anaerobic digestion system (Neczaj & Grosser 2019). While on the other hand, the mesophilic step is used as a polishing stage in order to produce better effluent quality as well as to increase process stability (Alonso et al. 2016).

There are quite a number of research studies on optimum temperature of TPAD system. A study by Qin et al. (2017) performed experimental investigation to compare modified TPAD process (hyperthermophilic-mesophilic; 70-35 °C) with conventional thermophilic-mesophilic type (55-35 °C). It was reported that in hyperthermophilic condition, the systems exhibited superior VS removal compared to conventional single stage mesophilic anaerobic digestion as well as conventional thermophilic-mesophilic AD process. However, AD system with high temperature is financially unfeasible for full-scale application. Among many TPAD studies, very few incorporate anaerobic digestion at 45 °C as a first step of the TPAD system. This is due to the assumption that limited activity of microorganisms occurs within the intermediate zone of 40 to 50 °C. It was believed 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 (Kahar et al. 2018; Li et al. 2015). Despite of that, a study by Shi et al. (2019) has shown slight similarity in the selection of temperature for their system. The system consisted of a series of digester operated at elevated temperature of 45 °C as a first selection, 38 °C as a second selection while mesophilic reactor was run at 36 °C throughout the study. It was found that at elevated temperature, higher abundance of functional microbes and genes were observed, which consequently lead to higher hydrolysis and acidogenesis, therefore, resulted in better biogas production rate and VS removal. While the study presented the analysis of thermophilic and methanogen that were dominant in their TPAD's first stage and second stage reactors, the study did not provide a side by side comparison with the other TPAD that operated at different temperature or SRT.

Considering these observations, it is apparent that by replacing the conventional 55 °C thermophilic with 45 °C system in TPAD, a comparable effluent quality is possibly achievable. Therefore, this research is initiated with the aim to evaluate the effectiveness of TPAD system with 45 °C digester system as a first stage thermophilic, in combination with a conventional mesophilic as a second stage system, with a hope that this strategy can be considered as one of the most economical modification of the conventional anaerobic digestion system.

MATERIALS AND METHODS

ANAEROBIC DIGESTION SYSTEMS

In this study, four TPAD batch experiments were carried out. Two of the TPAD systems were the conventional combinations of thermophilic (55 ℃) digester followed by mesophilic (35 ℃) digester, labelled as 55 ℃ TPAD 2.5/10 and 55 ℃ TPAD 7.5/10. The SRTs for the first stage of the systems were 2.5 and 7.5 days, respectively. The other two TPAD systems used 45 ℃ digester as a first stage followed by mesophilic (35 ℃) digester as a second stage. The systems were labelled as 45 ℃ TPAD 2.5/10 and 45 ℃ TPAD 7.5/10. The SRTs for the first stage of the systems were 2.5 and 7.5 days, respectively. The SRTs for the second stage remained similar at 10 days for all systems. Additionally, three single-stage digesters, which were mesophilic (35 °C) digester, 45 °C digester and thermophilic (55 °C) digester, with SRTs of 10 days, were used as a control. They were labelled as 35 °C Control AD, 45 °C Control AD and 55 °C Control AD. The characteristics of all systems were presented in Table 1.

TABLE 1. The characteristics of TPAD and single-stage control systems

	45 °C TPAD 2.5/10 (Total SRT = 12.5 d)	45 °C TPAD 7.5/10 (Total SRT = 17.5 d)	55 °C TPAD 2.5/10 (Total SRT = 12.5 d)	55 °C TPAD 7.5/10 (Total SRT = 17.5 d)	35 °C Mesophilic Control AD (Total SRT = 10 d)	45 °C Control AD (Total SRT = 10 d)	55 °C Thermo-philic Control AD (Total SRT = 10 d)
<u>Stage 1</u>							
Temperature (°C)	45	45	55	55	35	45	55
Volume (L)	15	15	15	15	13	15	15
Flow (L/day)	6.0	2.0	6.0	2.0	1.3	1.5	1.5
SRT (days)	2.5	7.5	2.5	7.5	10	10	10
Total Solids (%)	6.5 ± 0.2	6.5 ± 0.2	6.5 ± 0.2	6.5 ± 0.2	5.0 ± 0.4	6.5 ± 0.2	6.5 ± 0.3
Volatile Solids (%)	5.2 ± 0.3	5.2 ± 0.3	5.2 ± 0.3	5.2 ± 0.3	4.1 ± 0.4	5.2 ± 0.3	5.2 ± 0.4
OLR (kg VS/m^3/day)	20.88 ± 0.72	6.96 ± 0.45	20.88 ± 0.72	6.96 ± 0.45	4.07 ± 0.36	5.22 ± 0.58	5.22 ± 0.65
<u>Stage 2</u>							
Temperature (°C)	35	35	35	35	NA	NA	NA
Volume (L)	12	12	12	12	NA	NA	NA
Flow (L/day)	1.2	1.2	1.2	1.2	NA	NA	NA
SRT (days)	10	10	10	10	NA	NA	NA
Total Solids (%)	4.4 ± 0.4	4.14 + 0.3	5.03 ± 0.6	4.91 + 0.5	NA	NA	NA
Volatile Solids (%)	2.9 ± 0.5	2.6 ± 0.4	3.5 ± 0.7	3.4 ± 0.5	NA	NA	NA
OLR (kg VS/m^3/day)	2.93 ± 0.54	2.64 ± 0.38	3.47 ± 0.71	3.36 ± 0. 47	NA	NA	NA

EXPERIMENTAL APPARATUS

The digesters were made of high-density polyethylene (HDPE) 25 L brewery tanks of Hobby Beverage Equipment Company (Temecula, CA, USA). 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 aluminium foil and temperature adjustable heating tape was placed on top of the foil. The aluminium 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. Gas mixing was applied to each digester by circulating the headspace gas to the bottom of digesters using a peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA). 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 (Nashville, TN, USA) to measure the volume of gas production. Figure 1 shows a schematic diagram of the anaerobic digestion system.

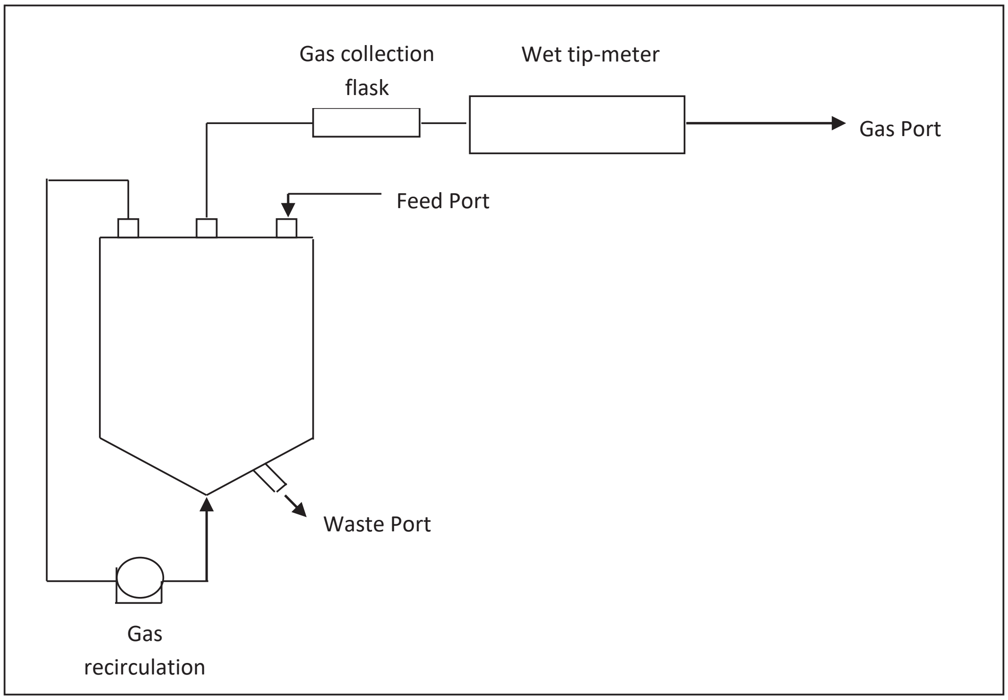

FIGURE 1. A schematic diagram of the anaerobic digestion system

SEED AND ANAEROBIC INOCULA

The seed sludge used for the inoculation of mesophilic digester was collected from the well-operated mesophilic anaerobic digester at Alexandria Wastewater Treatment Plant (operated by city of Alexandria, VA, USA). While, the anaerobic culture used as a thermophilic inoculum was obtained through a gradual temperature change from mesophilic condition of 35 to 45 °C and 55 °C.

The raw feed sludge for the system was collected from Blue Plains Advanced Wastewater Treatment Plant (operated by DC Water and Sewer Authority (DC Water), DC, USA). The raw sludge was the effluent after the thickening process and was collected once every two weeks and stored at 4 °C prior to its use as feed. The average total solids (TS) content of the raw sludge was approximately 13%. Prior to daily feeding routine, the raw sludge was diluted with tap water to make a feed of the desired concentration. The feed sludge was then acclimated to 35, 45 or 55 °C by incubating them at the desired temperature for approximately 2 h.

OPERATION

The anaerobic digestion systems were initiated with three single-stage mesophilic (35 °C) digesters operated in parallel to each other. The empty digesters were first inoculated with seed sludge collected from the mesophilic anaerobic digester at Alexandria Wastewater Treatment Plant. 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. During this initial phase, no effluents are taken out as the microorganisms need to have ample time to establish themselves within the new environment. 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. The effluent of the first stage digester was used as feed for the second stage digester. After three cycles of SRT, the analytical tests were initiated for the digester effluent as well as for the influent.

The first single-stage mesophilic (35 °C) digester was operated continuously at 35 °C for the whole duration of the study, which was 48 months. The second single-stage mesophilic (35 °C) digester was initially operated at 35 °C for 6 months before the temperature was gradually increased to 45 °C and remained at this temperature for the rest of the study duration. Similarly, the third mesophilic (35 °C) digester was operated at 35 °C for 6 months, then at 45 °C for 8 months, before gradually increased to 55 °C for the rest of the study duration. All digesters were operated as a single stage for at least 12 months before the TPAD systems were started. At this point, it was believed that the systems had already achieved their stability and maturity stage. Additionally, to ensure that steady-state conditions had been re-established following a change of SRT, a period of operation equal to three SRTs was allowed to elapse before new experimental analyses were begun. The steady-state condition was assumed to be achieved once constant effluent characteristic values in terms of total solids (TS), volatile solids (VS), gas production and composition, and volatile fatty acids (VFA) concentrations, were ensured. Each 45 °C TPAD system was allowed to run for at least 8 months before starting the new TPAD sequence. As for thermophilic (55 °C) TPAD, the system was allowed to run for at least 11 months, longer than 45 °C TPAD system, because of its tendency towards instability.

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-NH3 B & C, respectively (APHA 2005). 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 °C, and the injector and flame ionization detector temperature was 250 °C. 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 (EPA, 2003).

RESULTS AND DISCUSSION

VOLATILE SOLIDS (VS) REDUCTION

Figure 2 shows the VS reduction achieved by the TPAD systems and control digesters. It can be seen that all systems except 55 °C Thermophilic Control AD, with VS reduction of only 30%, were able to exceed the required average VS reduction efficiency of 38% for Class A biosolids criterion for vector attraction reduction (US EPA 2003). In fact, TPAD system with the least efficiency and highest loading of 20.88 kg VS/m^3/day (Stage 1) and 3.47 kg VS/m^3/day (Stage 2), 55 °C TPAD 2.5/10, was still able to achieve comparable VS reduction as 35 °C Mesophilic Control AD and 45 °C Control AD. Among all, 45 °C TPAD 7.5/10 achieved the highest VS reduction at 58%, 10% more than single-stage 45 °C Control AD. Furthermore, if 45 °C TPAD 7.5/10 and 45 °C TPAD 2.5/10 were to be compared with another TPAD systems with similar OLR that signifies similar SRT, that is 55 °C TPAD 7.5/10 and 55 °C TPAD 2.5/10, the total VS production of 45 °C system remained considerably higher. Conclusively, although the SRT of 45 °C TPAD's first stage digesters diminished from 7.5 days to only 2.5 days, and the first stage's VS reductions decreased from 48 to 40%, the overall reductions were still exceeding the recommended 38%.

A greater solids reduction in all systems except single-stage thermophilic digester implied an increase in sludge reduction as well as sufficient sludge stabilization. While in thermophilic digester, less solids removal indicated an inhibited activity which caused incomplete solids stabilization within the system. In TPAD systems, comparison of the solids reduction between the first and second stages demonstrated that, most of the solids biodegradation activity occurred during the first stage of the system, or approximately 27-48% of total VS reductions. Therefore, the system performance was heavily dependent on the performance of the elevated temperature reactor, while the mesophilic reactor improved the effluent quality by consistently achieving additional 7-19% VS reductions.

VOLATILE FATTY ACIDS (VFA) DESTRUCTION

The concentrations of individual and total VFAs in TPAD and control digesters were shown in Figure 3. A moderate accumulation of VFA, particularly acetate, was observed in 45 ℃ TPAD 7.5/10 with a concentration of 525 mg/L. While high VFA concentration in the first stage of 45 ℃ TPAD 2.5/10, at 1549 mg/L, was associated to its short SRT that had washed out the required methanogens needed for the degradation of VFAs (Han & Dague 1997). Through the subsequent mesophilic stage, the acetate levels in 45 ℃ TPAD 7.5/10 was successfully reduced to a low level of 127 mg/L, comparable to the amount of acetate found in the individual mesophilic system. While in 45 ℃ TPAD 2.5/10, though leaving more acetate compared to the other system, was still able to reduce the concentration to a moderate level of 583 mg/L. In comparison to a single-stage 45 ℃ Control AD (10d), the final concentrations of all types of VFAs were much lesser. As for the comparison of two different systems of similar OLR and SRT with different temperature (45 ℃ TPAD 7.5/10 vs 55 ℃ TPAD 7.5/10; 45 ℃ TPAD 2.5/10 vs 55 ℃ TPAD 2.5/10), the accumulation of acetate and other VFAs was very significant at higher temperature. These observations were very typical for thermophilic anaerobic digesters (Fernández-Rodríguez et al. 2016; Mohd et al. 2015). However, the final VFA concentrations of the TPAD systems were considerably lower than the single-stage 55 ℃ Thermophilic Control AD (10d) indicated that the mesophilic stage of the TPAD system were able to degrade the massive amount of VFA generated during the thermophilic stage. The mesophilic stage reduced VFA concentrations in the thermophilic effluent by 10 to 32%.

From the findings, it was very apparent that, VFA concentrations, especially acetate, were at the highest in 55 °C system, followed by 45 °C system and 35 °C system. The large reduction of acetate concentration in 35 °C system 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 °C is an optimum temperature for the acetate-utilizing methanogens (Westerholm et al. 2010). Furthermore, at this temperature, the digestion of proteinaceous materials did not create a large amount of free ammonia that can be inhibitory to the methanogens, therefore explaining the high reduction of acetate within the stress-free system (Aboudi et al. 2017).

On the other hand, 45 °C system produced high acetate accumulation. The final concentrations of acetate were significantly higher compared to those in 35 °C system and in some cases, they were even higher than the feed itself, even though methane was continuously produced throughout the process. 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. Third, at 45 °C, 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 inoculated. 45 and 55 °C digesters were started 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. Thus, the operating temperature of 45 °C 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 °C system. The higher the temperature, the higher the rate of the proteinaceous materials breakdown (De Prá et al. 2019). In addition to that, as 55 °C 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. These observations had further supported the fact that microorganisms responsible for the degradation of acetate, namely methanogens, are the most sensitive to the environmental changes and the one that is most likely responsible for unstable digesters (Wang et al. 2019; Westerholm et al. 2012).

Aside from that, it was also observed that VFA concentrations in the effluent of the first stage digesters increased when the SRT decreased, regardless the temperature (Figure 3). Thus, indicated that, other than temperature, SRT was also another important factor contributed to the accumulation of the VFA.

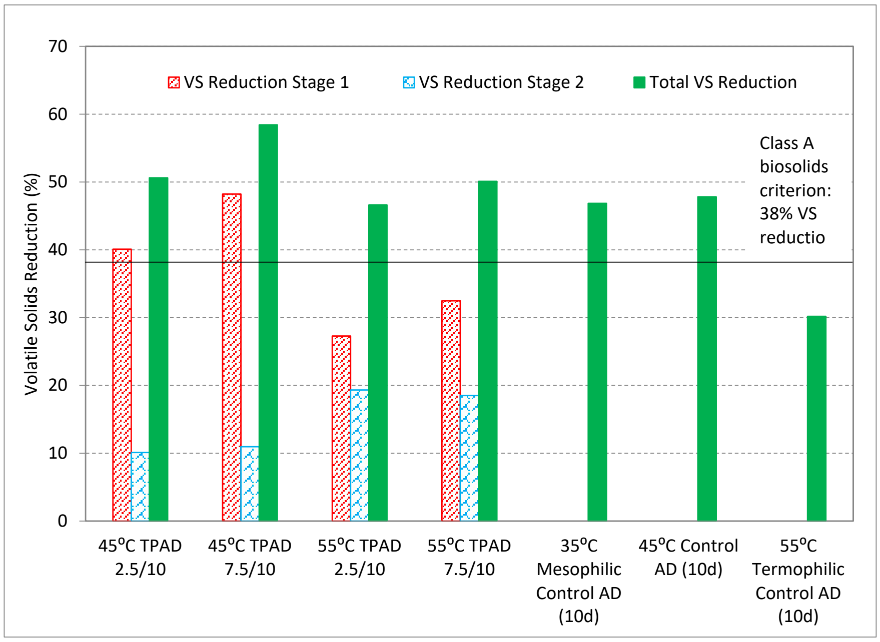

FIGURE 2. Volatile solids reductions (expressed as %) for TPAD and single-stage control systems

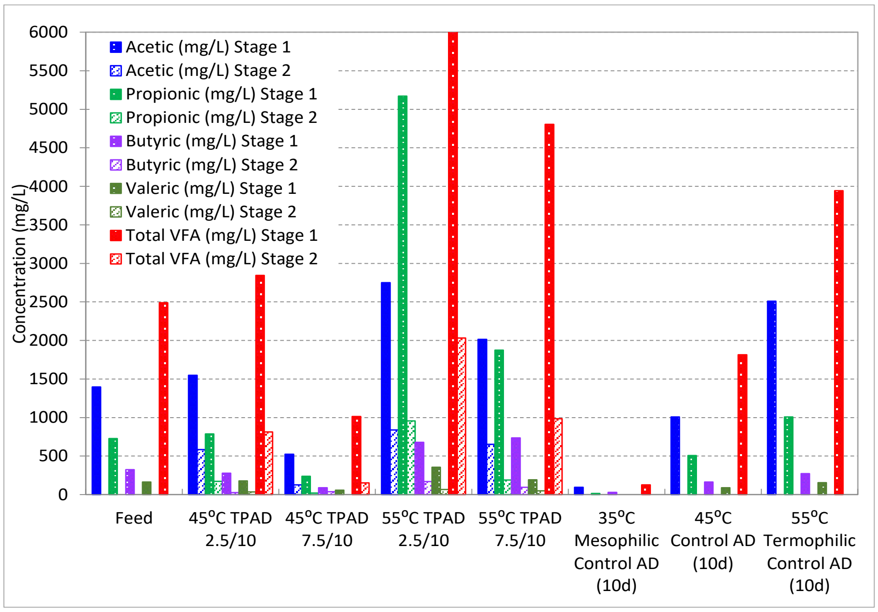

FIGURE 3. VFA concentrations (expressed as mg/L) for TPAD and single-stage control systems

pH, VFA-TO-ALKALINITY RATIO

Higher VFA in the first stage of TPAD system was also apparent, in that the pH was slightly lower in all first stage digesters compared to the second stage digesters. The pH was 7.32, 7.40, 7.28 and 7.36 for 45 ℃ TPAD 2.5/10, 45 ℃ TPAD 7.5/10, 55 ℃ TPAD 2.5/10 and 55 ℃ TPAD 7.5/10, respectively. During the mesophilic second stage, the pH increased to 7.40, 7.57, 7.39, and 7.51, respectively. Additionally, pH was significantly lower in the TPAD with the shorter SRT. In comparison to single stage systems, the pH was considered to be significantly similar. The distribution of pH in the TPAD systems was illustrated in Figure 4.

This finding was in agreement with the one observed by Rattanapan et al. (2019) and can be taken as indicative of a hydrolytic-acidogenic performance of the digesters. This observation was further confirmed by the fact that the alkalinity was also reduced as the pH gets lower (Tangkathitipong et al. 2017). McCarty suggested that an anaerobic digestion system requires an alkalinity of at least 1500 mg/L in the presence of biogas containing about 30% carbon dioxide (McCarty 1964). Within our TPAD 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 that increased alkalinity within the digester (Jang et al. 2014). In a digester, these salts produce natural buffers, which normally remain fairly constant at about 3,000 to 4,000 mg/L. Additionally, other than ammonia and salts, the increase in alkalinity was also attributed to the high concentration of VFA observed in all TPAD systems.

As VFA concentrations began to increase, they were neutralized by the bicarbonate alkalinity, thus, forming volatile acid alkalinity (McCarty 1964). Therefore, instead of only having bicarbonate alkalinity, which is normally observed in mesophilic systems, the 45 and 55 °C TPAD systems now had total alkalinity composed of bicarbonate alkalinity and volatile acid alkalinity.

In addition to that, the VFA-to-alkalinity ratio was higher in the first stage of all TPAD systems. Clearly, the higher ratio was primarily a result of the higher VFA concentration. The high ratio was apparent in all TPAD systems except 45 ℃ TPAD 7.5/10. All values were higher than 0.4, thus, clearly indicated that the digester does not contain a good equilibrium between acidogenic and methanogenic microbiota (Zhao & Kugel 1996). However, after the following mesophilic second stage, the high VFA-to-alkalinity ratio values had successfully been reduced 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.

Overall, pH values of all digesters were stable throughout the operation period and lie within the optimum pH range of 6.7-7.0 (Zhang et al. 2014). These findings demonstrated that, despite high VFA accumulation observed in the systems, it had not reduced the pH to the levels that would cause digester failure.

METHANE GAS PRODUCTION, SPECIFIC METHANE YIELD AND METHANE COMPOSITION

The performances of TPAD systems were compared in terms of methane production. Figure 5 illustrates daily methane production of all systems for a specific period of operation. The results showed that methane was produced at all temperatures, with 55 ℃ Thermophilic Control AD (10d) demonstrated the lowest methane production at 6.03 L/day. The limited amount of methane produced within the system 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 (Yenigün & Demirel 2013). When combined with mesophilic digester for the second stage, 45 °C TPAD systems generated more methane of approximately 17-26 L/day in comparison to a single stage 45 °C AD and single-stage mesophilic AD. While 55 °C TPAD systems generated 13-23 L/day more methane in comparison to a single stage 55 °C Thermophilic AD. Methane production from the first stage composed of 47-83% of total methane production and this observation was in concordance with higher VS destruction achieved during the first stage of the systems. Among all TPAD systems, 45 °C TPAD 2.5/10 generated the most methane at 47.24 L/day followed by 45 °C TPAD 7.5/10 at 38.65 L/day, 55 ℃ TPAD 2.5/10 at 29.47 L/day and 55 ℃ TPAD 7.5/10 at 19.35 L/day. With the exception of 55 ℃ TPAD 7.5/10, all other systems generated more methane than control AD. As for comparison between different temperatures, 45 °C TPAD systems produced considerably more methane than 55 °C TPAD systems. Furthermore, when the systems are compared based on its similar OLR that signifies similar SRT, it was apparent that systems with shorter SRT produced relatively more methane as the systems have higher organic loading rate (OLR).

However, in terms of specific methane yield, systems with higher OLR and shorter SRT, 45 °C TPAD 2.5/10 and 55 °C TPAD 2.5/10 produced significantly low yield at 0.166 and 0.190 m^3 CH_4/kg VS removed in contrast to

0.580 and 0.490 m^3 CH$_4$/kg VS removed for 45 °C TPAD 7.5/10 and 55 °C TPAD 7.5/10, respectively. Nonetheless, none of the TPAD system was able to achieve comparable methane yield as 35 °C Mesophilic Control AD. The significantly low methane yield amidst the amount of methane generated in the system was possibly associated to the washout of methanogens at very short SRT (Jang et al. 2014). Seneesrisakul et al. (2018) stated that a relatively short SRT was more favourable for hydrogen-utilizing methanogens rather than acetate-utilizing methanogens, therefore, explaining low methane yield at low SRT. However, in spite of lower yield in the first stage of the digesters, an increase in methane yield was observed in the following mesophilic stage. This probably due to the fact organic matter was hydrolysed into the VFA in the first stage and the VFA was then converted into methane during the second stage. Through this way, the TPAD process achieved higher values of methane yield and when it is at the optimum SRT, which was 7.5 days, the yield was higher than the yield in a single stage digester. The total specific methane yield for 45 °C TPAD 7.5/10 was 0.580 m^3 CH$_4$/kg VS removed in comparison to 0.564 m^3 CH$_4$/kg VS removed in 45 °C Control AD (10d). In 55 °C TPAD 7.5/10, the total specific methane yield was 0.490 m^3 CH$_4$/kg VS removed compared to 0.249 m^3 CH$_4$/kg VS removed in 55 °C Thermophilic Control AD (10d).

As for the biogas composition, there were apparent differences in the methane content especially for the thermophilic systems. The methane composition for the first stage of 55 °C TPAD 2.5/10 and 55 °C TPAD 7.5/10 were 57.38 and 56.87%, respectively. These values were similar to the composition of the single stage thermophilic digester at 58.97%. However, these values were less than the other TPAD and control systems. Methane composition of the other systems ranged between 63 and 69%, the levels that were considered as normal for a typical anaerobic digestion system (Riau et al. 2010).

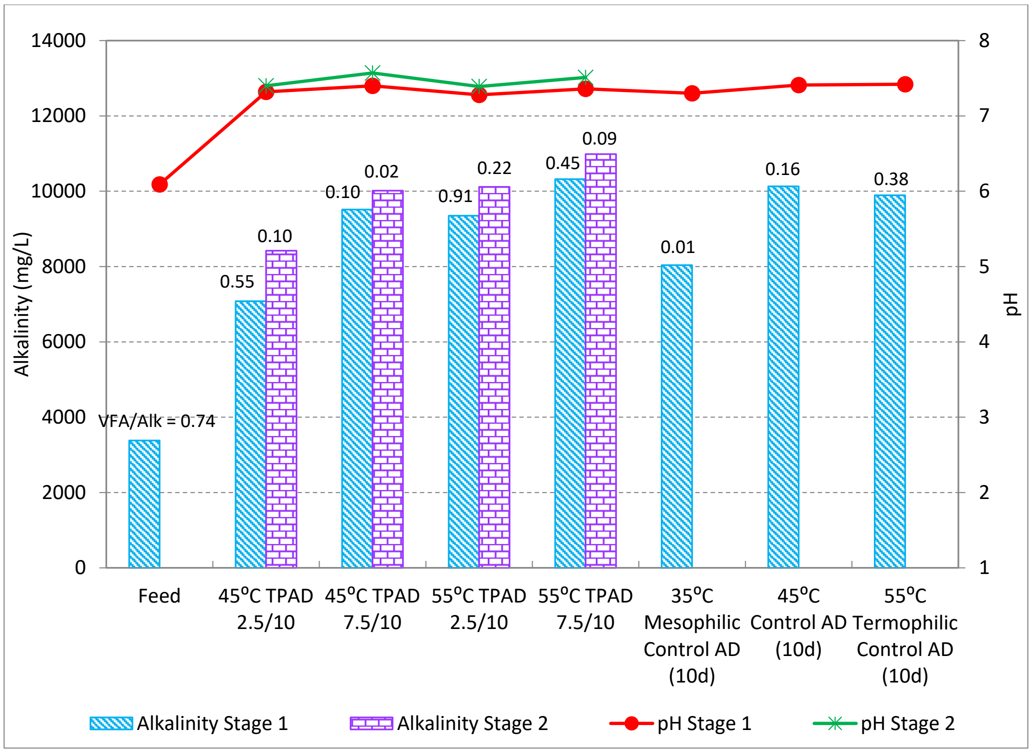

FIGURE 4. pH, Alkalinity (expressed as mg/L) and VFA to alkalinity ratio for TPAD and single-stage control systems

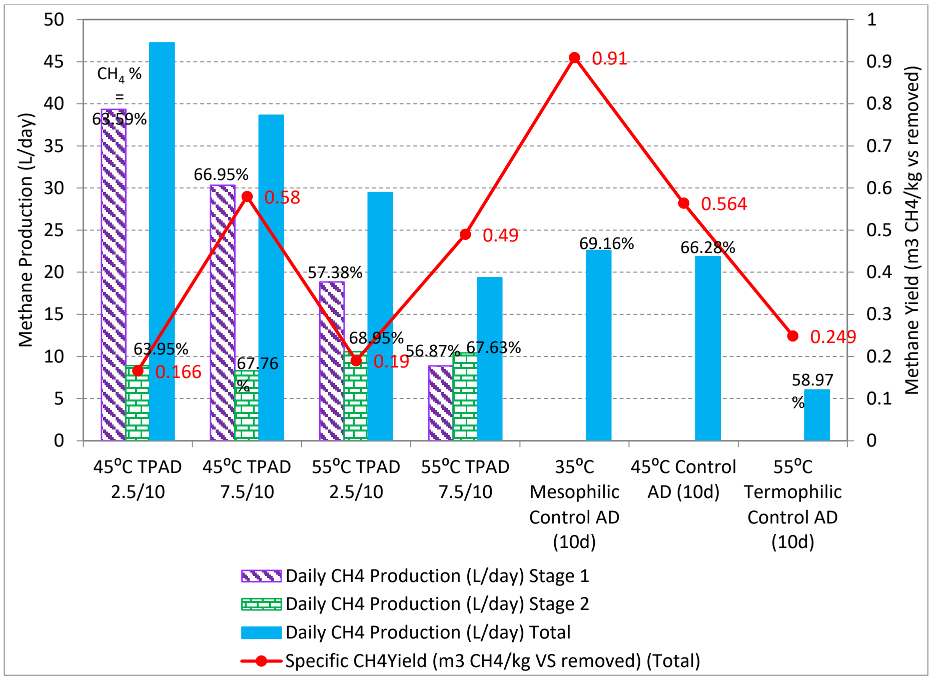

FIGURE 5. pDaily CH_4 production (expressed as L/day), specific CH_4 yield (expressed as m^3 CH_4/kg VS removed) and CH_4 content (expressed as %) for TPAD and single-stage control systems

TOTAL AMMONIACAL NITROGEN (TAN) AND FREE AMMONIA CONTENT

TAN and free NH_3 concentrations for each digester were presented in Figure 6. All mesophilic second stage digesters operated at higher TAN concentration than the first stage digesters because of the additional volatile solids or protein breakdown and consequent conversion of organic nitrogen to ammonia in mesophilic digesters (Bi et al. 2019). Due to the same reason, it was also observed that TPAD system generated more TAN compared to the single stage control systems. Among all, the best performance was observed in 45 °C TPAD 7.5/10 with final TAN content of 1923 mg/L. While both TPAD systems at the lowest SRT, 45 °C TPAD 2.5/10 and 55 °C TPAD 2.5/10 generated the highest TAN concentrations. This was most likely attributed to the high OLR sustained by the low SRT system. Additionally, it was also observed that, in all TPAD systems, the TAN concentrations were higher than 35 °C mesophilic system though remain less than the inhibitory level of 3000 mg/L. 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 thermophilic population contained less versatile protein degraders in comparison to the mesophilic population, hence, leaving more protein undegraded, thus explaining the higher TAN concentrations at higher temperature.

In contrast to TAN concentrations, final free ammonia concentrations were all lower than the concentrations in the first stage digesters and even less than those reported in single-stage systems (Figure 6). Though the final TAN concentrations were considerably higher, the dissociation constant was less at mesophilic temperature and the pH was small enough so as not to cause free ammonia to increase tremendously. In addition to that, all free ammonia levels were below the inhibitory envelope of 100-150 mg/L reported by Braun et al. (1981), thus, eliminating the possibility of the existence of ammonia-stress condition in any of the mesophilic digesters. Free ammonia concentration was at the lowest in 45 °C TPAD 7.5/10 at 67.5 mg/L (Braun et al. 1981). As for 55 °C thermophilic digesters, high TAN at high temperature, 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 (Shi et al. 2017). However, significant inhibition was not observed, as the digesters have been operated for more than 2 years with sufficient VS destruction and methane production, as well as stable pH distribution.

COD REDUCTION

Figure 7 illustrates the sCOD and tCOD destruction efficiency of TPAD system as well as single stage system at different SRT. Clearly, the highest removal efficiency was demonstrated by TPAD system with longer SRT and lower OLR, regardless of its temperature, 45 °C TPAD 7.5/10 and 55 °C TPAD 7.5/10. The total sCOD and tCOD reductions were 74 and 55% for 45 °C TPAD 7.5/10, and 75 and 57% reductions for 55 °C TPAD 7.5/10, respectively. Though the effluent quality in terms of sCOD did not show equivalent removal efficiency as what was produced by individual mesophilic system at approximately 81%, the tCOD removal efficiency was significantly similar at 56%. The other TPAD systems reduced lesser amount of sCOD and tCOD, but the reductions were still greater than the individual 45 and 55 °C thermophilic systems. Similar to what was observed in VFAs and solids removal, majority of the constituents were removed during the first stage of the system, with 50 to 70% of the total removal of both types of COD. In general, despite high COD concentration found in the first stage of all TPAD systems, the subsequent mesophilic stage had successfully reduced the concentration, thus producing a comparable quality of final effluent.

The superior capability of mesophilic digester in COD destruction in comparison to the digesters at the other two temperatures was in agreement with the observation reported by Wei et al. (2019). The finding 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 °C and the efficiencies decreased as the temperature increased (Wang et al. 2019). 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 °C digester had more affinity towards the substrate compared to the thermophilic colonies found in higher temperature digesters (Jung et al. 2019)'. 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 concentration with the increase of temperature was consistent with trends in VFA concentrations found in higher temperature digesters observed in other study as well (Wang et al. 2019). This was due to the fact that sCOD is usually composed of up to 90% of VFAs (Bolzonella et al. 2005). A similar increasing trend of tCOD concentrations over the increase of temperature was also observed in all systems. Overall, this finding implied that the degradation of complex organic solids was highly affected by temperature.

PATHOGEN DEACTIVATION

Another important factor to consider, if the sludge is to be used as a fertilizer or soil amendment, is the content of pathogenic bacteria. For pathogen destruction, all systems except 35 °C Mesophilic Control AD (pathogen count 2.55 × 10^5 MPN/g dry solids) showed near complete removal of fecal coliform, thus meeting the Class A biosolids requirement of less than 1000 MPN/g dry solids (Figure 8) (EPA 2003). In TPAD systems, most of the indicator organism removal was achieved in the first stage digesters. Regardless of the SRT and OLR, subsequent mesophilic digesters provided little additional pathogen destruction. The final pathogen counts were 431, 710, 420, and 353 MPN/g dry solids for 45 °C TPAD 2.5/10, 45 °C TPAD 7.5/10, 55 °C TPAD 2.5/10 and 55 °C TPAD 2.5/10, respectively. However, despite of sufficient destruction of fecal coliform, the recurrence of fecal coliform in second stage was observed in 45 °C TPAD 7.5/10. This was probably because of the incomplete destruction of fecal coliform during the thermophilic stage, and mesophilic temperature in the second stage had altered the culturable stage of the pathogens, making them regrow rather than killing them (Fu et al. 2014).

These results have shown that 45 and 55 °C digesters can be operated at a short SRT of 2.5 days with the same degree of coliform reduction achieved at the longer SRT, hence implying that high pathogen destruction was attributed to its operating temperature of 45 and 55 °C. However, although temperature was believed to be the primary factor responsible for the inactivation of pathogens, it has been reported that high VFA concentrations, low pH, high free ammonia content and long retention time may also cause substantial inactivation of the pathogen (Akgul et al. 2016; Avery et al. 2014).

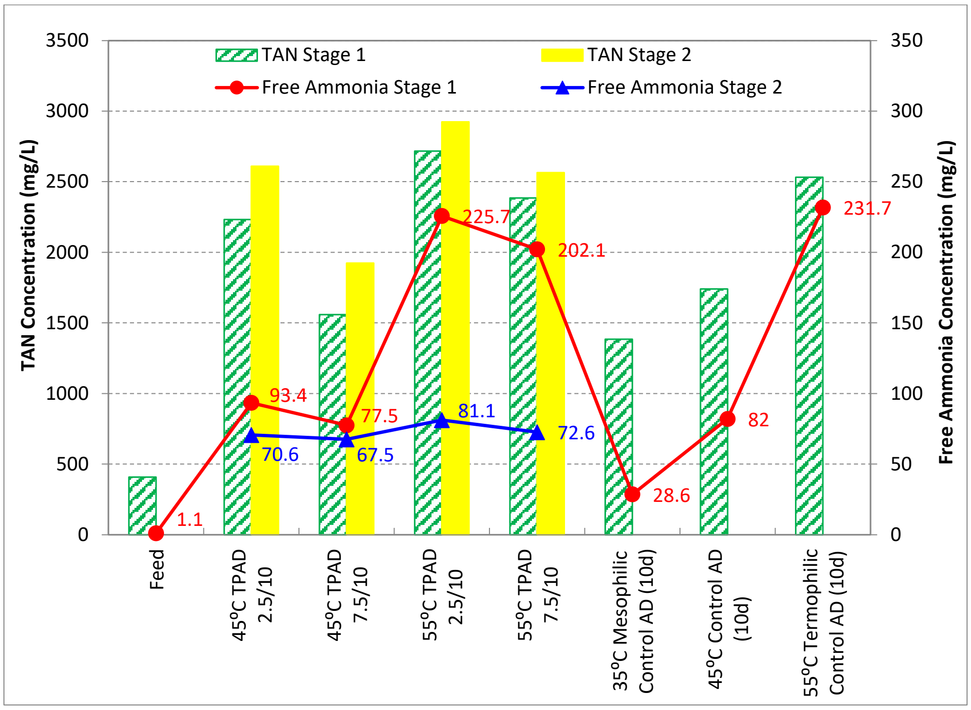

FIGURE 6. Total Ammoniacal Nitrogen (expressed as mg/L) and free ammonia concentration (expressed as mg/L) for TPAD and single-stage control systems

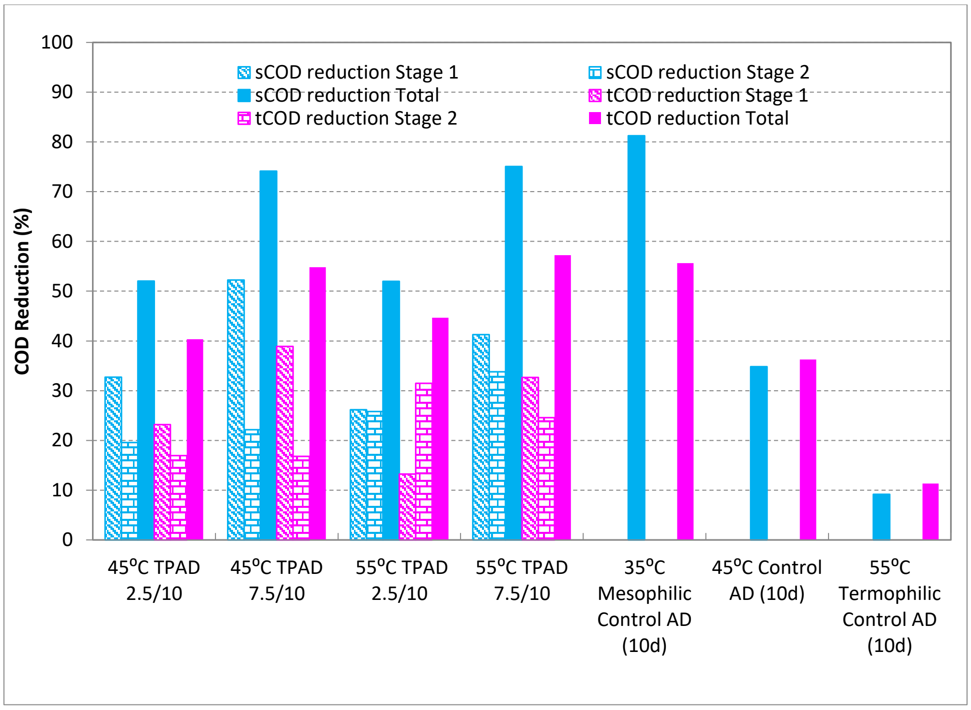

FIGURE 7. sCOD and tCOD reduction (expressed as %) for TPAD and single-stage control systems

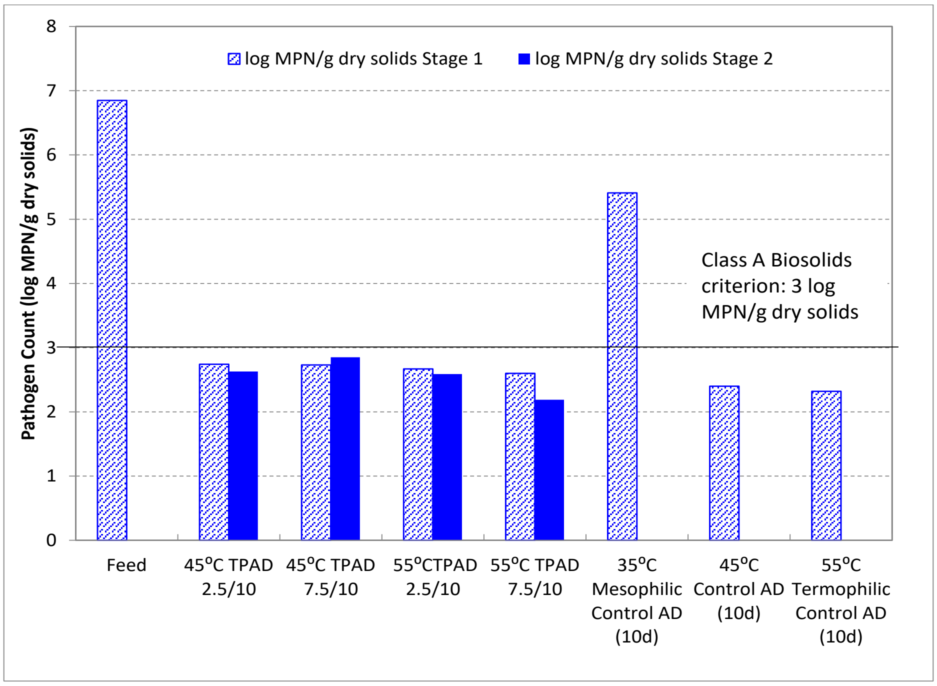

FIGURE 8. Pathogen count (log MPN/g dry solids) for TPAD and single-stage control systems

CONCLUSION

TPAD systems showed better performances and process stability in comparison to the single-stage systems at the similar elevated temperature. Apparently, its overall performances depended heavily on the performance of the thermophilic first stage digester, while effluent quality depended on the operation of the following mesophilic digester.

Among all systems, 45 °C TPAD 7.5/10 was found to be the best system. Its best performances were attributed to its high VS destruction (58%), minimal acetate accumulation (127 mg/L), high methane yield (0.58 m^3 CH_4/kg VS removed), high COD destruction (sCOD; 74% and tCOD 54%) and minimal free NH_3 content (67.5 mg/L). As for its stability, it was contributed by its stable pH distribution, high alkalinity content and low VFA to alkalinity ratio, therefore, indicated a well-buffered system. Additionally, the system had also able to produce class A biosolids.

Conclusively, it was very apparent that TPAD system operated at the intermediate zone of 45 °C can perform better than the conventional TPAD system operated at 55 °C, hence, highlighting its economic advantage.

ACKNOWLEDGEMENTS

This study was funded by a grant from USAID under the Pakistan-US Science and Technology Cooperation Program and University of Malaya Grant RF020A-2018.

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Received: 5 February 2020
Accepted: 7 November 2020