ENVIRONMENTAL ENGINEERING SCIENCE
Volume 25, Number 9, 2008
© Mary Ann Liebert, Inc.
DOI: 10.1089/ees.2007.0242

Two-Phase Thermophilic Acidification and Mesophilic Methanogenesis Anaerobic Digestion of Waste-Activated Sludge

Goksel N. Demirer[1*] and Maazuza Othman[2]

[1]*Department of Environmental Engineering, Middle East Technical University, 06531, Ankara, Turkey*
[2]*School of Civil, Environmental and Chemical Engineering, RMIT University, Victoria 3001, Australia*

Received: August 29, 2007; Accepted in revised form: December 2, 2007

Abstract

Biological pretreatment methods of waste-activated sludge (WAS) have recently received attention because of their efficiency and relatively low investment. The application of hydrolysis/acidification step before methanization in anaerobic digestion or the two-phase anaerobic digestion process constitutes one of these methods. Two phase (thermophilic acidification and mesophilic methanogenesis) anaerobic digestion of WAS was investigated and reported to perform better in terms of chemical oxygen demand (COD) solubilization, volatile solids destruction, gas production, and pathogen indicator reduction. The solids loading rates (SLR) used in these studies were significantly high (6.5–28.9 g volatile solids (VS)/L · day). It is well-known that high SLRs along with low retention times lead to higher acidification in two-phase systems. However, WAS from different wastewater treatment plants is likely to have different concentrations due to different process configurations and operational practices that lead to lower solids loading rates. Therefore, this study investigated the performance of thermophilic anaerobic preacidification of WAS prior to conventional mesophilic (methanogenic) anaerobic digestion at low SLRs (0.98–3.93 g VS/L · day). To this purpose, continuous anaerobic acidogenic reactors with no recycle were operated at two different HRT (or SRT) values of 2 and 4 days, and three different SLR values of 0.98, 1.97, and 3.93 g VS/L · day. After the acidification reactors reached to steady-state conditions, the preacidified WAS samples (or the effluents of thermophilic acidogenic anaerobic reactors) and raw WAS samples (or the feeds to the thermophilic acidogenic anaerobic reactors) were subjected to biochemical methane potential (BMP) assay. Results indicated that thermophilic preacidification led to a 20.9–34.8% increase in dissolved (soluble) COD concentration and 18.2–33.3% CODt reduction in acidogenic reactors. When the CODt removals observed for preacidification and BMP were both considered, preacidification (or phase separation) has led to 26.2–49.4% additional CODt removal. It is apparent from these results that beneficial effect of preacidification on CODt removal is sustained for low SLRs (0.98–3.93 g VS/L · day).

Key words: anaerobic digestion; waste activated sludge; phase separation; acidification

Introduction

Waste-activated sludge (WAS) is generated by operation of the conventional biological wastewater treatment plants. WAS treatment and disposal is receiving increasing attention as sludge volumes are becoming higher and higher as a consequence of more stringent criteria for wastewater treatment plant effluent and due to the building of new treatment facilities (Bolzonella *et al.*, 2007). The disposal of WAS poses a significant challenge to wastewater treatment because sludge handling represents 30–40% of the capital cost and about 50% of the operating cost of many wastewater treatment facilities (Vlyssides and Karlis, 2004; Choi *et al.*, 2006).

Anaerobic digestion is the most widely used method of WAS disposal due to its high performance in volume reduction and stabilization and the production of biogas that makes the process profitable. However, biological hydrolysis, which is the rate-limiting step for the anaerobic degradation of WAS (Li and Noike, 1992; Tiehm *et al.*, 2001) has to be improved to enhance the overall process performance as well as the associated cost. Several mechanical, thermal, chemical, or biological pretreatment methods have been considered to improve hydrolysis and anaerobic digestion performance. These pretreatments result in the lysis or disintegration of cells (Delgenes *et al.*, 2003) and release of intracellular matter that becomes more accessible to anaero-

---

*Corresponding author: Department of Environmental Engineering, Middle East Technical University, 06531, Ankara, Turkey. *Phone:* +90-312 210 58 67; *Fax:* +90-312 210 26 46; *E-mail:* goksel@metu.edu.tr

bic micro-organisms (Bougrier *et al.*, 2005), thus improving anaerobic digestion (Tiehm *et al.*, 2001).

The commonly used pretreatment methods for WAS are thermal (Li and Noike, 1992), alkaline (Ray *et al.*, 1990; Lin *et al.*, 1997), ultrasonic (Tiehm *et al.*, 2001; Bougrier *et al.*, 2005), mechanical (Nah *et al.*, 2000; Choi *et al.*, 2006), thermal-alkaline (Vlyssides and Karlis, 2004), thermochemical (Tanaka *et al.*, 1997), microwave (Park *et al.*, 2004; Kennedy *et al.*, 2007), and ozone (Weemaes *et al.*, 2000; Yeom, 2002) pre-treatment. Provided that these methods are energy-intensive and costly (Takashima *et al.*, 1996), biological pretreatment methods of WAS has recently received attention because of their efficiency and relatively low investment (Bolzonella *et al.*, 2007). Two-phase anaerobic digestion process or the application of hydrolysis/acidification step before methanization constitutes one of these methods (Gavala *et al.*, 2003; Lu *et al.*, 2005; Bolzonella *et al.*, 2007).

The different growth rate and pH optima for acidogenic and methanogenic anaerobic organisms and, thus, different requirements regarding reactor conditions, has led to the development of the two-phase AD process (Borchardt, 1971; Ghosh and Klass, 1977; Ghosh, 1987; Guerrero *et al.*, 1999). The two-phase configuration has several advantages over conventional one-phase processes, such as selection and enrichment of different bacteria in each phase, increased stability of the process, preventing pH shock to the methanogenic population, etc. Thus, the process can be smaller and more cost-efficient (Ghosh and Klass, 1977; Fox and Pohland, 1994; Speece, 1996; Solera *et al.*, 2002; Demirer and Chen, 2004 and 2005).

Bhattacharya *et al.* (1996) studied the volatile solids reduction in two-phase and conventional anaerobic sludge digestion of WAS. They reported that the volatile solid (VS) reduction efficiency in the two-phase system was 8.7% higher than in conventional system. In another study, Shana *et al.* (2005) investigated the two-phase anaerobic digestion of a mixture of primary sludge and WAS first in bench and then in full scale. Both the acidogenic and methanogenic phases were operated at a mesophilic temperature range with hydraulic retention times (HRTs) of 2.4 and 10 days, respectively. The performance of the two-phase configuration was assessed by comparing it with that of a one phase conventional anaerobic sludge digestor. The results indicated that the two-phase process performed better than the control digester in terms of volatile solids destruction, gas production, and pathogen indicator reduction.

Bolzonella *et al.* (2007) reported the main findings in recent literature with particular attention to two-phase anaerobic digestion of WAS (Table 1). Data reported in Table 1 indicates that level of COD solubilization and biogas production are significantly increased for pretreatment (acidification) at thermophilic temperatures over 60°C and HRTs of 2–3 days. It has to be noted here that the concentrations and thus the solids loading rates (SLR) used in the pretreatment (acidification) studies were significantly high, namely 6.5–28.9 g VS/L · day (Shana *et al.*, 2005; Bolzonella *et al.*, 2007). Considering that high SLRs and/or low retention times lead to higher acidification in two-phase systems (Ghosh, 1987; Shin *et al.*, 2001), the high performance presented in Table 1 is mainly due to the high SLRs applied (6.5–28.9 g VS/L · day) in these studies. However, it is well known fact that WAS from different wastewater treatment plants is likely to have different concentrations due to different process configurations and operational practices which lead to lower SLRs.

Based on the previous studies and the above discussion, this study investigated the performance of thermophilic anaerobic preacidification prior to conventional mesophilic (methanogenic) anaerobic digestion or two-phase anaerobic digestion of waste activated sludge (WAS) at low SLRs, namely 0.98–3.93 g VS/L · day. The objective was simply investigating whether the advantages of anaerobic phase separation can still be exploited at low SLRs. To this purpose, continuous anaerobic acidogenic reactors with no recycle were operated at two different HRT (or SRT) values of 2 and 4 days, and three different SLR values of 0.98, 1.97, and 3.93 g VS/L · day. Then, after the acidification reactors reached steady-state conditions, the preacidified WAS samples (or the effluents of thermophilic acidogenic anaerobic reactors), and raw WAS samples (or the feeds to the thermophilic acidogenic anaerobic reactors) were subjected to biochemical methane potential (BMP) assay.

TABLE 1. EXPERIMENTAL RESULTS FROM PREVIOUS STUDIES ON TWO-PHASE ANAEROBIC DIGESTION OF SLUDGE

Pretreatment (acidification) step temperature (°C)	*Pretreatment (acidification) step HRT (day)*	*Dissolved COD (%)*	*Increase in gas production (%)*	*Reference*
35	2	4.8	—	Bhattacharya *et al.*, 1996
47	2	22.4	69	Watts *et al.*, 2005
54	2	25.3	81	Watts *et al.*, 2005
55	0.2	—	29.5	Roberts *et al.*, 1999
55	3.5	11.8	—	Lu and Ahring, 2005
60	5.5	—	16.6	Oles *et al.*, 1997
60	2	38.9	98	Watts *et al.*, 2005
62	1	—	26.9	Cheunbarn and Pagilla, 2000
70	4	—	144.6	Gavala *et al.*, 2003
70	3.5	27.8	—	Lu and Ahring, 2005
75	3.5	29.5	—	Lu and Ahring, 2005
80	3.5	30.5	—	Lu and Ahring, 2005

COD, chemical oxygen demand; HRT, hydraulic retention time.

Materials and Methods

Waste activated sludge and anaerobic seed cultures

Unthickened WAS was collected from the Sunburry Wastewater Treatment Plant in Melbourne, Australia. It was filtered through a screen of 1.0-mm mesh size and stored at 4°C prior to use. The characterisation of the WAS is given in Table 2. The mixed anaerobic culture used as seed was obtained from the anaerobic sludge digesters of the Eastern Wastewater Treatment Plant in Melbourne, Australia. It was filtered through a screen of 1.0-mm mesh size and concentrated by settling before being used as inoculum. It was stored at 4°C prior to use. The characterisation of the seed is given in Table 3.

Experimental setup

Thermophilic Anaerobic Acidification of WAS. Twenty daily-fed continuously mixed acidogenic anaerobic reactors (Supelco, Bellefonte, PA), with no recycle were operated at two different HRT (or SRT) values of 2 and 4 days, and three different SLR values of 0.98, 1.97, and 3.93 g VS/L · day. Duplicate reactors were operated for each HRT (or SRT) and SLR pair. The effective and total volumes of the reactors were 50 and 120 mL, respectively. Reactor operation involved daily feeding of raw WAS and wasting of corresponding reactor contents (Table 4). Solids and hydraulic retention times (SRT and HRT) applied to each reactor (Table 4) was the same because no recycle of the effluent was practiced. Initially, 25 mL of concentrated anaerobic seed was added to all the reactors except Blank (B) reactors (Table 4). The control reactors received seed but not WAS. The reactors with HRT (or SRT) of 2 and 4 days were fed with 25 and 12.5 mL of original (undiluted, see Table 2) or $^1/_2$ diluted WAS every day, respectively. Namely, reactors B1, T1, B2, and T2 were fed with undiluted WAS. While reactors B3, T3, B4, and T4 were fed with WAS diluted by $^1/_2$.

The Control (C) reactors were fed with water (Table 4). Then, the reactors were flushed with 100% N_2 gas for 5 min, sealed with natural rubber sleeve stoppers, and maintained in a shaking water bath (Stuart Scientific, Staffordshire, UK) at 60 ± 1°C and 100 rpm. Daily feeding and wasting were conducted as seen in Table 4. The daily effluents from all the acidogenic reactors were used for the analysis during the operation of the reactors. Furthermore, the effluents from the reactors on the last day (day 15) of operation were collected and subjected to biochemical methane potential (BMP) assay along with corresponding raw (not subjected to anaerobic acidification) WAS and WAS($^1/_2$) samples of 25 (HRT or SRT of 2 days) and 12.5 mL (HRT or SRT of 2 days) in separate reactors.

Table 2. Characterization of the WAS Used in the Study

Parameter	Concentration
Total COD	14,550 ± 250 mg/L
Soluble COD	280 ± 20 mg/L
Total Solids	8,180 ± 35 mg/L
Volatile Solids	7,860 ± 27 mg/L
Total P	873.75 ± 8.75 mg PO_4^{3-}/L
Soluble TP	65.5 ± 1.0 mg PO_4^{3-}/L
[PO_4^{3-}] Total	64.2 ± 3.0 mg PO_4^{3-}/L
Total N	500 ± 25 mg N/L
Soluble Total N	37.5 ± 2.5 mg N/L
NH_3-N	7.5 ± 0.5 mg NH_3/L
pH	6.82

COD, chemical oxygen demand; P, phosphorus; TP, total phosphorus.

Table 3. Characterization of the Anaerobic Seed Used in the Study

Parameter	Concentration
Total COD	30,900 ± 0 mg/L
Soluble COD	540 ± 0 mg/L
Total Solids	19,370 ± 230 mg/L
Volatile Solids	12,860 ± 160 mg/L
Total P	2,104 ± 124 mg PO_4^{3-}/L
Soluble TP	1,013 ± 75 mg PO_4^{3-}/L
[PO_4^{3-}] Total	371.5 ± 38.5 mg PO_4^{3-}/L
Total N	1,662.5 mg N/L
Soluble Total N	625 ± 125 mg N/L
NH_3-N	468.5 ± 56.5 mg NH_3/L
pH	7.50

See table 2 for abbreviations.

Biochemical methane potential (BMP) assay. In order to determine the anaerobic biodegradability and biogas production from raw and anaerobically preacidified WAS, BMP experiments (Owen *et al.*, 1979; Erguder *et al.*, 2000; Gungor-Demirci and Demirer, 2004; Demirer and Chen, 2004) were performed.

Thirty-two batch methanogenic anaerobic reactors (Supelco) were operated. Duplicate reactors were fed with the daily effluent of each reactor from the thermophilic anaerobic acidification phase as well as their corresponding feeds [or WAS and WAS($^1/_2$)]. The effective and total volumes of the reactors were 50 and 120 mL, respectively. Initially, 25 mL of concentrated anaerobic seed was added to all the reactors except Blank (B) reactors (Table 5). The Control (C) reactors contained seed but were fed with water (Table 5). Then, the reactors were flushed with 100% N_2 gas for 5 min, sealed with natural rubber sleeve stoppers, and maintained in a shaking water bath (Stuart Scientific, Staffordshire, UK) at 35 ± 1°C and 100 rpm and daily gas production was monitored by a water displacement device in each reactor. At the end of the BMP assay (day 30), the reactor contents were analysed for chemical oxygen demand (CODt), CODs, and volatile acid (VA).

Analytical methods

The measurement of pH was made using calibrated pH meter (ThermoOrion, Model 550A). The total solids (TS) and VS were measured gravimetrically according to the Standard Methods (American Public Health Association, 1998). The measurements of COD, VA, total phosphorus (P), total N, PO_4^{3-}, and NH_4^+ were made by colorimetric techniques using HACH Spectrophotometer (DR/4000). The content of methane in the biogas was determined as follows. A known volume of the headspace gas (*V*1) produced in a serum bot-

Table 4. Experimental Setup for Acidogenic Reactors

Reactor	*Seed addition*	*Daily feeding with*	*HRT or SRT (days)*	*Daily feeding/wasting volume (mL)*	*SLR (g VS/L day)*
C1-Acid	+	Water	2	25	0
B1-Acid	−	WAS	2	25	3.93
T1-Acid	+	WAS	2	25	3.93
C2-Acid	+	Water	4	12.5	0
B2-Acid	−	WAS	4	12.5	1.97
T2-Acid	+	WAS	4	12.5	1.97
B3-Acid	−	WAS($^1/_2$)	2	25	1.97
T3-Acid	+	WAS($^1/_2$)	2	25	1.97
B4-Acid	−	WAS($^1/_2$)	4	12.5	0.98
T4-Acid	+	WAS($^1/_2$)	4	12.5	0.98

VS, volitile solids; WAS, waste-activated sludge.

tle used in the biochemical methane production (BMP) experiments was syringed out and injected into another serum bottle which contained 20 g/L KOH solution. This serum bottle was shaken manually for 3–4 min so that all the CO_2 and H_2S were absorbed in the concentrated KOH solution. The volume of the remaining gas ($V2$), which was 99.9% CH_4, in the serum bottle was determined by means of a syringe. The ratio of $V2/V1$ provided the content of CH_4 in the headspace (Erguder *et al.*, 2000). The methane content of biogas was determined in six duplicate samples and found to be 62.4 ± 4.8%.

Results and Discussion

Thermophilic anaerobic acidification of WAS

Figure 1 depicts the pH profile in all the acidogenic reactors during the course of operation. After the onset of the operation, the pH of all test reactors decreased with varying rates and extent, which was determined by the combination of HRT (or SRT) and SLR applied.

Table 5. Experimental Setup for BMP Assay

Reactor	*Seed addition*	*Fed with*	*Feed volume (mL)*	*Seed (mL)*
C1-Acid	+	Eff	25	25
B1-Acid	+	Eff	25	25
T1-Acid	+	Eff	25	25
C2-Acid	+	Eff	12.5	25
B2-Acid	+	Eff	12.5	25
T2-Acid	+	Eff	12.5	25
B3-Acid	+	Eff	25	25
T3-Acid	+	Eff	25	25
B4-Acid	+	Eff	12.5	25
T4-Acid	+	Eff	12.5	25
B1-Meth	−	WAS	25	0
B2-Meth	−	WAS	12.5	0
C1-Meth	+	WAS	25	0
C2-Meth	+	WAS	12.5	0
C3-Meth	+	WAS($^1/_2$)	25	0
C4-Meth	+	WAS($^1/_2$)	12.5	0

Eff., Effluent of the corresponding acidifying reactor; WAS, undiluted raw (not subjected to anaerobic acidification) waste-activated sludge; WAS($^1/_2$): raw (not subjected to anaerobic acidification) waste activated sludge diluted by $^1/_2$.

For the same HRT, the rate of pH drop during the operation was increased with the increase in the SLR (Fig. 1) as expected. While for the same SLR, the pH drop was inversely proportional with the increase in HRT of the reactor (Fig. 1). This is an expected observation because it is a well-known fact that low retention times and high SLRs lead to higher acidification in two-phase systems (Ghosh, 1987; Shin *et al.*, 2001). The extent of pH drop observed for all the reactors did not vary too much and the minimum pH value obtained in reactor T1 was 6.40 (Fig. 1). The relatively high pH values observed can be explained by the alkalinity generated by the anaerobic biodegradation of nitrogenous organic compounds (Speece, 1996) contained in the WAS used in this study. Similar observations were reported for dairy manure which was also rich in nitrogenous compounds (Demirer and Chen 2004/2005; Yilmaz and Demirer, 2008). The pH drop in the control reactors (Fig. 1) is due to the low pH of the tap water (6.36) with which these reactors were fed. The pH profile in all test reactors relative to their blanks (containing no anaerobic seed) was an indication of anaerobic acidification. However, this phenomenon had to be confirmed with further parameters and analysis. Therefore, degree of solubilization and acidification and VA production were determined in the acidifying reactors.

Figure 2 depicts the total and soluble COD concentrations in the reactors as well as the degree of solubilisation (or percentages of soluble to total COD). As seen in Table 4, due to presence or absence of seed, different volumes and/or strengths of WAS used (undiluted or $^1/_2$ diluted), the initial total and soluble COD values (Fig. 2a and b) for acidogenic reactors are different. All the reactors except controls were fed by either original or $^1/_2$ diluted WAS. Therefore, the influent total and soluble COD concentrations for reactors B1, T1, B2, and T2 were 14,550 ± 250 mg/L and 280 ± 20 mg/L, respectively. While the influent total and soluble COD concentrations for reactors B3, T3, B4, and T4 were 7,275 ± 125 mg/L and 140 ± 10 mg/L, respectively. Discarding the microbial conversion, which will be discussed below, the daily feeding of the reactors (except controls) with WAS or WAS($^1/_2$) would converge the COD concentrations in the re-

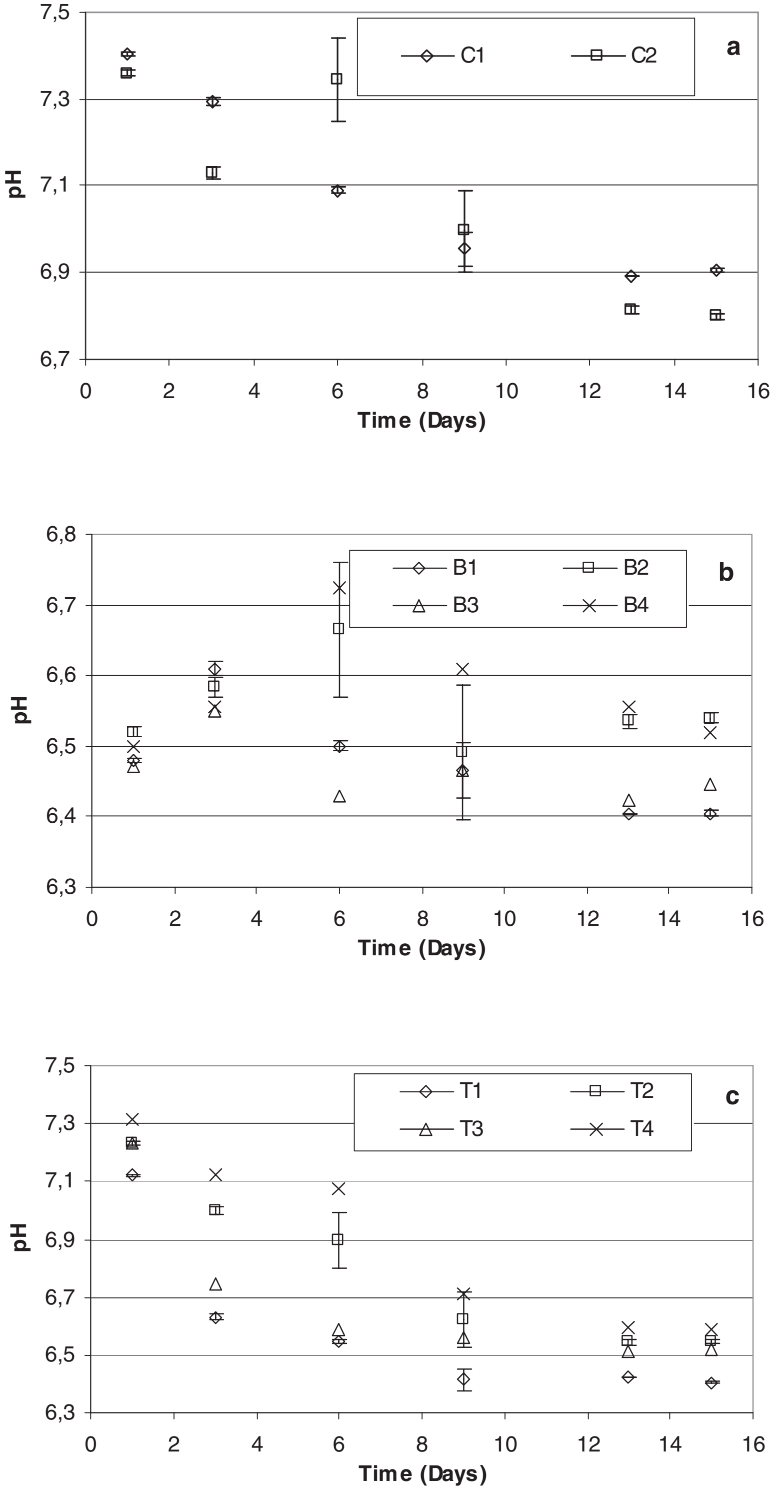

FIG. 1. pH profiles in acidifying reactors.

actors to the corresponding influent COD concentrations at a rate determined by the HRT (or SRT) applied to the reactors.

When a high solids-containing waste is introduced to an anaerobicly acidifying reactor, the particulate organic matter is liquefied through hydrolysis along with acidification (Demirer and Chen, 2004). This mechanism can be quantified with monitoring the soluble COD concentration as well as the ratio of soluble to total COD in the acidogenic reactors. When the soluble COD (CODs) values for the reactors are considered (Fig. 2b), it is seen that there has been a dramatic increase in the CODs at varying levels. For example CODs for T1 increased from the initial value of 412 mg/L to 3,040 and 3,380 mg/L on days 6 and 14, respectively. For the

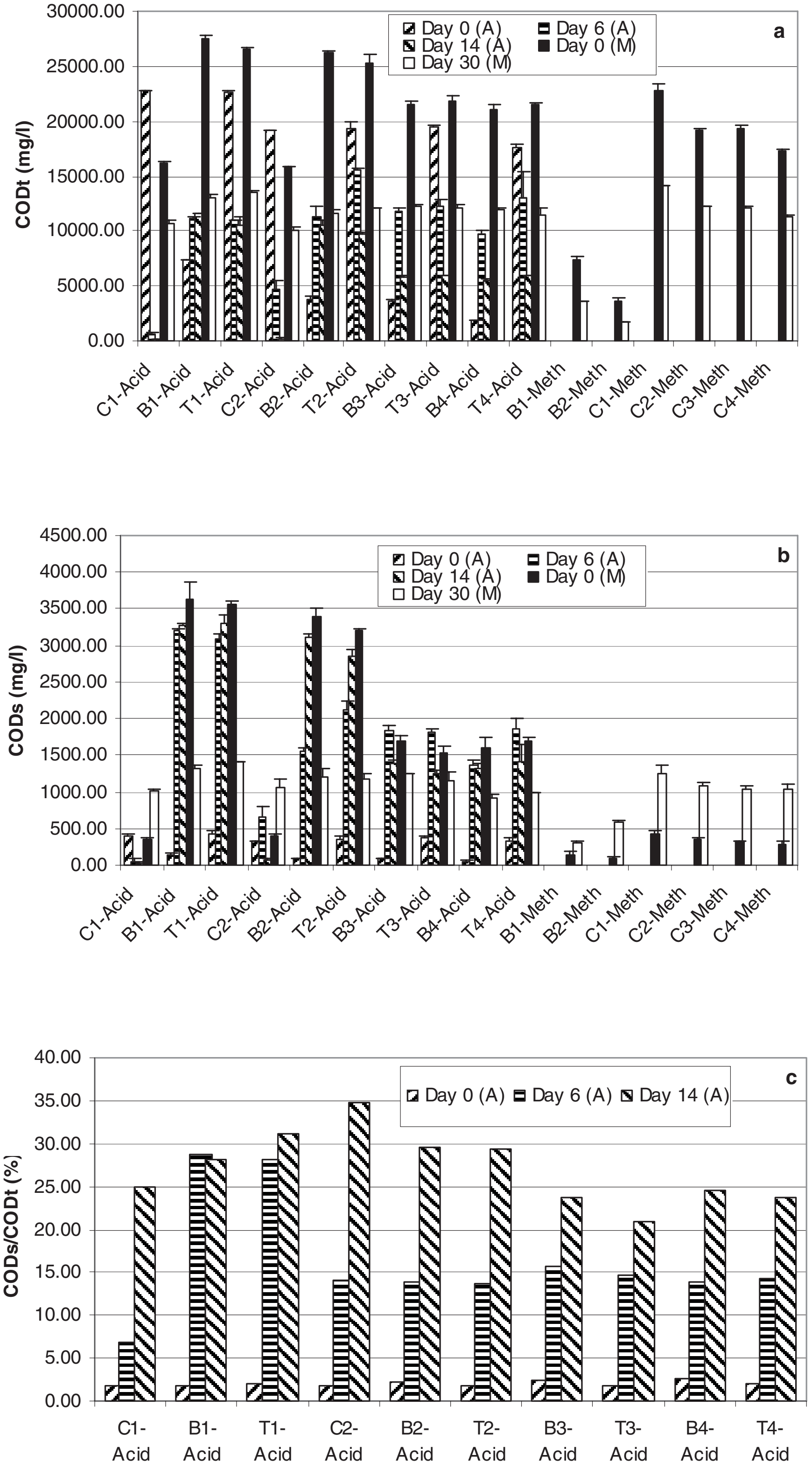

FIG. 2. Total and soluble chemical oxygen demand (COD) concentrations and the percentages of soluble to total COD in acidifying reactors.

reactors with an HRT (or SRT) of 2 days, most of the solubilization was achieved within the first 6 days due to high solids loading rates which accelerated the acidification rate. However, for the reactors with an HRT (or SRT) of 4 days, the rate solubilization was relatively slower. For example, CODs for T2 increased from the initial value of 393 mg/L to 2,208 and 2,910 mg/L on days 6 and 14, respectively.

It has to be noted at this point that even though anaerobic acidification increases the CODs values through hydrolysis, there is also parallel microbial CODs consumption due to the incomplete separation of acidogenic and methanogenic phases as will be discussed below. Therefore, the CODs values reported in Fig. 2b is the result of these two main microbial mechanisms.

The headspace gas in acidogenic reactors contained 7–22% methane. Ideally, the methane content in the headspace gas produced in acidifying reactors should be negligible. In practice, however, varied amounts of methane of up to 30% have been detected in acid-phase digesters (Eastman *et al.*, 1981; Ghosh, 1987; Shana *et al.*, 2005; Yilmaz and Demirer, 2008). This may be due to either incomplete separation of the two phases, which results in the coexistence of heterotrophic methane producers, or the presence of certain fast-growing autotrophic methanogenic organisms such as *Methanobacterium*, or both. The HRT (or SRT) values applied in this study (2 and 4 days) were not favorable for the most sensitive anaerobic bacteria type known as methanogens. However, the methane production at such low SRTs could be explained by unintentional extended retention times of micro-organisms in the reactors due to very high solids concentration and thus lack of homogeneity during daily wasting of sludge (Ghosh, 1985, 1987).

A simple completely stirred tank reactor (CSTR) model was used to demonstrate the CODt reduction as well as the microbial hydrolysis of the particulate organic matter in the acidifying reactor. In this CSTR model, each reactor was assumed as operating under feeding and wasting process without any microbial production/consumption of substrate in terms of COD (Fig. 3). This model was not used to predict the experimental COD concentrations in the reactor. On the contrary, the difference between the model output and the experimental data indicate the reduction of CODt (Fig. 3a) and the increase of CODs due to hydrolysis (Fig. 3b) in the acidifying reactor. As an illustrative example, the model was run for T1. The model outputs as well as the total and soluble COD concentrations are depicted in Fig. 3. The model output predicts that the initial CODt concentrations of 22,410 and 22,690 mg/L for duplicate T1 reactors, are reduced at a rate determined by the HRT of the reactor (2 days) and reaches their steady state value of 14,550 ± 250 mg/L (or the CODt of the feed) in around 8 days. However, the effluent COD values observed for these reactors on days 6 and 14 are considerably lower than what the model predicted. On average, the effluent COD concentration in T1 was 10,550 ± 590 mg/L on day 14. This value corresponds to 27.5% reduction in CODt. Meanwhile, when Fig. 3b is considered, when no microbial interaction of the substrate is considered, the CODs value on day 14 would be 280 mg/L. However, the experimental data indicated that the average CODs value in reactors T1 on day 14 was 3,290 ± 90 mg/L.

As mentioned above, the increase in CODs (3,290 mg/L) is through the hydrolysis in the acidifying reactor. When the CODt reduction in duplicate reactors of T1 is considered along with the presence of methane in the headspace gas produced, it was concluded that there was an unintentional methanogenic activity in the acidogenic reactors due to the incomplete separation of phases. This observation is parallel to the relevant literature (Eastman *et al.*, 1981; Ghosh, 1987; Yilmaz and Demirer, 2008). The CODt reductions in all acidogenic reactors ranged between 18.2 and 33.3% (Table 6).

VA production is another important parameter indicating the performance of acidifying cultures. Therefore, VA analyses were conducted in the effluents of the acidifying reactors as well their feeds namely WAS and WAS($^1/_2$) on day 13 of the operation (Fig. 4). As it is clear from Fig. 4, the VA for-

TABLE 6. CODT REDUCTION IN ACIDOGENIC AND BMP REACTORS

Reactor	*CODt removal (%)*	
	Preacidification	*BMP assay*
B1-Acid	20.1	52.2
T1-Acid	27.5	49.2
B2-Acid	27.6	55.4
T2-Acid	33.3	52.4
B3-Acid	20.0	43.5
T3-Acid	18.2	45.0
B4-Acid	25.2	43.2
T4-Acid	18.6	46.7
C1-Meth		38.4
C2-Meth		36.3
C3-Meth		37.0
C4-Meth		34.8

COD, chemical oxygen demand.

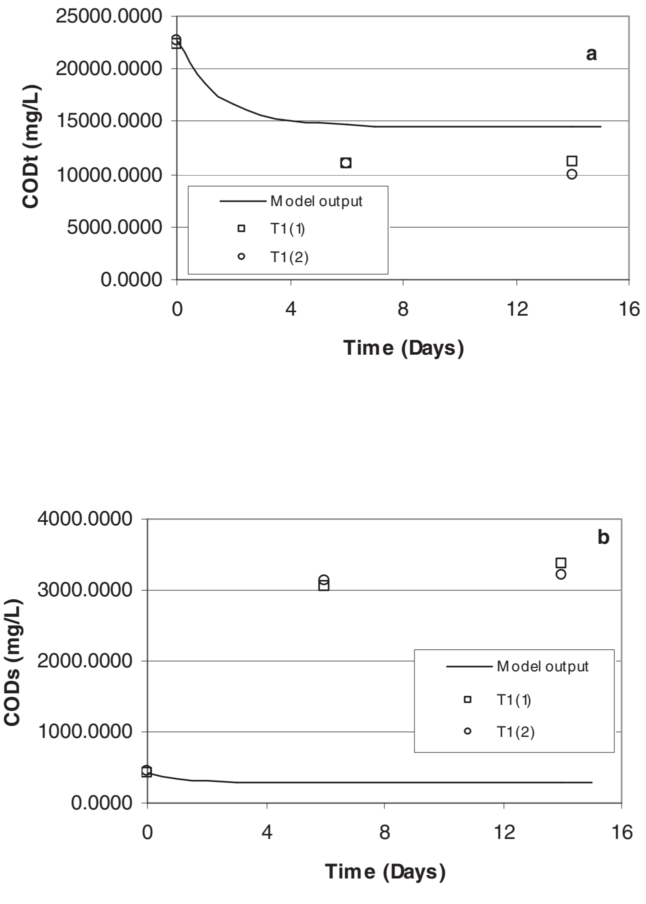

FIG. 3. Change in (**a**) total and (**b**) soluble COD concentrations in duplicate T1 reactors.

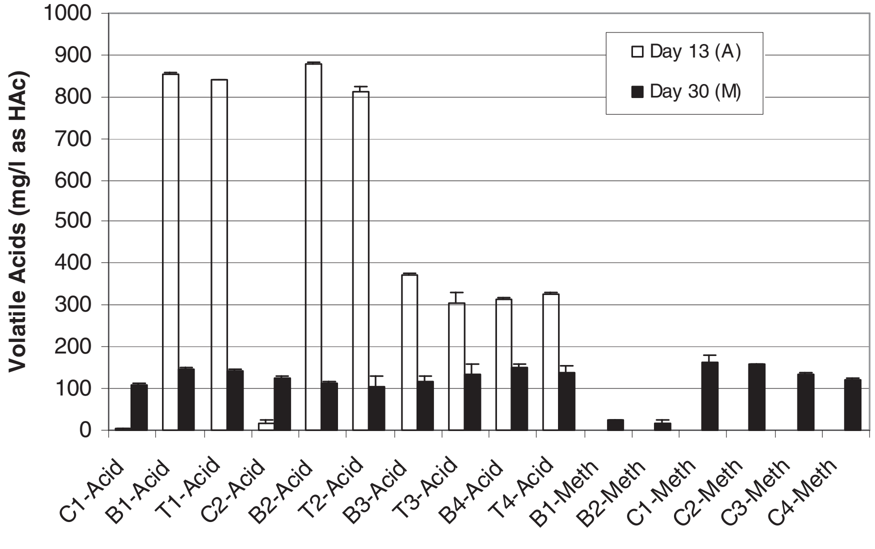

FIG. 4. Volatile acids concentrations.

mation is due to the acidifying activity because the VA concentration in the control reactors were insignificant (less than 24 mg/L). In all the other reactors, the VA formation (281–881 mg/L) was much higher than that of the feed WAS solutions (52–86 mg/L). This indicated that anaerobic acidification increased the VA concentration in the WAS and WAS($^1/_2$) by 6.3 and 9.8 times, respectively. The VA formation in reactors T1 and T2 was considerably higher (799–881 mg/L) than in reactors T3 and T4 (281–375 mg/L) (Fig. 4). This observation, which was similar with the CODs/CODt ratios (Fig. 2c) was expected for the reactors with both higher SLRs and lower HRTs (or SRTs) (Ghosh, 1987; Shin *et al.*, 2001). However, despite its higher HRT, reactor T2 had higher VA (799–881 mg/L) concentration than reactor T3 (281–375 mg/L). If the feeds for these reactors are considered (Table 4), it will be seen that reactor T2 was fed with undiluted WAS while reactor T3 was fed with $^1/_2$ diluted WAS. In order words, the influent COD concentration for reactor T2 was twice as much compared to that of T3. Therefore, it can be stated that higher influent substrate strength applied to T2 resulted in a higher acidification thus VA formation relative to T3 (Fig. 4). Hence, it must be underlined that the solids loading rate applied to both of these reactors (1.97 g VS/L · day) was relatively low and a similar observation may not necessarily be observed for higher solids loading rates.

The degree of acidification is also commonly used (Dinopoulou *et al.,* 1988; Fang and Yu, 2001; Demirel and Yenigun, 2004; Yilmaz and Demirer, 2008) to assess the extent of acidification. Acidifying reactors and their acidification performances were also compared in terms of the degree of acidification and depicted in Fig. 5. The degree of acidification was calculated by taking the ratio of COD-equivalent of VAs produced and the total COD of the influent wastewater (Dinopoulou *et al.*, 1988). The degree of acidification values (4.48–6.43%) obtained in this study is lower than stated in the relevant literature. For example, in a study of two-phase anaerobic digestion of a mixture of fruit and vegetable wastes, Bouallagui *et al.* (2004) reported acidification yields of 38.9–44.4% for acidifying reactors that were operated at an HRT of 3 days. Dinopoulou *et al.* (1988) investigated the influence of various operational parameters the anaerobic acidification of a complex substrate based on beef extract. The initial COD concentrations and hydraulic reten-

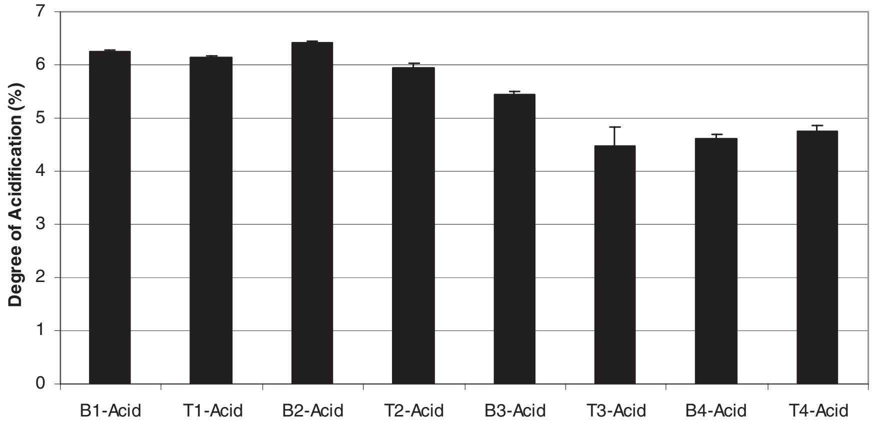

FIG. 5. Degree of acidification in acidogenic reactors.

tion times identified as 3 g/L and 6 h, respectively, the degree of acidification achieved was between 30 and 60%. Furthermore, Demirel and Yenigun (2004) reported the degree of acidification values for the acidification of dairy wastewater as 20–60%. The reason of obtaining these low acidification values is thought to be the low SLRs used in the study.

Similar to the CODs/CODt ratios (Fig. 2c) and VA concentrations (Fig. 4), the degree of acidification values are higher for reactors T1 and T2 (5.85–6.29%) than for reactors T3 and T4 (4.48–4.76%) (Fig. 5).

Biochemical methane potential (BMP) assay

After the acidification reactors reached to steady-state conditions, the preacidified WAS samples (or the effluents of thermophilic acidogenic anaerobic reactors) and raw WAS samples (or the feeds to the thermophilic acidogenic anaerobic reactors; see Table 4) were subjected to BMP assay (Table 5).

The BMP assay was run for 30 days, at the end of which all the reactors were analyzed for CODt, CODs, and VA (Figs. 2a, b and 4). When the CODt results are considered (Table 6), it is observed that all the preacidified WAS samples (T1-Acid, T2-Acid, T3-Acid, and T4-Acid) has led to increased CODt removals than their corresponding feeds (C1-Meth, C2-Meth, C3-Meth, and C4-Meth). The difference between the CODt removals of preacidified and raw WAS samples were more pronounced for reactors T1 and T2 (13.8 and 16.1%, respectively) than T3 and T4 (8.0 and 11.9%, respectively). This observation agrees with higher VA and CODs/CODt ratios observed for the same reactors as discussed above.

The increase in the CODt removal by up to 16.1% observed during the BMP assay is an important enhancement of the anaerobic biodegradability of WAS. However, when the CODt removal observed for the same samples during acidogenesis along with the methane production is considered, the net effect of preacidification on the process can better be evaluated. This is in agreement with Ghosh (1987), who suggested that the methane from acidification phase could be transmitted to methanogenic phase of the system to increase the overall system efficiency. So, when the total CODt removals are considered (Table 6), it will be seen that preacidification has led to 38.3, 49.4, 26.2, and 30.5% additional CODt removals in reactors T1, T2, T3, and T4, respectively, relative to reactors fed with nonpreacidified WAS. It is apparent from these results that beneficial effect of preacidification on CODt removal (Table 6) is sustained for low SLRs (0.98–3.93 g VS/L · day). The corresponding values obtained are comparable to the studies reported in Table 1.

Conclusions

This study investigated the performance of thermophilic anaerobic preacidification of WAS prior to conventional mesophilic (methanogenic) anaerobic digestion. The aim was to observe whether the advantages of anaerobic phase separation which was clearly seen for high SLRs (6.5–28.9 g VS/L · day) can still be exploited at low SLRs (0.98–3.93 g VS/L · day). The following conclusions can be drawn based on the experimental results of this study:

Thermophilic preacidification of WAS at 0.98–3.93 g VS/L · day of SLR and 2–4 days of HRT resulted in 18.2–33.3% CODt reduction and 20.9–34.8% increase in dissolved (soluble) COD concentration in acidogenic reactors. This corresponded to 6.3–9.8 times higher acidification levels relative to unacidified WAS feed samples. When the preacidified WAS samples were subjected to BMP assay along with unacidified WAS samples, it was observed that 8–16.1% additional CODt removal or gas production was observed for preacidified samples. When the CODt removals observed for preacidification and BMP were both considered, preacidification (or phase separation) has led to 26.2–49.4% extra CODt removal.

It is apparent from these results that beneficial effect of preacidification on CODt removal is sustained for low SLRs (0.98–3.93 g VS/L · day).

Acknowledgments

The Department of Education, Science and Training of the Australian Government is appreciated to support Dr. Goksel N. Demirer's visit to the RMIT University through the Endeavour Research Fellowship. The authors would also like to thank Dr. Sibel Uludag-Demirer for her assistance in the analyses. At the time of the study, the first author was affiliated with the School of Civil, Environmental and Chemical Engineering, RMIT University, Victoria 3001, Australia.

Author Disclosure Statement

The authors declare that no competing financial interests exist.

References

American Public Health Association. (1998). *Standard Methods for the Examination of Water and Wastewater* (19th ed.). Washington, DC: United Book Press.

Bhattacharya, S.K., Madura, R.L., Walling, D.A., and Farrell, J.B. (1996). Volatile solids reduction in two-phase and conventional anaerobic sludge digestion. *Water Res.* 30, 1041.

Bolzonella, D., Pavan, P., Zanette, M., and Cecchi, F. (2007). Two-phase anaerobic digestion of waste activated sludge: Effect of an extreme thermophilic prefermentation. *Ind. Eng. Chem. Res.*

Bouallagui, H., Torrijos, M., Godon, J.J., Moletta, R., Ben-Cheikl, R., Touhami, Y., Delgenes, J.P., and Hamdi, M. (2004) Two-phase anaerobic digestion of fruit and vegetables waste: bioreactors performance. *Biochem. Eng. J.* 21, 193.

Borchardt, J.A. (1971). Anaerobic phase separation by dialysis technique. In *Anaerobic Biological Treatment Processes, Advances in Chemistry Series No 105.* Washington, DC: American Chemical Society, p. 108.

Bougrier, C., Carrere, H., and Delgenes, J.P. (2005). Solubilisation of waste-activated sludge by ultrasonic treatment. *Chem. Eng. J.* 106, 163.

Cheunbarn, T., and Pagilla, K.R. (2000). Anaerobic thermophilic/mesophilic dual stage sludge treatment. *J. Environ. Eng.* 126, 796.

Choi, H., Jeong, S.W., and Chung, Y. (2006). Enhanced anaerobic gas production of waste activated sludge pretreated by pulse power technique. *Bioresource Technol.* 97, 198.

Delgenes, J.P., Penaud, V., and Moletta, R. (2003). Pretreatments for enhancement of anaerobic digestion of solid waste. In J. Mata-Alvarez Ed., *Biomethanization of the Organic Fraction of Municipal Solid Wastes,* Chapter 8. London: IWA Publishing, p. 201.

Demirel, B., and Yenigun, O. (2004). Anaerobic acidogenesis of dairy wastewater: The effects of variations in hydraulic retention time with no pH control. *J. Chem. Technol. Biotechnol.* 79, 755.

Demirer, G.N., and Chen, S. (2004). Effect of retention time and organic loading rate on anaerobic acidification and biogasification of dairy manure. *J. Chem. Technol. Biotechnol.* 79, 1381.

Demirer, G.N., and Chen, S. (2005). Two-phase anaerobic digestion of unscreened dairy manure. *Proc. Biochem.* 40, 3542.

Dinopoulou, G., Rudd, T., and Lester, J.N., (1988). Anaerobic acidogenesis of a complex wastewater: I. The influence of operational parameters on reactor performance. *Biotechnol. Bioeng.* 31, 958.

Eastman, J.A., and Ferguson, J.F. (1981). Solubilisation of organic carbon during the acid phase anaerobic digestion. *J. WPCF* 53, 352.

Ergüder, T.H., Güven, E., and Demirer, G.N. (2000). Anaerobic treatment of olive mill wastes in batch reactors. *Process Biochem.* 36, 243.

Fang, H.H.P., and Yu, H.Q. (2001). Acidification of lactose in wastewater. *J. Environ. Eng.* 127, 825.

Fox, P., and Pohland, F.G. (1994). Anaerobic treatment applications and fundamentals: substrate specificity during phase separation. *Water Environ. Res.* 66, 716.

Gavala, H.N., Yenal, U., Skiadas, 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. *Water Res.* 37, 4561.

Ghosh, S. (1985). Solid-phase methane fermentation of solid wastes. *Trans. ASME* 107, 402.

Ghosh, S. (1987). Improved sludge gasification by two-phase anaerobic digestion. *J. Environ. Eng.* 113, 1265.

Ghosh, S., and Klass, D.L. (1977). Two phase anaerobic digestion. US Patent No 4,022,665.

Ghosh, S., Ombregt, J.P., and Pipyn, P. (1985). Methane production from industrial wastes by two-phase anaerobic digestion. *Water Res.* 19, 1083.

Guerrero, L., Omil, F., Mendez, R., and Lema, J.M. (1999). Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein. *Water Res.* 33, 3281.

Gungor-Demirci, G., and Demirer, G.N. (2004). Effect of initial COD concentration, nutrient addition, temperature and microbial acclimation on anaerobic treatability of broiler and cattle manure. *Bioresource Technol.* 93, 109.

Kennedy, K.J., Thibault, G., and Droste, R.L. (2007). Microwave enhanced digestion of aerobic SBR sludge. *Water SA* 33, 261.

Li, Y.Y., and Noike, T. (1992). Upgrading of anaerobic digestion of waste activated sludge by thermal pretreatment. *Water Sci. Technol.* 26, 857.

Lin, J.G., Chang, C.N., and Chang, S.C. (1997). Enhancement of anaerobic digestion of waste activated sludge by alkaline solubilization. *Bioresource Technol.* 62, 85.

Lu, J., and Ahring, B.K. (2005). Effects of temperature and hydraulic retention time on thermophilic anaerobic pretreatment of sewage sludge. In *Proceedings Anaerobic Digestion of Solid Waste*, Copenhagen (Denmark), Aug 31–Sept 2, p. 159.

Nah, LW., Kang, Y.W., Hwang, K.Y., and Song, W.K. (2000). Mechanical pretreatment of waste activated sludge for anaerobic digestion process. *Water Res.* 34, 2362.

Park, B., Hahn, J., and Hwang, S. (2004). Use of microwave pretreatment for enhanced anaerobiosis of secondary sludge. *Water Sci. Technol.* 50, 17–23.

Oles, J., Dichtl, N., and Niehoff, H.H. (1997). Full scale experience of two stage thermophilic/mesophilic sludge digestion. *Water Sci. Technol.* 36, 449.

Owen, W.F., Stuckey, D.C., Healey, J.B., Young, L.Y., and Mccarty, P.L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Res.* 13, 485.

Ray, B.T., Rajan, R.V., and Lin, J.G. (1990). Low-level alkaline solubilization for enhanced anaerobic digestion. *Res. J. WPCF* 62, 81–87.

Roberts, R., Le, S., and Forster, C.F. (1999). A thermophilic/mesophilic dual digestion system for treating waste activated sludge. *J. Chem. Technol. Biotechnol.* 74, 445.

Shana, A., Asaadi, M., Morchoisne, T., and Marsh, P. (2005). *Acid Phase Digestion Pre-treatment and Its Impact on Conventional Mesophilic Anaerobic Digestion.* Swindon: Thames Water. http://www.thameswater.co.uk/en_gb/Downloads/PDFs/Paper_treatment_3_Ashan a.pdf

Shin, H.S., Han, S.K., Song, Y.C., and Lee, C.Y. (2001). Performance of UASB reactor treating leachate from acidogenic fermenter in the two-phase anaerobic digestion of food waste. *Water Res.* 35, 3441.

Solera, R., Romero, L.I., and Sales, D. (2002). The evolution of biomass in a two-phase anaerobic treatment process during start-up. *Chem. Biochem. Eng. Q.* 16, 25.

Speece, R.E. (1996). *Anaerobic Biotechnology for Industrial Wastewaters.* Barnaby, Canada: Archae Press.

Takashima, M., Kudoh, Y., and Tabata, N. (1996). Complete anaerobic digestion of activated sludge by combining membrane separation and alkaline heat post-treatment. *Water Sci. Technol.* 34, 477.

Tanaka, S., Kobayashi, T., Kamiyama, K.L, and Bildan, M.L.N.S. (1997). Effects of thermochemical pretreatment on the anaerobic digestion of waste activated sludge. *Water Sci. Technol.* 35, 209.

Tiehm, A., Nickel, K., Zellhorn, M., and Neis, U. (2001). Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. *Water Res.* 35, 2003.

Vlyssides, A.G., and Karlis, P.K. (2004). Thermal-alkaline solubilization of waste activated sludge as a pre-treatment stage for anaerobic digestion. *Bioresource Technol.* 91, 201.

Watts, S., Hamilton, G., and Keller, J. (2005). Two-stage thermophilic-mesophilic anaerobic digestion of waste activated sludge from a biological nutrient removal plant. *Water Sci. Technol.* 53, 149.

Weemaes, M., Grootaerd, H., Simoens, F., and Verstraete, W. (2000). Anaerobic digestion of ozonized biosolids. *Water Res.* 34, 2330.

Yeom, I.T. (2002). Effects of ozone treatment on the biodegradability of sludge from municipal wastewater treatment plants. *Water Sci. Technol.* 46, 421.

Yilmaz, V., and Demirer, G.N. (2007). Improved anaerobic acidification of unscreened dairy manure. *Environ. Eng. Sci.* 25, 309.