

A Precise Experimental Study on Key Dissimilarities between Mesophilic and Thermophilic Anaerobic Digestion of Waste Activated Sludge

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ABSTRACT: Key dissimilarities between thermophilic and mesophilic anaerobic digestion of waste activated sludge (WAS) were experimentally studied in this research. Typical WAS with total solids (TS) concentrations of 30 and 60 g/L were digested anaerobically in a batch digester at mesophilic and thermophilic temperatures. Solids reduction, total COD changes, the production of different volatile fatty acids (VFAs), pH variation, the quality and quantity of the produced biogas, an energy audit, pathogen inactivation and sludge dewaterability during anaerobic digestion were investigated and compared for mesophilic and thermophilic processes in this research. Only the thickest sludge (TS concentration 60 g/L) provided auto-thermality under mesophilic conditions. The mesophilic digestion took place after 32 to 36 days with slightly more methane production and removal of organics than thermophilic digestion. The results showed that there was no significant difference between thermophilic and mesophilic digestion with respect to the gas composition. Among the VFAs (key intermediates), only propionate accumulated during sludge anaerobic digestion at both the mesophilic and thermophilic temperatures. Thermophilic anaerobic digestion imparts improved dewaterability. The required time for sludge pathogen inactivation under mesophilic conditions was more than one month.

Key words: Solid reduction, Volatile fatty acids, Energy audit, Dewaterability, Pathogen inactivation

INTRODUCTION

WAS, is generated as a by-product in large and increasing quantities (for example, in Iran, 25000 tons/year is generated) (Abduli and Azimi, 2010; Firdaus and Ahmad, 2010). Different studies have considered the potentials of waste degradation and possible reuse (Mehrdadi *et al.*, 2007, Rasapoor *et al.*, 2009, Uemura, 2010, Nwabanne *et al.*, 2009). Anaerobic digestion is an appropriate technique for the treatment of WAS before final disposal and it is employed worldwide as the oldest and most important process for sludge stabilization (Dohányos and Zábranská, 2001, Rahmani *et al.*, 2009). However, WAS anaerobic digestion is difficult and with the technologies available nowadays, only approximately 20–30% of the sludge TS is mineralized (Rulkens, 2008).

In general, mesophilic anaerobic digestion of WAS is more widely used than thermophilic digestion, mainly because of the lower energy requirements and the higher stability of the process. However, the thermophilic

anaerobic digestion process is usually characterized by accelerated biochemical reactions, higher growth rates of microorganisms and accelerated interspecies hydrogen transfer, resulting in an increased methanogenesis potential at lower retention times (Zábranská *et al.*, 2000; Nwabanne *et al.*, 2009). Also, thermophilic anaerobic digestion of WAS can lead to the EPA's class A sludge, which is suitable for subsequent land application (Watanabe *et al.*, 1997). Studies undertaken by several researchers (Ahn and Forster, 2000; Kim *et al.*, 2002; Song *et al.*, 2004) showed that thermophilic systems were capable of treating higher organic loadings and had a higher specific growth rate as compared to their mesophilic counterparts. The yield of microorganisms per unit amount of substrate for thermophilic systems is also lower. The lower growth yield of thermophilic anaerobes could be due to their increased decay rate, which is double that of mesophilic cultures, because the cells have a tendency to lyse quickly under thermophilic conditions; it may also be due to their

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higher energy requirement for maintenance or the specific molecular properties of enzymatic reactions at thermophilic temperatures (Kim *et al.*, 2002; Amani *et al.*, 2010).

The main problems with thermophilic WAS digestion (as compared to mesophilic digestion) include energy requirements, instability, and a highly polluted supernatant, all of which prevent this technique from being widely used and commercialized (Zábranska *et al.*, 2000; Zupančič and Roš, 2003; Gavala *et al.*, 2003). The biogas produced can be used as a heat source for the digester and to generate power. To decrease the energy requirements of anaerobic thermophilic digestion, Zupančič and Roš (2003) evaluated the ability of the combined heat and power (CHP) unit performance to compensate for the excess heat requirements of a thermophilic digester. Their results showed that a CHP unit can produce excess heat that could be utilized elsewhere. The problem of process stability can be avoided by combining the thermophilic and mesophilic digestion processes into one; reaping the benefits of both while eliminating the problems associated with these systems when operated independently (Han and Dague, 1997).

Thermophilic anaerobic digestion was investigated by many researchers (Kim *et al.*, 2002; Gavala *et al.*, 2003; Song *et al.*, 2004; Mottet *et al.*, 2009; Rubio-Loza and Noyola, 2010), but all the aspects of the thermophilic anaerobic digestion of WAS and its dissimilarities with anaerobic mesophilic digestion still need to be studied precisely and comparatively. The main objective of this research was to investigate the performance of the anaerobic digestion of a typical WAS with respect to VSS reduction, total COD reduction, VFA concentrations, pathogens inactivation,

pH variations, gas composition in terms of methane content, gas production rate and dewaterability of the sludge in order to experimentally determine the main differences between mesophilic (35 °C) and thermophilic (55 °C) anaerobic digestion of WAS. Also, the energy audits for both of conditions were carried out.

MATERIALS & METHODS

WASTE ACTIVATED SLUDGE

Undigested WAS (secondary sludge) was collected from one of the clarifier bottoms from the Delhi Jal Board sewage treatment plant. Table 1 shows the characteristics of the initial WAS. The amount of volatile dissolved solids (VDS) shown in Table 1 indicates that only a small amount of non-cellular organics was present in the WAS. Also, the volatile suspended solids (VSS) were never observed to increase with further aeration. Therefore, the sludge could be considered a typical WAS. Thickened sludge samples, having TS concentrations of 30 and 60 g/L, were generated from the original sludge. For obtaining total suspended solids (TSS) up to 30 g/L, simple gravity thickening was applied. However, centrifugation was used to thicken the sludge further.

ANAEROBIC BATCH DIGESTER

Four bench scale batch anaerobic digesters (20 L), as illustrated in Fig.1. were set up for the studies and operated in parallel. An electrical heating tape (200 cm and 200 Ohms) was wound on the outside surface of each digester vessel and a thick layer of glass wool was installed to insulate them from the surrounding. This was augmented with a layer of thermo-col. Finally, a thin layer of aluminum foil was applied to the outside surface. The tape was energized using 230 V alternating

Table 1. Initial characteristics of undigested secondary sludge

Solids (± 0.1 g/L)	Indicator Organisms (CFU/L)		pH	SVI (mL/g)	SCST [s/(g/L)]	COD (mg/L)
TS	8.0	TC	4.8×10^8	6.7	44	12
TSS	7.6	FC	1.0×10^8			
TDS	0.4	FS	2.7×10^7			
TVS	6.1					
VDS	0.1					
VSS	6.0					

Note: TS: total solids, TSS: total suspended solids, TDS: total dissolved solids, TVS: total volatile solids, VDS: volatile suspended solids, VSS: volatile suspended solids, SCST: specific capillary suction time, SVI: sludge index volume, COD: chemical oxygen demand. TC: total coliforms, FC: fecal coliforms, FS: fecal streptococci.

current (AC) connected through an auto-transformer and a Watt meter. Gas produced by the digestion process could vent out from the digester through a connecting pipe and was measured at fixed times each day by the water displacement method as specified in Standard Methods (APHA, 1992). The gas collector was not insulated and it was exposed to an ambient temperature. To avoid gas pressure build-up inside the vessel, the collected gas was measured and discharged frequently. In each experiment, 15 L of sludge were digested anaerobically. For keeping the digester's temperature under thermophilic conditions (55°C in this work), 3.0 Watts of electrical power energy input was required.

INOCULUMS

The mesophilic upflow anaerobic sludge blanket (UASB) reactors were inoculated with 2 L of granular sludge harvested from a mesophilic lab-scale UASB reactor. Also, the inoculums were taken from a thermophilic lab-scale UASB for the thermophilic digesters in this research. Both lab-scale UASB reactors had been operating for 1 year and treating dairy wastewater.

ANALYTICAL METHODS

Biogas samples were collected using a gas sampling injector and a sample of 100–200 µmL was used for each run. The biogas composition ($\text{CH}_4 + \text{CO}_2$) was determined using a gas chromatograph (Nucon 5700)

equipped with a thermal conductivity detector (GC-TCD) and stainless steel column that was 6 ft long with a 1/4 inch OD and 2 mm ID and contained Porapak Q 100 that had a mesh range from 80–100. The carrier gas was N_2 , and the analysis was carried out at a carrier gas flow rate of 30 mL/min with the injector, column, and detector temperatures at 120, 90, and 120 °C, respectively. The gas quality was checked 2 to 4 times a day.

To ensure the efficient transfer of the intermediates and to release gas bubbles trapped in the medium, mixing was performed for 5 min every 2 h using a magnetic stirrer. Twice a day, 2–3 mL of slurry was taken from the sampling port of the digester and immediately acidified by adding 1 to 2 drops of concentrated hydrochloric acid to stop more anaerobic digestion. The samples were kept at 4 °C until the measurement of the concentration of VFAs. The VFAs were measured by a gas chromatograph (Nucon 5765) equipped with a flame ionization detector (GC-FID) using a D-BFFAP megabore column (2 m × 0.536 mm ID). The carrier gas was N_2 (3.5 mL/min) and a sample size of 2 µL was used. The initial column temperature of 100 °C was increased at the rate of 3 °C/min to a temperature of 160 °C. It was then increased at the rate of 20 °C/min until a temperature of 220 °C was reached.

Sludge dewaterability was measured using the procedure described in Standard Methods (APHA, 1992) in terms of the capillary suction time (CST). The results were reported as specific capillary suction time

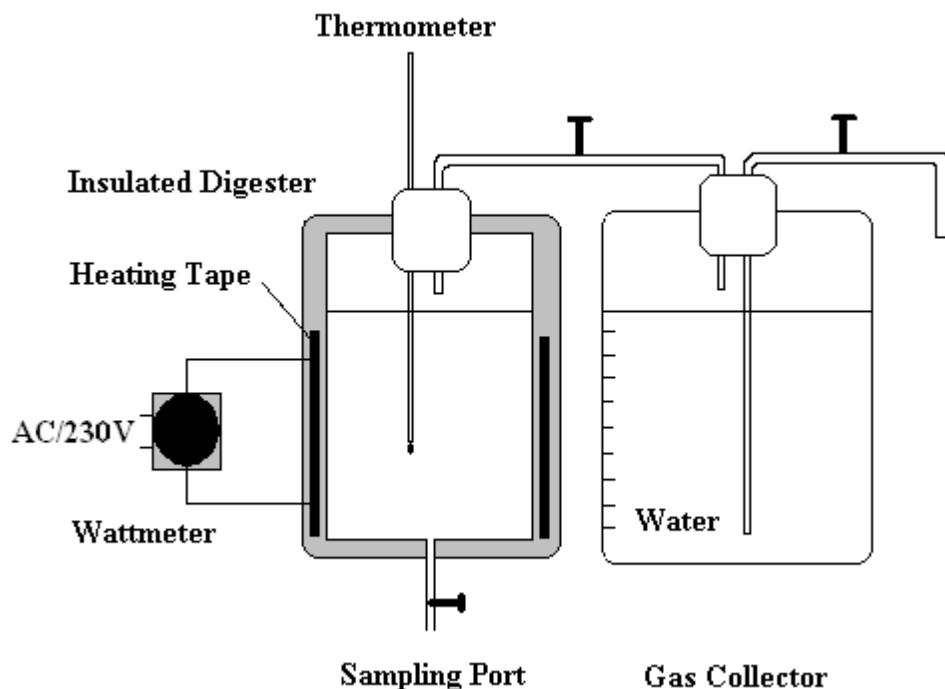


Fig. 1. Schematic of the bench scale batch anaerobic digester

(SCST) (Zhou *et al.*, 2002). Experimental investigations of the removal of indicator organisms were performed at 35°C and 55°C. Total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) as standard pathogen indicators were counted by using the colony forming units (CFU) technique (APHA, 1992). The pH of the anaerobic slurry (sludge) was measured using a digital pH meter, which had an accuracy of ± 0.1 pH unit. Total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), total volatile solids (TVS), volatile suspended solids (VSS) and chemical oxygen demand (COD) were determined according to Standard Methods (APHA, 1992).

RESULTS & DISCUSSION

To study the effect of solid concentration on the performance of the anaerobic digestion process, WAS with initial concentrations of TS = 30 and TS = 60 g/L (VSS = 25 and VSS = 40 g/L) at mesophilic and thermophilic temperatures were digested. Fig. 2a shows the VSS reduction during anaerobic digestion process. Under thermophilic conditions, the VSS concentration was gradually decreased, then its reduction rate was increased sharply and finally reached a constant value (within 15 days) and the anaerobic digestion process was apparently stopped. No sharp zone of VSS reduction was observed for the

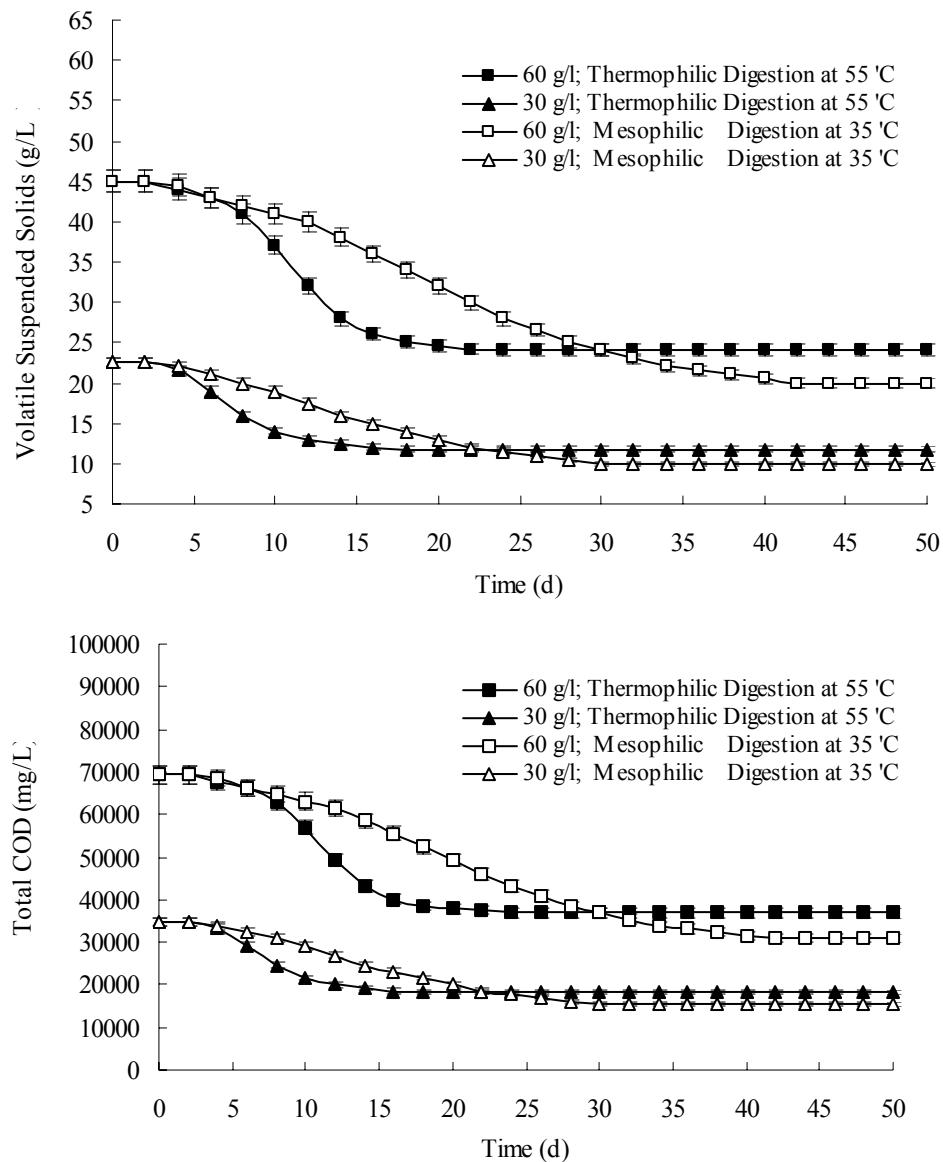


Fig. 2. Sludge (a) VS reduction and (b) total COD reduction during anaerobic digestion under mesophilic and thermophilic conditions

mesophilic digestion. The kinetic profiles of VSS reduction for the mesophilic and thermophilic processes were different; however, in the mesophilic process, more solids were eliminated at the end (within 30 to 40 days). In Fig. 2b, changes in the sludge COD during the mesophilic and thermophilic anaerobic digestion processes are illustrated. Similar to the VSS reduction, total COD reduction rate under thermophilic conditions is significantly higher than that under mesophilic conditions. As can be seen from Fig. 2b, no more total COD removal was observed for the thermophilic digestions after two weeks of the anaerobic process.

The efficient anaerobic oxidation of acetate, propionate and butyrate (VFA) affect the overall performance of the process (Kida *et al.*, 1993; Amani *et al.*, 2010b; Amani *et al.*, 2010a). The profiles of VFA production and their degradation during sludge anaerobic digestion at mesophilic and thermophilic temperatures are presented in Fig. 3 (a,b&c). At the beginning of sludge anaerobic digestion, the concentrations of VFAs were increased and reached some maximum amounts; then, they were consumed by the acetogenic and methanogenic microorganisms and their concentrations decreased. However, VFA accumulations could be the main reason for unsteadiness in the anaerobic process (Amani *et al.*, 2010b). In the present study, acetate and butyrate concentrations were decreased during anaerobic digestion and they diminished at the end of the process for sludge. On other hand, propionate was not consumed by the anaerobic microorganisms completely and accumulated partly in the digester. Accordingly, in some previous studies, propionate has been reported as the main reason for instabilities in the anaerobic digestion processes (Wang *et al.*, 1999; Schink and Stams, 2005; Tatara *et al.*, 2008). Propionate accumulation in the thermophilic process was more critical than during the mesophilic process. It seems that enzymatic issues or microbial spatial proximity are the main reasons for natural ambiguity of propionate degradation. A high rate of production for acetate and butyrate occurred at the beginning of the anaerobic digestion (initially reached up to 5000 and 7000 mg/L, respectively); however, they also disappeared quickly after digestion process proceeded. The issue for propionate here is that less was produced (maximum 800 mg/L) but that it degraded slowly. Fig. 3b shows that around 40 to 60 percent of the propionate could not be degraded in the digester. Although acetate and butyrate were degraded faster under thermophilic conditions, they also would be degraded totally under mesophilic conditions but at a lower rate. However, mesophilic degradation of propionate is slightly better than that of the thermophilic process.

The presence of VFAs in the anaerobic digester leads to a reduction in pH and the growth of anaerobes; methanogen growth is especially strongly inhibited (Turovskiy and Mathai, 2006). Therefore, pH values in the digester can affect the production of biogas and methane composition. Fig. 4a and 4b show the pH variations during the sludge anaerobic digestion and the methane content in the effluent gas, respectively. As seen, the pH dropped at first due to the VFAs production and then gradually increased; finally, it reached a level about 8. An increase in pH led to enhanced methane production (Fig. 4a and 4b). In this study, mesophilic digestion took place in 32 to 36 days with slightly more methane production and a slightly higher removal of organics. Fig. 4c confirmed this property of mesophilic digestion over thermophilic digestion; also, it was previously reported by other investigators (Buhr and Andrews, 1977; Kalia *et al.*, 2000). The study of methane content (Fig. 4b) shows that there is no significant difference between the effects of thermophilic and mesophilic digestion on this property. The pH variations (Fig. 4a), and VFA concentration profiles (Figs. 3) show that thermophilic conditions do not accelerate the activity of acidogens; furthermore, some results reported inhibitory effects of high temperatures on the growth of acidogens (Buhr and Andrews, 1977; Roy and Sreekrishnan, 2003). This issue is also approved thermodynamically (de Bok *et al.*, 2004).

Fig. 5 illustrates the dewaterability of anaerobic sludge under mesophilic and thermophilic conditions. In general, anaerobic digestion provides better sludge dewaterability compared to aerobic digestion (Nosrati *et al.*, 2007). Furthermore, as seen in these results, thermophilic anaerobic digestion causes improved dewaterability (lower SCSTs). This may be because sludge dewaterability is reported to be associated with the existence of substances (e.g. extracellular polymers, ECPs) produced during the anaerobic digestion of intracellular materials (polysaccharides and proteins). There is an equilibrium balance achievable between production and destruction of these substances, causing poor dewaterability under thermophilic conditions.

Pathogen inactivation of sludge during anaerobic digestion under thermophilic and mesophilic conditions is illustrated in Table 2. Thermophilic digestion at 55 °C provided complete pathogen inactivation. Satisfactory pathogen inactivation also was observed during mesophilic digestion; however, the required time for this sanitary achievement (or satisfactory pathogen inactivation) was more than one month (35 days). Fecal streptococci are the easiest indicator to remove (Table 2).

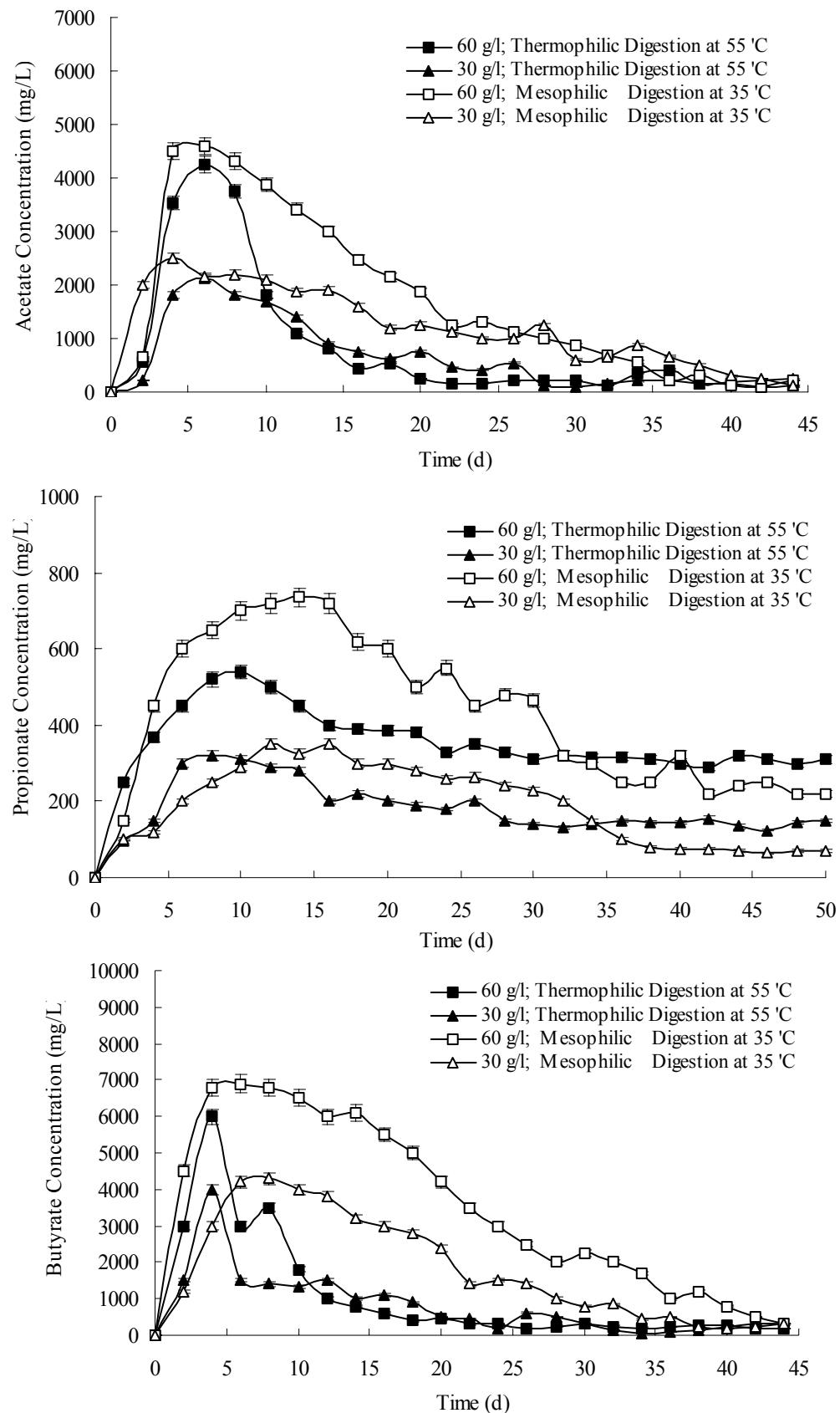


Fig. 3. Concentrations of (a) acetate, (b) propionate and (c) butyrate during sludge anaerobic digestion under mesophilic and thermophilic conditions

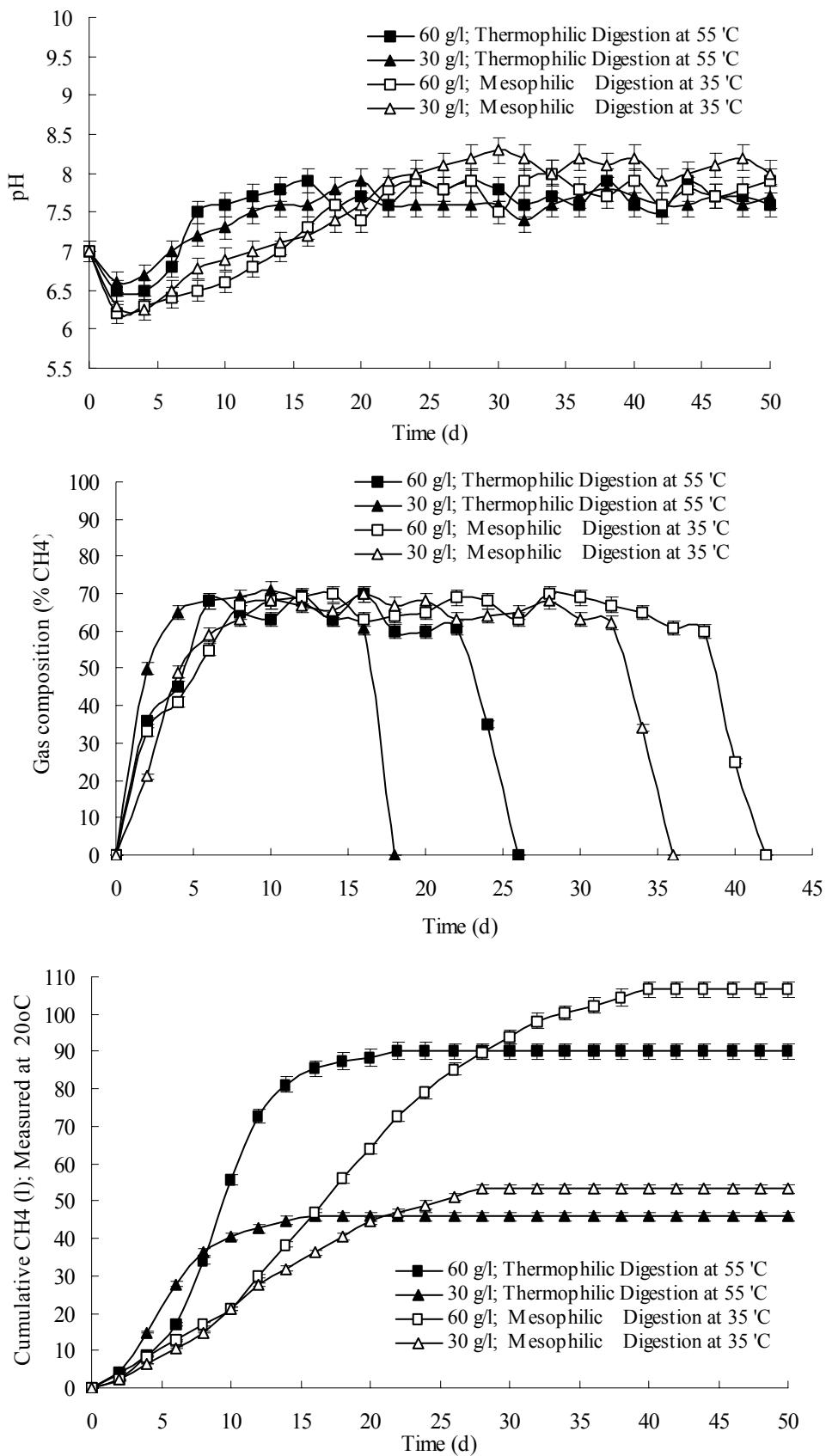


Fig. 4. (a) pH variations, (b) purity of methane produced and (c) accumulative volume of methane (measured at 20 °C) produced during sludge anaerobic digestion under mesophilic and thermophilic conditions

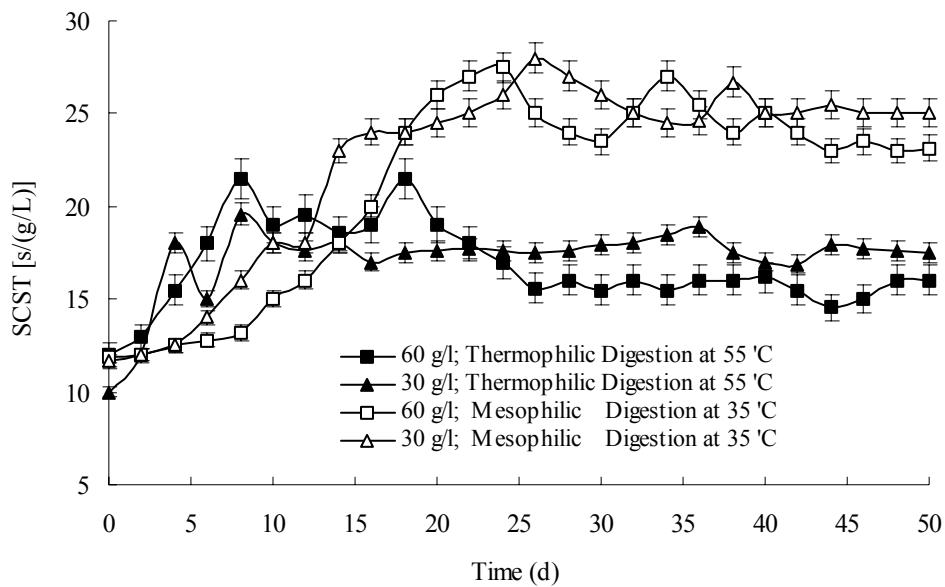


Fig. 5. Dewaterability during anaerobic digestion under mesophilic and thermophilic conditions

Table 2. Pathogen removal from sludge in thermophilic and mesophilic anaerobic digestion

TS ₀ (g/L)	Temperature(°C)	Time(day)	TC(CFU/g dried sludge)	FC(CFU/g dried sludge)	FS(CFU/g dried sludge)
60	55	15	0.0×10 ⁰	0.0×10 ⁰	0.0×10 ⁰
30	55	15	0.0×10 ⁰	0.0×10 ⁰	0.0×10 ⁰
60	35	35	1.3×10 ³	4.2×10 ²	3.0×10 ¹
30	35	35	1.0×10 ³	3.7×10 ²	1.0×10 ¹

Note: TS₀: initial total solids, TC: total coliforms, FC: fecal coliforms, FS: fecal streptococci.

Table 3. The energy audit for sludge thermophilic and mesophilic anaerobic digestion

TS ₀ (g/L)	Temperature(°C)	Time(day)	E _{Pre-heating} (kJ)	E _{Lost} (kJ)	E _{Methane} (kJ)	E _{Balance} (kJ)
60	55	15	1643	7290	2993	-5940
30	55	15	1643	7290	1553	-7380
60	35	35	705	2498	3540	+338
30	35	35	705	2498	1800	-1403

The energy audits for sludge thermophilic and mesophilic anaerobic digestion are presented in Table 3. Reactor body heat loss at 55 and 35 °C were 3.0 and 0.4 Watts, respectively. Sludge pre-heating and energy loss under thermophilic conditions were significantly more than those of mesophilic anaerobic digestion. Therefore, only the thickest sludge (TS = 60 g/L) provided auto-thermality (energy production from the biogas produced) under mesophilic conditions; however, for this treatment, a batch time around 35 days was required (Table 3). Because more retention time is required for the digestion of the same mass of sludge in a continuous digester, the pre-heating process was essential.

CONCLUSION

The following conclusions can be drawn based on the data generated in this research.

- 1.Mesophilic anaerobic digestion of sludge takes 30 to 40 days to eliminate around 50 % of the initial mass of the sludge; for the same results under thermophilic digestion, 11 to 14 days is required.
- 2.The hydrolysis step in sludge anaerobic digestion takes place very slowly; this causes a limitation of methanogen growth. Therefore, thermophilic anaerobic digestion of sludge could not be operated as an independent auto-heated process due to the high energy requirements (in the sludge pre-heating step) and the digesters' heat loss.
- 3.Under mesophilic conditions, in spite of longer detention time, the produced biogas could be sustained auto-thermally (energy production from the biogas produced) with respect to covering the pre-heating energy as well as the power loss from the digester during the anaerobic treatment.
- 4.Propionate is generated in low concentrations and consumed slowly but accumulates in both anaerobic digestion processes (mesophilic and thermophilic). The accumulation of propionate could not be overcome by the methanogenic archaea (even partially). Accumulated concentrations of propionate during the thermophilic process were, to some extent, greater than the concentrations seen during the mesophilic process.
- 5.Mesophilic anaerobic digestion of WAS with an initial concentration of TS = 60 g/L could be operated auto-thermally. Consequently, highly concentrated WAS could be auto-thermally digested anaerobically.
- 6.The thermophilic anaerobic digestion causes more improved sludge dewaterability, but little less degradation.
- 7.Satisfactory pathogen inactivation was observed during mesophilic and thermophilic digestions.

REFERENCES

- Abduli, M. A., and Azimi, E. (2010). Municipal waste reduction potential and related strategies in Tehran. *Int. J. Environ. Res., 4 (4)*, 901-912.
- Ahn, J. H., and Forster, C. F. (2000). A comparison of mesophilic and thermophilic anaerobic upflow filters. *Biores. Technol., 73*, 201-205.
- Amani, T., Nosrati, M., and Sreekrishnan, T. R. (2010a). Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects – a review. *Environ. Rev., 18*, 255–278.
- Amani, T., Nosrati, M., and Mousavi, S. M. (2010). Using enriched cultures for elevation of anaerobic syntrophic interactions between acetogens and methanogens in a high-load continuous reactor. *Bioresource Technology, Accepted Paper. DOI: 10.1016/j.biortech.2010.11.111*
- APHA, (1992). Standard methods for the examination of water and wastewater, 18th ed. Washington, DC: American Public Health Association.
- Buhr, H. O. and Andrews J. F. (1977). The thermophilic anaerobic digestion process – A review. *Wat. Res., 11*, 129–143.
- Dohányos, M., and Zábranská, J. (2001). Sludge into biosolids: processing, disposal, and utilization. IWA publishing Chapter, **13**, 223–241.
- de Bok, F. A. M., Plugge, C., and Stams, A. J. M. (2004). Interspecies electron transfer in methanogenic propionate degrading consortia. *Wat. Res., 38*, 1368–1375.
- Firdaus, G. and Ahmad, A. (2010). Management of urban waste pollution in developing countries. *Int. J. Environ. Res., 4 (4)*, 795–806.
- Gavala, H. N., Yenal, U., Skidas, I. V., Westermann, P., and Ahring, B. K. (2003). Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature. *Wat. Res., 37*, 4561-4572.
- Han, Y., and Dague, R. R. (1997). Laboratory studies on the temperature-phased anaerobic digestion of domestic wastewater sludge. *Wat. Environ. Res., 69*, 1139-1143.
- Kalia, V. C., Sonakya, V., and Raizada, N. (2000). Anaerobic digestion of banana stem waste. *Biores. Technol., 73*, 191-193.
- Kida, K., Morimura, S. and Sonoda, Y. (1993). Accumulation of propionic acid during anaerobic treatment of distillery wastewater from barleyshchu making. *J. Fermen. Bioeng., 75*, 213–216.
- Kim, M., Ahn, Y. H. and Speece, R. E. (2002). Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. *Wat. Res., 36*, 4369–4385.
- Mehrdadi, N., Joshi, S. G., Nasrabadi, T. and Hoveidi, H. (2007). Aplication of Solar Energy for Drying of Sludge from Pharmaceutical Industrial Waste Water and Probable Reuse, *International Journal of Environmental Research, 1 (1)*, 42-48.

- Mottet, A., Steyer, J. P., Délérès, S., Vedrenne, F., Chauzy, J. and Carrère, H. (2009). Kinetics of thermophilic batch anaerobic digestion of thermal hydrolysed waste activated sludge. *Bioch. Eng. J.*, **46**, 169–175.
- Nosrati, M., Sreekrishnan, T. R., and Mukhopadhyay, S. N. (2007). Energy audit, solids reduction, and pathogen inactivation in secondary sludges during batch thermophilic aerobic digestion process. *J. Environ. Eng.*, **133**, 477–484.
- Nwabanne, J. T., Onukwuli, O. D., and Ifeakandu, C. M. (2009). Biokinetics of anaerobic digestion of municipal waste. *Int. J. Environ. Res.*, **3** (4), 511-516.
- Rahmani, K., Mahvi, A. H., Vaezi, F., Mesdaghinia, A. R., Nabizade, R. and Nazmara, Sh. (2009). Bioremoval of Lead by Use of Waste Activated Sludge, *Int. J. Environ. Res.*, **3** (3), 471-476.
- Rasapoor, M., Nasrabadi, T., Kamali, M. and Hoveidi, H. (2009). The effects of aeration rate on generated compost quality, using aerated static pile method. *Waste Management*, **29**, 570-573.
- Roy, S. and Sreekrishnan, T. R. (2003). Anaerobic degradation using thermophilic methanogenesis M. Tech Project, Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology (IIT), New Delhi, May.
- Rubio-Loza, L. A. and Noyola, A. (2010). Two-phase (acidogenic-methanogenic) anaerobic thermophilic/mesophilic digestion system for producing Class A biosolids from municipal sludge. *Biores. Technol.*, **101**, 576-585.
- Rulkens, W. (2008). Sewage sludge as a biomass resource for the production of energy: overview and assessment of the various options. *Ener. Fuel.*, **22**, 9-15.
- Schink, B. and Stams, A. J. M. (2005). Syntrophism among prokaryotes. In: Dworkin M. (Ed.), *The prokaryotes: an evolving electronic resource for the microbiological community*, 3rd ed. Springer, New York.
- Song, Y. C., Kwon, S. J. and Woo, J. H. (2004). Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic- and thermophilic digestion of sewage sludge. *Wat. Res.*, **38**, 1653-1662.
- Tatara, M., Makiuchi, T., Uneo, Y., Goto, M. and Sode, K. (2008). Methanogenesis from acetate and propionate by thermophilic down-flow anaerobic packed-bed reactor. *Biores. Technol.*, **99**, 4786-4795.
- Turovskiy, I. S., and Mathai, P. K. (2006). *Wastewater sludge processing*. New York: Wiley.
- Uemura, Sh. (2010). Mineral Requirements for Mesophilic and Thermophilic Anaerobic Digestion of Organic Solid Waste, *Int. J. Environ. Res.*, **4** (1), 33-40.
- Watanabe, H., Kitamura, T., Ochi, S. and Ozaki, M. (1997). Inactivation of pathogenic bacteria under mesophilic and thermophilic conditions. *Wat. Sci. Technol.*, **36**, 25-32.
- Wang, Q., Kuninobu, M., Ogawa, H. I. and Kato, Y. (1999). Degradation of volatile fatty acids in highly efficient anaerobic digestion. *Biom. Bioener.*, **16**, 407-416.
- Zábranská, I., Štpová, J., Wachtl, R., Jeníček, P. and Dohányos, M. (2000). The activity of anaerobic biomass in thermophilic and mesophilic digesters at different loading rates. *Water Sci. Technol.*, **42**, 49-56.
- Zhou, J., Mavinic, D. S., Kelly, H. G. and Ramey, W. D. (2002). Effects of temperature and extracellular proteins on dewaterability of thermophilically digested biosolids. *J. Environ. Eng. Sci.*, **1**, 409-415.
- Zupančič, G. D. and Roš, M. (2003). Heat and energy requirements in thermophilic anaerobic sludge digestion. *Ren. Ener.*, **28**, 2255-2267.