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Mixing effect on thermophilic anaerobic digestion of source-sorted organic fraction of municipal solid waste

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ABSTRACT

This paper examines the effect of mixing on the performance of thermophilic anaerobic digestion of source-sorted organic fraction of municipal solid waste during the start-up phase and in the absence of an acclimated seed. For this purpose, two digesters were used under similar starting conditions and operated for 235 days with different mixing schemes. While both digesters exhibited a successful startup with comparable specific methane yield of 0.327 and 0.314 l CH_4/g VS, continuous slow stirring improved stability by reducing average VFA accumulation from 2890 to 825 mg HAc/l, propionate content from 2073 to 488 mg/l, and VFA-to-alkalinity ratio from 0.32 to 0.07. As a result, the startup with slow mixing was faster and smoother accomplishing a higher loading capacity of 2.5g VS/l/d in comparison to 1.9g VS/l/d for non-mixing. Mixing equally improved microbial abundance from 6.6 to 10 g VSS/l and enhanced solids and soluble COD removal.

1. Introduction

Food waste is an attractive source of renewable energy if micro-organism-based bioenergy technologies such as anaerobic digestion (AD) can successfully convert it to methane. While early work in AD has focused on mesophilic temperature regimes (35–40 °C), considerable efforts are targeting thermophilic temperatures (55–60 °C) to increase organic loading rate, gas production, and resistance to foaming, as well as enhance biodegradation of organics (Zabranska et al., 2002). However, thermophilic AD is hindered by operational difficulties and instability problems often connected with poor startup (Angelidaki et al., 2006). Indeed, successful and fast start-up is considered a challenging obstacle because of the lack of acclimated thermophilic seeds often leading to instability and failure of the system. Even where anaerobic systems are well established, the number of thermophilic digesters is still limited and it is relatively difficult to acquire sufficiently large quantities of acclimated seed for starting up new thermophilic digesters (Suwannoppadol et al., 2011).

At the micro-environment level, successful startup and stability of anaerobic digesters are highly affected by: (1) the degree of contact between the microbial consortia and the substrate, and (2) the interaction between methanogens and their syntrophs. Both are primarily a function of the mixing scheme in the reactor (Karim et al., 2005). However, the effect of mixing on microbial dynamics and digester parameters is still unclear (Ward et al., 2008). Consequently, the need for adequate mixing has been supported by many researchers (Bridgeman, 2012; Conklin et al., 2008; Elnekave et al., 2006; Gerardi, 2003; Halalsheh et al., 2011; Karim et al., 2005; Zabranska et al., 2002) and, concurrently, questioned by many others (Gomez et al., 2006; Ike et al., 2010; Kaparaju et al., 2008; Kim et al., 2002; Steinberg and Regan, 2011; Stroot et al., 2001; Suwannoppadol et al., 2011; Ward et al., 2008). The impact of mixing on startup of mesophilic co-digestion systems and thermophilic digesters fed with acetate was addressed by Stroot et al. (2001) and Suwannoppadol et al. (2011), respectively. However to date, mixing effects during startup of thermophilic digesters relying entirely on source-sorted organic fraction of municipal solid waste (SS-OFMSW), in the absence of an acclimated seed have not been reported. Therefore, the objective of this study is to assess the effect of mixing on the performance of thermophilic AD treating SS-OFMSW during the start-up phase, using cattle manure as a seeding source.

2. Methods

2.1. Waste collection and preparation

The SS-OFMSW was collected from restaurants and food markets in two consecutive batches. The waste was ground and

Abbreviations: AD, anaerobic digestion; HRT, hydraulic retention time; IA, intermediate alkalinity; OLR, organic loading rate; OFMSW, organic fraction of municipal solid waste; PA, partial alkalinity; TA, total alkalinity; TDS, total dissolved solids; TS, total solids; VFA, volatile fatty acids.

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homogenized in a food processor, then stored at −20 °C in 150 ml bottles for subsequent use. The feed was prepared in two consecutive batches: Batch 1 and Batch 2 (Table 1). The purpose of early collection and storage of FW samples is to reduce fluctuations in substrate composition. Prior to use, the food samples were thawed and diluted with distilled water to a total solids (TS) content of 7.5–8.0%.

2.2. Seeding and operation

Fresh manure samples were collected from a cattle rearing farm a few hours after excretion and transported immediately on ice to the lab. Two 14-L digesters (Bioflo 110, New Brunswick Scientific Co.) with 9L working volume were inoculated with fresh manure and the temperature was raised to 55 °C on the same day. While a gradual increase in temperature can induce gradual acclimation of the non-thermophilic inoculums, a one-step temperature increase was reported to be suitable to adapt mesophilic media/seed to thermophilic conditions and to select real thermophiles rather than thermo-tolerant mesophilic organisms (Bouskova et al., 2005). In order to ensure similar initial seed in both digesters, 1.5 kg of manure (TS = 19.8%, VS = 92.1% TS) was mixed with deionized water to 8 l in one digester, incubated for 1 week with daily low feeding rate (15 g waste/day ~0.32 g VS/l/d) then split equally into two digesters (A and B). The digesters were started under similar operational conditions but different mixing schemes. Digester A was run under continuous mixing of 100 rpm to provide a mild stirring power, while digester B was operated at mostly stagnant conditions and mixed for few minutes after feeding and prior to wasting. The organic loading rate (OLR) was step-increased and the hydraulic retention time (HRT), which is equal to solids retention time (SRT) in stirred systems, was step-decreased concomitantly. Increases in OLR and/or decreases in HRT were made slowly (about once every 3 weeks) as long as the methane content in the biogas remains above 50%. Upon disturbance, the feeding was suspended or reduced and HRT was increased (Table 1). Semi-continuous feeding mode was adopted, consisting of batch feeding the reactors (three times per week except in periods of instability) with an amount equal to the wasted volume.

2.3. Monitoring and control methods

Temperature and pH inside the digesters were monitored continuously via built-in probes connected to a data acquisition system. Total biogas yield and gas composition (CH_4 and CO_2) were measured on a daily basis using the water displacement method and a dual wavelength infrared cell with reference channels (GEM-2000 monitor, Keison Products, UK), respectively. Specific methane yield, which was calculated as daily methane produced divided by the amount of VS fed to the digester, was used to monitor the efficiency of the digesters.

Table 1
Physico-chemical characteristics of SS-OFMSW.

Parameter	Batch 1	Batch 2
TS (%)	18.6	19.3
TVS (%)	92.6	95.6
COD_{Total} (mg/l)	276,500	293,250
$COD_{Soluble}$ (mg/l)	119,750	117,600
C (% TS)	44.09	43.15
N (% TS)	3.01	2.57
C:N	15	17
COD:N	2.02	1.69
Phosphorus (% TS)	0.35	0.37
Carbohydrates (% TS)	59	52

In addition, physico-chemical parameters were monitored on a weekly basis including soluble and total COD, using the modification of Standard Methods 5220D procedure using HACH high-range (HR and HR+) COD kits (HACH Company, Loveland, Colorado); total, suspended, dissolved and volatile solids using Standard Methods 2540B and 2540E procedures; ammonia content by spectrophotometry method; and total, partial and intermediate alkalinity by titration (Ripley et al., 1986).

Individual VFAs (acetate, propionate and butyrate) were measured, one to three times per week, using a gas chromatograph (GC) equipped with a flame ionization detector and a 30 m, 0.25 mm, 0.25 μm capillary column (TR-FFAP). Phosphoric acid was added to decrease the pH to 2.0 to avoid losing VFAs and samples were diluted to avoid exceeding the saturation limit of the column. The heating protocol was adopted from Zhang et al. (2009). Nitrogen was used as a carrier gas with a flow rate of 2.6 ml/min. Calibration curves for acetate, propionate, and butyrate were constructed prior to each test. Calibration stock solution and dilutions were performed according to Standard Methods for the Analysis of Water and Wastewater (APHA, AWWA and WPCF, 1998). Total VFAs in anaerobic digesters are composed mainly of acetate, propionate and butyrate, with minor or negligible amounts of the remaining VFAs (Angelidaki et al., 2006). Accordingly, VFA content in the digesters was calculated as the sum of acetate, propionate and butyrate concentrations expressed as mg of acetic acid equivalent per liter (mg HAc/l). The VFA-to-alkalinity (α) ratio was calculated as total VFAs/total alkalinity expressed in equivalents of acetic acid/equivalents of calcium carbonate.

2.4. Preparation of samples for physico-chemical analysis

Samples were centrifuged at 10,000 rpm and the supernatant was filtered over 1.2 μm pore-size filters for the determination of TDS/VDS. The TSS/VSS contents were calculated by subtracting TDS/VDS from TS/VS. The liquid passing the 1.2 μm pore-size filter was further filtered through 0.45 μm syringe filters for the analysis of VFAs, ammonia and soluble COD. All other tests were performed with non-filtered samples. The volatile solids were measured as total solids minus the ash content remaining after complete combustion at 540 °C.

3. Results and discussion

3.1. Startup process and loading conditions

Both digesters were seeded with 4 l of incubated manure solution and filled with distilled water to a volume of 7 l. They were batch-fed at an OLR of 0.45–0.46 g VS/l/d without wasting, until they were filled (operating volume = 9L) on day 32. Then, equal wasting and feeding were initiated. Methane production peaked quickly in both digesters to reach a maximum of ~2 l CH_4/g VS in digester B (days 19 and 20) and ~1 l CH_4/g VS in digester A (days 13–18). The overall average methane production during the first month was higher in digester A (0.60 compared to 0.45 l CH_4/g VS in B). Then production dropped necessitating the reduction of OLR.

Digester A was gradually loaded from 0.28 g VS/l/d (HRT = 240d) on day 33 (i.e. at the initiation of equal wasting/feeding) to 2.5 g VS/l/d (HRT = 47d) on day 159, with no instability occurrences. On day 167, VFAs increased drastically from an average of 825 to about 10,319 mg HAc/l. The specific biogas and methane yields dropped to 0.28 and 0.10 l/g VS, respectively, and the CO_2-to-CH_4 ratio increased from an average of 0.4 to 1.32 indicating a system upset. Accordingly, feeding was suspended for 5 days then resumed at a low rate (0.31 g VS/l/d) and high retention time (173d) for 2 weeks. The high VFAs content persisted with a noticeable increase in acetate, from 1270 mg/l on day 174 to 6333 mg/l on day 191. Feeding was suspended once again for 16 days and resumed on day 203 with an OLR of 0.24 g VS/l/d, then reduced further to 0.10 g VS/l/d due to the persisting high VFAs. For the last 31 days, the average specific methane yield reached a low of 0.11 l/g VS necessitating the termination of the experiment on day 235 (Fig. 1a; Table 1).

Digester B was initially loaded at the same rate as digester A. On day 125, the methane content in the biogas dropped from an average of 52% (OLR = 1.35 g VS/l/d) to 43% and the VFA concentration increased to about 4464 mg HAc/l, leading to a drop in pH from 7.40 to 7.17. Even though the pH value was still within the optimum range for AD, the pH drop and VFA rise reflects potential instability due to VFA accumulation. Therefore, the feed was stopped for 2 days and resumed at a lower rate (0.8 g VS/l/d). As a result, digester B remained lagging, in terms of OLR, with respect to digester A for the rest of the experiment. The loading rate was increased again in a step-wise mode until reaching a value of about 1.9 g VS/l/d at an HRT of 46 days. When the load was further increased to 2.1 g VS/l/d, the VFA concentration almost doubled and the specific methane yield decreased to its lowest value since the initiation of incremental loading, about 4 months earlier, which necessitated stopping the feeding on day 190. After 12 days, the feeding was resumed at a low rate of 0.8 g VS/l/d, which was further reduced to 0.6 g VS/l/d for 25 days due to persisting high VFA levels. The experiment was terminated on day 242 (Fig. 1b, Table 1).

3.2. Reactors behavior prior to overload

3.2.1. Biogas composition

The specific methane yield peaked in both digesters during the first month after seeding (i.e. corresponding to the filling period) to reach 0.60 and 0.45 l CH_4/g VS in digesters A and B, respectively, as a consequence of the degradation of organic material in the initial seed and accumulated substrate during the filling process. Upon initiation of wasting/feeding, the specific methane yield in digester A decreased to 0.21 l CH_4/g VS and then progressively increased to a maximum average of 0.36 l CH_4/g VS at OLR of 0.84 g VS/l/d and an HRT of 130 days on days 89–110 and gradually decreased afterwards (Fig. 1c). In digester B, biogas decreased to 0.26 3 CH_4/g VS upon initiation of the wasting/feeding process then decreased further to about 0.18 l CH_4/g VS upon system upset and load suspension. Afterwards, methane production increased progressively to reach a maximum of 0.35 l CH_4/g VS at OLR of 0.79 g VS/l/d and HRT of 87 days on days 128–144 and gradually decreased afterwards (Fig. 1d). The maximum methane production rate was 5.29 and 5.10 l/d in digesters A and B, respectively, at OLR ~1.9 g VS/l/d.

In digester A, the ratio of CO_2-to-CH_4 increased continuously throughout the experiment, except for periods of low- or no-loading (Fig. 1c). Similarly, the CO_2-to-CH_4 profile of digester B is consistent with the loading pattern. It increased continuously during progressive loading and dropped upon suspension of feeding on day 126. When loading was resumed, the CO_2-to-CH_4 ratio increased again until reaching a maximum of 0.83 prior to the second suspension on day 190, then dropped again (Fig. 1d).

3.2.2. Volatile fatty acids

In digester A, total VFA concentrations ranged between 174 and 2505 mg HAc/l, with an average of 825 mg HAc/l. While there is no consensus about inhibitory VFA limits, thermophilic digesters treating OFMSW have been successfully operated at concentrations as high as 3000 mg HAc/l (Angelidaki et al., 2006). Therefore the VFAs content was considered acceptable as long as average methane in the biogas does not drop below 50%. Acetate and propionate concentrations ranged from 115 to 936 mg/l and 57 to 1951 mg/l,

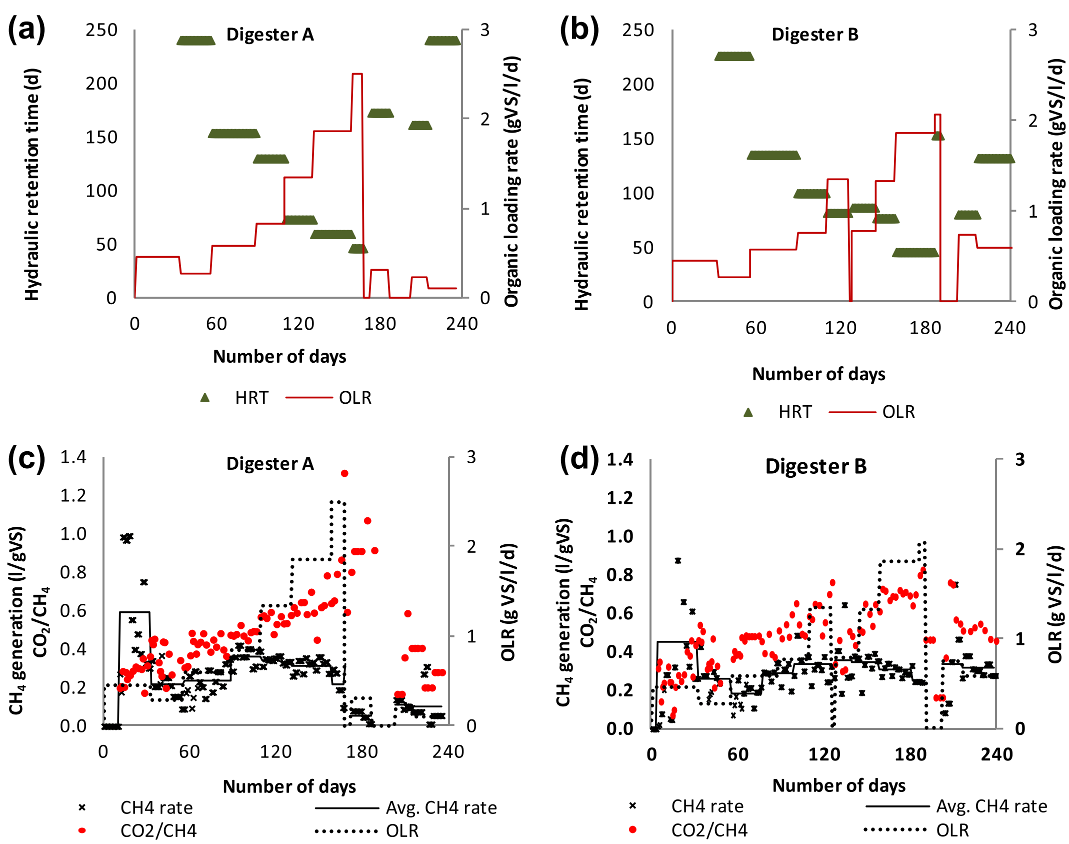

Fig. 1. Loading conditions and gas composition.

respectively (Fig. 2a). Butyrate was present at low concentrations (5–138 mg/l).

VFA concentrations in digester B were significantly higher than those in digester A, with propionic acid being the major contributor – typical under thermophilic conditions (Banks et al., 2008; Ike et al., 2010; Kim et al., 2002). As such, the propionate-to-acetate ratio was relatively high (average = 1.7). However, alkalinity levels were equally high (average = 10,500 mg $CaCO_3$/l) to buffer the large VFA concentration, resulting in a pH between 6.8 and 7.7 (average = 7.4). Furthermore, at low OLR (below 1.9 g VS/l/d), the high VFA levels (average = 2890 mg HAc/l, maximum = 8729 mg HAc/l) did not inhibit gas production or biodegradation which is consistent with the findings of Banks et al. (2008) who reported that as long as the pH remains within a specific range, inhibition as a result of high VFA does not occur. This is similarly explained by Angelidaki et al. (1993) who reported that as long as VFA accumulation is within the system's tolerance, equilibrium is maintained whereby VFA remains dissociated at the higher pH. This is equally applicable to digester B with a pH of 7.4 and a VFA tolerance of about 5000 mg HAc/l.

3.2.3. Volatile suspended solids

Volatile suspended solids (VSS) concentration is commonly used as an indicator of microbial abundance (Wu et al., 2008; Zhang et al., 2006) and biomass production in different reactors (Hawkes et al., 2007; Kim et al., 2006). At low OLR (0.58 g VS/l/d), VSS content averaged 0.5–0.55% in both digesters. In digester A, VSS steadily increased with the increasing load, indicating an increase in biomass production until reaching a maximum value of about 1% (10 g VSS/l) at the highest OLR (2.5 g VS/l/d) (Fig. 3a). However, in digester B, the VSS pattern was less dependent on the loading rate with an almost constant value of 0.66% (6.6 g VSS/l) on average (Fig. 3b). This can be attributed to the lack of stability and high VFA concentration in digester B resulting in a non-systematic response and hindering of microbial growth under higher OLRs.

3.2.4. Alkalinity

In digester A, the total alkalinity was initially high with an average of 11,501 mg $CaCO_3$/l (Fig. 2c). Similar ranges of high alkalinity have been reported in digesters treating food waste (Banks et al., 2008). Average partial alkalinity (PA, defined as alkalinity due to HCO_3^- species) and intermediate alkalinity (IA, defined as alkalinity due to VFAs) were 7887 and 3614 mg as $CaCO_3$/l, respectively resulting in an average VFA-to-alkalinity (α) ratio of 0.07. The α ratio is commonly used as a stability indicator, with stable digesters operating at an $\alpha < 0.3$ (Sanchez et al., 2005). In general, PA, IA (Fig. 4a) and ammonia increased with the increasing loading rate (Fig. 3c). A statistically significant correlation ($R^2 = 0.8$) is evident between the intermediate-to-total alkalinity ratio (IA/TA) and total VFAs (Fig. 4c).

As to digester B, even though the average alkalinity level (10,479 mg $CaCO_3$/l) was comparable to that in digester A, no clear pattern could be discerned between PA and OLR (Fig. 4b) or between ammonia and OLR (Fig. 3d). Also, the correlation between the IA/TA ratio and total VFAs is not as significant ($R^2 = 0.37$, Fig. 4d) which can be attributed to the lack of stability in digester B reflected by an average α ratio of 0.32 despite the high alkalinity levels.

3.3. System's capacity

The highest efficiency was recorded in digester A at a loading rate of 0.84 g VS/l/d (day 89–109) with an ultimate methane yield of 0.363 l CH4/g VS (Fig. 1c). The maximum OLR reached was

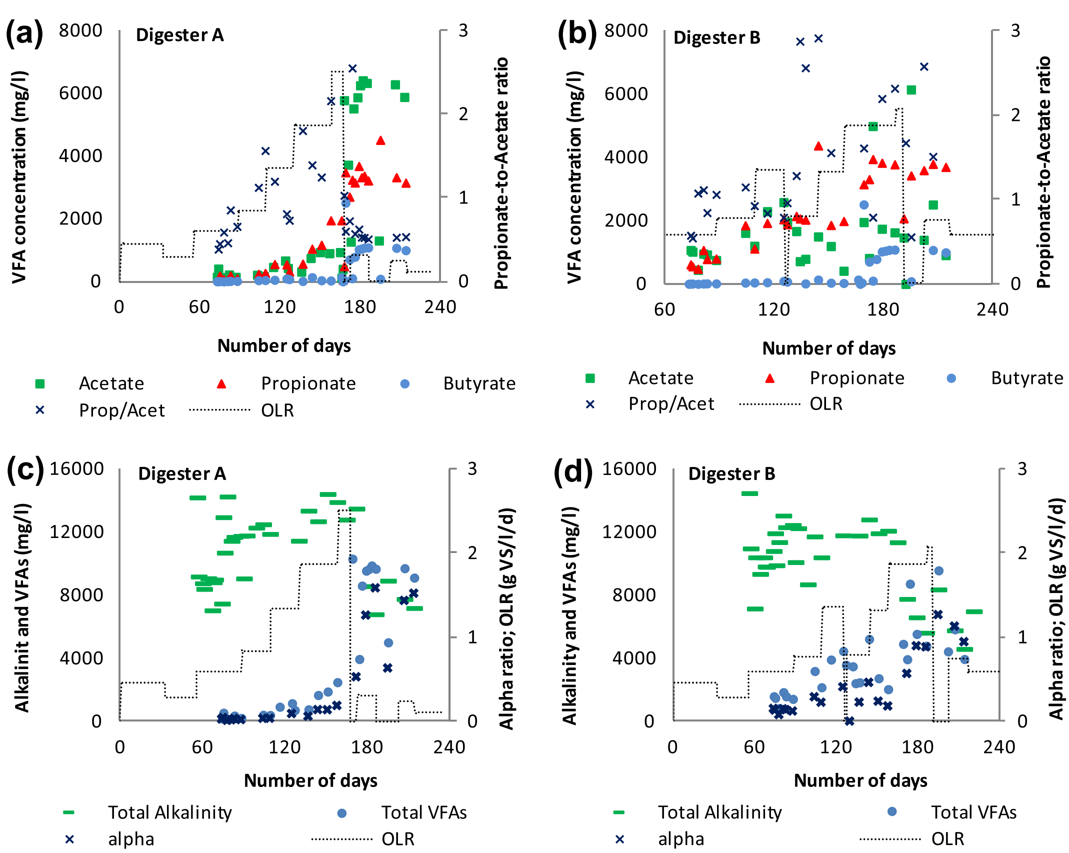

Fig. 2. VFAs, alkalinity and alpha ratio.

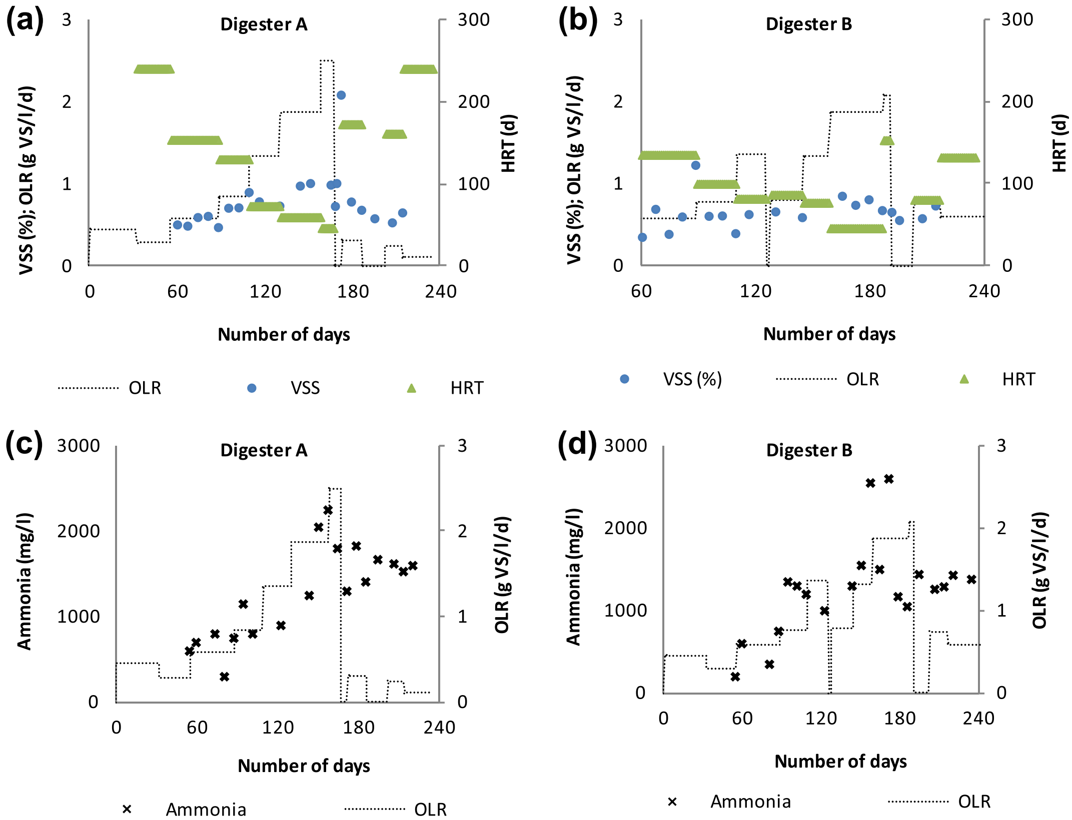

Fig. 3. Volatile suspended solids and ammonia.

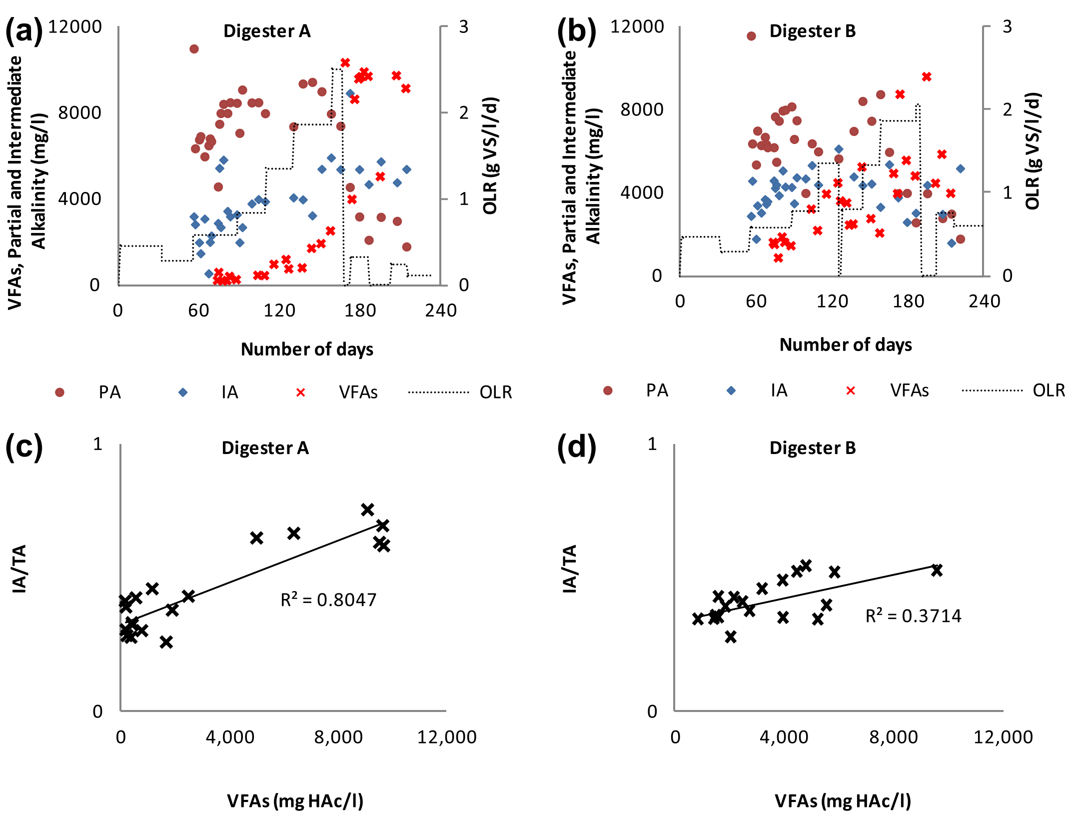

Fig. 4. (a and b) Partial and intermediate alkalinity, (c and d) intermediate-to-total alkalinity ratio (IA/TA) versus VFAs.

2.5 g VS/l/d at an HRT of 47 days, in digester A. However, this loading rate could not be sustained. Indeed, one-stage digesters treating SS-OFMSW have usually low limiting OLR which is commonly attributed to the high biodegradability of SS-OFMSW in comparison to MS-OFMSW (Pavan et al., 2000). The high TVS content of the feed (93–96%, Table 1) is an indication of the high biodegradability of the waste used in this experiment, resulting in fast generation and accumulation of VFAs, potentially leading to inhibitory effects. High ammonia concentrations associated with low C:N ratios, can be another inhibiting factor that limits maximum sustainable OLR (Banks et al., 2008). In this context, the initial C:N ratio of the waste (17:1 and 15:1, Table 2) is lower than the optimum 30:1 ratio resulting in total ammonia levels of 1815 mg/l (182 mg NH_3–N/l) and 1440 mg/l (134 mg NH_3–N/l) in digesters A and B, respectively. These values are close to the 1500 mg/l total ammonia threshold for safe (uninhibited) methanogenic activity in thermophilic AD (Sung and Liu, 2003) which could have induced moderate inhibition. In general, OLR of one-stage thermophilic AD of highly biodegradable substrates should remain low and reported rates vary between 1.6 and 4.3 g VS/l/d (Supporting information Table S1).

3.4. Impact of mixing

Digester A was slowly and continuously mixed at a rate of 100 rpm while digester B was not mixed, except for few minutes after feeding and prior to wasting. Their performance was compared based on methane yield, stability, loading capacity and treatment efficiency and interpreted in light of the mixing intensity and seeding source.

3.4.1. Methane yield

Both digesters exhibited a successful startup with comparable average methane yield of 0.327 l CH_4/g VS (2.819 l/d) and 0.314 l CH_4/g VS (2.504 l/d) for digesters A and B, respectively. This concurs with the findings of Bridgeman (2012) where, at low TDS similar to this experiment, slow mixing (100 rpm) did not improve nor impair the biogas yield. The impact of mixing on gas generation is perceivable only at TS concentrations >10%, with 10–30% higher biogas production in mixed digesters (Karim et al., 2005). Accordingly, the need for mixing in terms of energy input versus methane production becomes of less significance for wet processes, which constitute the majority of currently operating commercial systems.

Table 2
Operating conditions.

Digester A			Digester B		
Days	OLR (g VS/l/d)	HRT (day)	Days	OLR (g VS/l/d)	HRT (day)
0–32	0.45	N/A[a]	0–32	0.46	N/A[a]
33–55	0.28	241	33–55	0.28	227
56–88	0.59	154	56–88	0.58	136
89–109	0.84	130	89–109	0.77	100
110–130	1.35	73	110–125	1.35	82
136–158	1.86	60	126–127	0	–
159–167	2.50	47	128–144	0.79	87
168–172	0	–	145–158	1.32	77
173–186	0.31	173	159–186	1.87	46
187–202	0	–	187–190	2.07	153
203–214	0.24	162	191–202	0	–
215–235	0.10	241	203–216	0.75	81
			217–242	0.59	133

[a] During the first 32 days of the experiment, the feed was added without wasting to reach he target 9L volume.

3.4.2. Stability and loading capacity

While both digesters exhibited a comparable methane yield, differences in system behavior were evident. Prior to overloading, (i.e. day 168 for digester A and day 191 for digester B), the OLR reached a maximum of 2.5 g VS/l/d in digester A, compared to 1.9 g VS/l/d in digester B. Also, continuous slow stirring in digester A improved the system's stability through: (1) reduced accumulation of VFAs, with an average of ~825 mg HAc/l compared to ~2890 mg HAc/l in digester B, (2) lower propionate content, with an average of 488 mg/l in A versus 2073 mg/l in B, and (3) enhanced VFA-to-Alkalinity ratio (α) of about 0.07 in A compared to 0.32 in B. As a result, the startup of digester A was faster and smoother than that of digester B. A similar observation was reported by Pandey et al. (2011) where manure digesters exhibited a higher α ratio and higher VFA concentrations under no-stirring conditions. Accordingly, it is postulated that in the absence of an acclimated seed, slow mixing is helpful in improving the stability and loading capacity of thermophilic digesters treating SS-OFMSW. In fact, mixing is considered essential to (1) ensure adequate contact between nutrients and micro-organisms (Gomez et al., 2006; Ward et al., 2008); (2) to provide uniform heat distribution and efficient dispersion of metabolic waste (Gerardi, 2003); (3) reduce particle size by shear forces, increase waste surface area and improve hydrolysis (Halalsheh et al., 2011); and (4) reduce deposition of heavy particles (Bridgeman, 2012).

3.4.3. Treatment efficiency

Even thought the average TS removal was comparable in both digesters (82.4% in A and 79.9% in B), it was mostly higher (by up to 8%) under mixing conditions (Fig. 5a). Mixing reduced also the dissolved solids (Fig. 5a) by about 23% and soluble COD (Fig. 5b) by about 16%. In fact, Elnekave et al. (2006) and Karim et al. (2005) observed that interrupted mixing creates hydraulic dead zones which can reduce the effective HRT and induce adverse effects on the reaction kinetics. This is particularly true for low solids processes (TDS < 2.5%) where the efficiency of mixing is at its maximum and the volume of low velocity zones is minimum (Bridgeman, 2012). In fact, the work of Zabranska et al. (2002) showed that the absence of mixing reduced the effective digester volume to 40% due to lack of homogeneity and short-circuiting effect.

3.4.4. Mixing intensity

In contrast with the above, Kim et al. (2002) reported markedly superior performance in unmixed digesters treating primary sludge in comparison with continuously stirred reactors. Karim et al. (2005) showed that the absence of mixing shortens and stabilizes the startup stage but reduces biogas production afterwards. Also, Kaparaju et al. (2008) reported higher biogas production under minimal and intermittent mixing compared to continuous mixing and better performance of gently mixed over vigorously mixed systems treating manure. Stroot et al. (2001) addressed the startup and stability of mesophilic co-digestion of OFMSW and sludge under minimal and continuous mixing. They showed that interrupted mixing stabilizes the vigorously shaken digesters and improves gas production, with a concurrent rise in the abundance of methanogens. They concluded that mixing increases the distance between syntrophic partners and, thus, destroys their associations. In fact, propionate accumulation in unstable digesters is commonly linked to increased diffusion distance between syntrophs (Ward et al. (2008). Hence, it can be argued that the absence of mixing can be more beneficial than vigorous mixing. On the other hand, the slowly mixed digester (100 rpm) in this experiment exhibited an overall better startup than the non-mixed digester, which is consistent with the conclusion of Conklin et al. (2008) that acetoclastic activity is improved with the degree of shaking below 200 rpm and the findings of Gomez et al. (2006) who showed that slow mixing

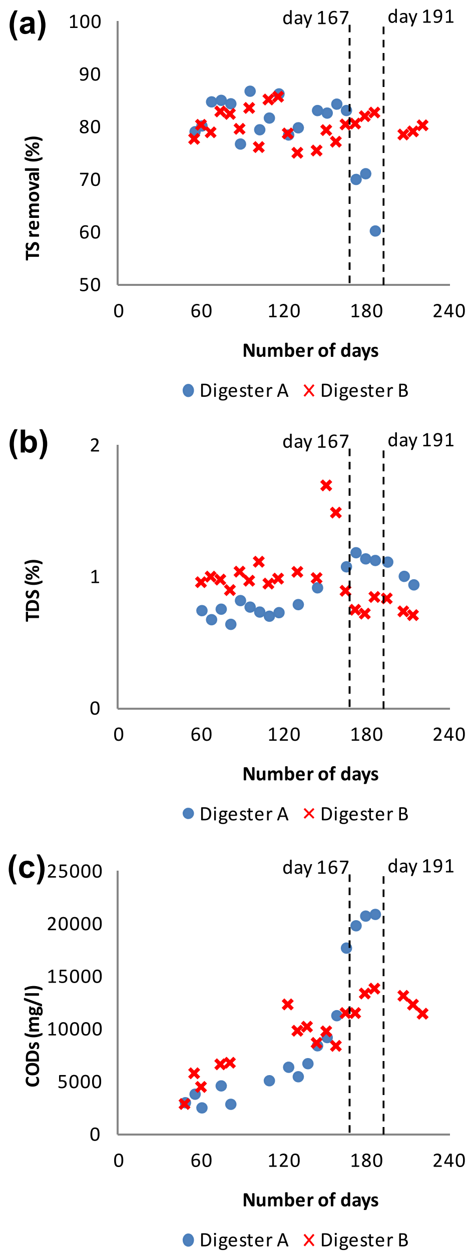

Fig. 5. (a) TS removal, (b) TDS content and (c) soluble COD.

(80 rpm) was better than both vigorous and no-mixing, in co-digestion of primary sludge and food waste. This highlights the need to define optimum mixing conditions and the relationship between mixing and microbial community structure and function.

3.4.5. Seeding source

The impact of mixing is expected to vary with the seeding source and composition (Pandey et al., 2011). In this study, digesters were inoculated with raw cattle manure and they both exhibited instabilities at minor disturbances, which could be due to inadequate diversity of thermophilic methanogens and lack of balance between hydrolytic/acetogenic Bacteria and methanogenic Archea. This was more pronounced in digester B where the high propionate-to-acetate ratio implies insufficient propionate degradation, commonly attributed to accumulation of inhibiting metabolic waste (hydrogen). This problem is likely to be due to low diversity/activity of hydrogen-consuming methanogens (hydrogenotrophs) in the initial seed that is more critical in digester B where hydrogen may accumulate due to insufficient dispersion during stagnant periods, resulting in buildup of propionate. In fact, manure is usually poor in methanogens with lack of organized juxtaposition (micro-colonies) of hydrogenotrops and syntrophic bacteria, namely propionate and butyrate degraders (Pandey et al., 2011). This implies that the diversity and spatial structure of the seed may influence the system's response to mixing. If the initial seed was rich in hydrogenotrophs, syntrophic interactions could be more recurrent and thus the syntrophic pathway could be more efficient in removing H_2, minimizing the need for mixing. This can be achieved by using a seeding mix that contains, in addition to manure, hydrogenotroph-rich seeds such as municipal waste compost and landfill leachate. For instance, methanogenic populations in compost and leachate are known to be dominated by thermophilic hydrogenotrophs (Chen et al., 2003; Neumann and Scherer, 2011). Therefore, they can both (i.e. compost and leachate) be considered for seed enrichment through hydrogenotrophic bioaugmentation. However, the impact of the initial seed on the overall startup process, specifically under mixing or no-mixing conditions, is still not sufficiently clear. While it is reported that seeds have a major impact on microbial diversity (Pandey et al., 2011), the changes in OLR (or HRT) during startup are expected to cause bacterial shifts that can lead to new communities that are considerably different from the initial seed (Chelliapan et al., 2011). Furthermore, operational conditions (digenster type, temperature, mixing and hydraulic/solids retention time) may select specific microbial communities, thus attenuating the impact of the initial seed enrichment.

3.5. Reactors disturbance and shutdown

Upon disturbance, methane production dropped gradually until reaching 0.21 l CH_4/g VS on day 167 in digester A and 0.24 CH_4/g VS on day 191 in digester B, which necessitated the suspension of feeding (Fig. 1d). The disturbances observed in digester A and digester B can be attributed to two different causes. While the high CO_2-to-CH_4 ratio in digester B dropped almost immediately upon load suspension (Fig. 1d), it persisted for a while after stopping the feeding in digester A (Fig. 1c). In digester B, OLR was increased beyond the digester's capacity without a decrease in HRT (Fig. 1b), causing a decrease in methane production and an overload condition on day 190. When the overload was stopped, the CO_2-to-CH_4 ratio decreased automatically. In comparison, digester A was subject to both an increase in OLR and a decrease in HRT (Fig. 1a), which led to a disturbance on day 167. When the load was stopped, both methane and carbon dioxide production decreased, thus maintaining a high CO_2-to-CH_4 ratio for a while indicating the inhibition of methanogenesis (low CH_4) accompanied by a general washout of the microbial flora (low CO_2 as well). The latter can be attributed to the decrease in HRT from 60 to 47 days.

3.5.1. Washout of digester A

Upon decreasing HRT (on day 167), all VFAs increased to high levels. Notably, acetate concentration peaked rapidly, thus reducing the propionate-to-acetate ratio from an average value near 1.0 to values close to 0.5 (Fig. 2a) and increasing the α ratio from an average of 0.07 to 0.53 (Fig. 2c), which reflects a loss of process stability. The concomitant increase in monitored VFAs (acetate, propionate and butyrate) indicates a loss of all VFA degraders further ascertaining the occurrence of washout conditions. In particular, the peak of acetate at high OLR (=2.5 g VS/l/d, day 167) can be attributed to the inhibition of acetate consumption due to excessive substrate (Penteado et al., 2011). Also, upon suspension of loading, a 68% decrease in PA occurred, with a concomitant 44% increase in IA (Fig. 4a). The increase in IA was expected due to the correlation between IA and total VFAs level (Ferrer et al., 2010). As a result, total alkalinity, which is the sum of PA and IA, dropped from an average of 11,501 to 7730 mg as $CaCO_3$/l (Fig. 2c).

Furthermore, when VSS content dropped in both reactors upon disturbance, the lag period was longer in digester A than in digester B indicating that the loss of the microbial flora was more pronounced in digester A due to the washout caused by a dilution rate faster than the microbial growth rate. Accordingly, it appears that the stability of the mixed digester is sensitive to the reduction in HRT and thus, a higher OLR can probably be achieved, under continuously mixing conditions, if the HRT of 60 days was maintained.

3.5.2. Overload of digester B

The high concentration of propionic acid in digester B increased further upon increased loading (beyond 1.9 g VS/l/d) to reach excessively high values (>3000 mg/l, Fig. 2c) while total VFAs did not increase proportionately. The propionate-to-acetate ratio reached levels as high as 2.5–3.0 (Fig. 2b). Even though the total VFAs increased slightly above the established tolerance, the stability of the system was compromised. Total alkalinity decreased drastically and the α ratio increased to values >1 (Fig. 2d) indicating a loss of stability which is often the main cause behind operational difficulties of thermophilic processes.

The propionate accumulation in digester B is consistent with the results reported by Kim et al. (2002), where thermophilic reactors exhibited propionate accumulation under overload, without an increase in other VFAs. In fact, propionic acid is hard to degrade and usually persists longer than other VFAs (Ferrer et al., 2010). The propionate-degrading bacteria are sensitive to high levels of their own metabolic waste: hydrogen. Hydrogenotrophic methanogens use H_2 and CO_2 as precursors of CH_4 and thus play a major role in reducing the H_2 level in the digester. Accordingly, propionate accumulation in digester B can be linked to poor microbial diversity, namely hydrogenotrophs, in the initial seed.

4. Conclusion

The maximum loading rate reached in digester A was ~20% higher than that reached in digester B. Digester A exhibited better treatment efficiency than digester B and was more stable in terms of reduced VFAs, lower propionate level and a lower α ratio. As such, slow mixing (100 rpm) enhanced the startup process, the digester's capacity and the system's stability and treatment efficiency. This can be attributed to inadequate hydrogenotrophic diversity in non-acclimated seed, thus increasing the importance of mixing to avoid buildup or accumulation of hydrogen.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2012.02.125.

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