Correlation between system performance and bacterial composition under varied mixing intensity in thermophilic anaerobic digestion of food waste

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Abstract

This study examines the stability and efficiency of thermophilic anaerobic digesters treating food waste under various mixing velocities (50 to 160 rpm). The results showed that high velocities (120 and 160 rpm) were harmful to the digestion process with 18 to 30% reduction in methane generation and 1.8 to 3.8 times increase in volatile fatty acids (VFA) concentrations, compared to mild mixing (50 and 80 rpm). Also, the removal rate of soluble COD dropped from 75-85% (at 50-80 rpm) to 20-59% (at 120-160 rpm). Similarly, interrupted mixing caused adverse impacts and led to near-failure conditions with excessive VFA accumulation (15.6 $g.l^{-1}$), negative removal rate of soluble COD and low methane generation (132 $ml.gVS^{-1}$). The best efficiency and stability were achieved under mild mixing (50 and 80 rpm). In particular, the 50rpm stirring speed resulted in the highest methane generation (573$ml.gVS^{-1}$). High-throughput sequencing of 16S rRNA genes revealed that the digesters were dominated by one bacterial genus (*Petrotoga*; phylym *Thermotogae*) at all mixing velocities except at 0 rpm, where the community was dominated by one bacterial genus (*Anaerobaculum*; phylum *Synergistetes*). The *Petrotoga* genus seems to have played a major role in the degradation of organic matter.

Keywords

Thermophilic anaerobic digestion, food waste, mixing, 16S rRNA gene sequencing

1. Introduction

Anaerobic digesters are often designed as continuously stirred reactors with the aim of providing efficient mixing for a homogeneous distribution of substrate and heating energy and to avoid settling of solids and short-circuiting as well as maintaining the design solid retention time (Lindmark *et al.* 2014b). In addition to physical effects, mixing can have direct impacts on the performance of the microbial system by dispersing inhibitory metabolic by-products, such as volatile fatty acids (VFA) and hydrogen (H_2) (Amani *et al.*, 2010). Accordingly, inadequate mixing in anaerobic digestion (AD) is often associated with various operational challenges, including non-uniform substrate distribution, grit deposition, stratification, formation of dead zones, and most notably, the inhibition of methanogenesis by accumulation of toxic metabolic by-products (Stroot *et al.*, 2001). Thus, adequate mixing was found essential to ensure a homogeneous temperature and an optimum digestion environment, leading to better methane generation, higher effective volume, improved removal of organic matter and enhanced tolerance to higher organic loading rates (OLR) (Lindmark *et al.*, 2014b; Elnekave *et al.*, 2006). However, reported results on mixing impact and optimal intensity remain inconclusive. While vigorous mixing has been suggested to improve the biodegradation of volatile solids by increasing their solubility and area of contact with bacteria (Halalsheh *et al.*, 2011), it has been equally reported to hinder the formation of flocks, where syntrophic microbial interactions can take place, and cause propionate accumulation resulting in lower removal efficiency (Suwannoppadol *et al.*, 2011). In comparison, systems with moderate mixing (80 rpm) seem to have high stability and are able to absorb the disturbance of a shock load, even when stratification occurs (Gomez *et al.*, 2006). Slower mixing at 15 and 25 rpm has also been reported to improve acidogenesis (Yu *et al.* 2017) and gas generation (Lindmark *et al.*, 2014a), respectively.

The need for mixing is equally controversial whereby some studies reported that no mixing may cause substrate shortcuts, ultimately decreasing the effective hydraulic retention time (HRT) and the overall efficiency of the digestion process, methane generation and pathogen removal (Elnekave *et al.*, 2006; Ghanimeh *et al.*, 2012). In contrast, the conversion of VFA to methane is inhibited by high concentration of hydrogen and close microbial contact has been found to be a solution for this limitation, which can be achieved by reduced or interrupted mixing. Similarly, the lack of mixing during startup, can reportedly shorten the startup period, stabilize the system, and increase methanogenic abundance (Stroot *et al.*, 2001) – associated with higher methane generation (Traversi*et al.*, 2012). Other work emphasized the importance of mixing during startup for VFA dissipation and stabilization, particularly in the absence of acclimated inocula (Ghanimeh *et al.* 2012).

Despite the divergence in reported findings, digesters operating at low TS feed or low OLR, appear to be less sensitive to the intensity of mixing or its absence. In low-TS systems, inhibiting byproducts are diluted and adequate mixing can be achieved by the slightest stirring effort, or even by the natural movement of the generated biogas (Wang *et al.*, 2017). In comparison, high-TS can result in high viscosity liquor that requires greater stirring effort to achieve the same level of mixing. The impact of mixing seems also to depend on the type of waste fed into the system as different substrate composition can lead to different microbial setups with varied tolerance, and different abundance of toxins and inhibitors (Lindmark *et al.*, 2014 b).

Based on the above, to this date, no optimum mixing pattern can be discerned from the literature (Kuczman *et al.*, 2017). The need for mixing in thermophilic digesters treating food waste, specifically in the absence of acclimated inocula, was previously demonstrated (Ghanimeh *et al.*, 2012), and the current study aims at examining the stability and efficiency of thermophilic anaerobic digesters treating food waste in response to varying the mixing

velocity. Furthermore, while microbial communities in anaerobic digesters have been characterized (Li 2015), studies addressing the effect of mixing on the microflora remain divergent and limited (Tian *et al.* 2014). Accordingly, this paper addresses the correlation between bacterial composition and mixing, on one hand, and system performance, on the other, in thermophilic digesters treating food waste. Four stirring velocities (50, 80, 120, 160 rpm) were tested, for one HRT (30 days) each. The change in stirring speed was initiated after completion of the startup period to avoid the impact of shock loads. The withdrawal of digestate took place after vigorous mixing to ensure constant SRT at all mixing speeds. A semi-continuous feeding mode was adopted because, compared to commonly reported batch systems, it has the advantage of simulating real-life applications. The performance of the reactors was evaluated in terms of methane generation, removal of total and soluble COD and VFA concentration. Also, the bacterial community composition at different mixing velocities was characterized by high-throughput 16S rRNA gene sequencing to correlate with the system's performance.

2. Materials and methods

2.1. Experimental setup and procedures

Two CSTR (9 liters working volume, Bioflo 110, New Brunswick Scientific Co.), referred to as digesters A and B, were operated at a HRT of 30 days under stable thermophilic temperature (55±1°C). The digesters were mechanically stirred by means of an internal impeller. The biogas was collected in gasometers using the water displacement method and was analyzed on a daily basis for CH_4, CO_2 and O_2 content. Prior to feeding, the digesters were vigorously mixed and samples were retrieved by pressure differential and tested on a weekly basis. The experiment lasted for 185 days (26 weeks) split between (a) the **startup** phase (87 days), during which the organic loading rate (OLR) was increased from 0.5 to 2 gVS/l/d at a mixing speed of 80 rpm,

and (b) the **varied mixing** period where three mixing schemes were applied to each system: 80, 120 and 0 rpm in digester A, and 80, 50 and 160 rpm in digester B. The fluid has a density of 980 kg.m^{-3} and a dynamic viscosity of 0.0195 Pa.s (Ghanimeh *et al.*, 2017). Accordingly, the highest velocity near the blade edges, at 50, 80, 120 and 160 rpm is around 0.19, 0.30, 0.45 and 0.60 m/s, respectively, resulting in a laminar to transitional flow with Reynolds numbers of 682, 1091, 1636 and 2181, respectively. Also, assuming minimum zero velocity near the cylinder wall, the average velocity gradient (G) at 50, 80, 120 and 160 rpm can be approximated at 2.4, 3.9, 5.8 and 7.7 sec^{-1}, respectively (Equation 1), requiring a power input of 0.1, 0.3, 0.7 and 1.2 W per cubic meter of digester volume (Equation 2).

$$G = \frac{(V_{max} - V_{min})}{gap} \qquad \textbf{(1)}$$

Where G is an approximated value of the average velocity gradient, V_{max} is the maximum velocity (i.e. velocity near the blade edge); V_{min} is the minimum velocity (assumed zero at the cylinder wall); *gap* is the clearance between the blade edge and the cylinder wall (7.8 cm).

$$G = \sqrt{\frac{P}{\mu V}} \qquad \textbf{(2)}$$

Where P is the power input (W); μ is the dynamic viscosity (Pa.s); and V is the volume of the compartment (m^3).

At the end of the startup phase, both digesters were run for one HRT under the same mixing speed (80 rpm) for stabilization purposes. Then, the mixer speed in digester A was increased to 120 rpm to achieve high-intensity mixing. The deterioration in the digester's performance necessitated the interruption of mixing (0 rpm). In comparison, the mixing speed in digester B was reduced to 50 rpm, in an attempt to improve the system's performance, prior to applying vigorous mixing (160 rpm). The minimum (50 rpm) mixing speed was experimentally identified, in the current study, as the lowest stirring speed at which stratification does not occur.

2.2. Inoculation and feeding

Both digesters were inoculated with 1.5 kg of fresh cow manure diluted to 3.5 L with de-ionized water. Slow feeding (low OLR) was initiated on the second day. By the second week, inoculation was completed with the addition of 0.5 kg of fresh compost diluted to 4 L with fresh leachate. Upon reaching the full (9 L) digester capacity, daily wasting and feeding was initiated. The feed consisted of a mixture of fruit and vegetable market waste and restaurant leftovers that were ground and mixed to ensure homogeneity, then stored at -20^{o}C to avoid fluctuation in characteristics. The waste had an initial TS of 33.1% that decreased to 6.5% after dilution with de-ionized water at 1:5 and an HRT of 30 days.

2.3. Analytical methods

Biogas composition (CH_4 and CO_2) was monitored on a daily basis using a dual wavelength infrared cell with reference channels (GEM-5000 monitor, Keison Products, UK). Total, suspended, dissolved and volatile solids were determined using Standard Methods 2540B and 2540E procedures (APHA, AWWA and WPCF, 2012). Chemical oxygen demand (COD) was tested using a modification of 5220D procedure of Standard Methods for the Analysis of Water and Wastewater (APHA, AWWA and WPCF, 2012) using the HACH high-range COD kit (HACH Company, Loveland, Colorado). Total and soluble COD (TCOD, SCOD) were determined in raw and filtered samples (using 1.2µm pore-size filters). Similarly, the ammonia-N concentration was measured using HACH spectrophotometry method.

Partial and total alkalinity (PA and TA) were determined by titration using hydrochloric acid (HCl, 0.2N) to pH 5.75 and 4.3, respectively, and then intermediate alkalinity (IA) was calculated by subtracting PA from TA (Ferrer et al. 2010).Volatile fatty acids (VFA) concentration was determined by titrating 50 ml of filtered sample using HCl (0.1 M) to

decrease the pH to 3.5.After 3 minutes of boiling, the pH is raised to 4 then 7 using NaOH (0.1M) and the resulting concentration is 120 times the volume (in ml) of NaOH added between pH 4 and 7.

2.4. Microbial community analysis

Samples for microbial analysis were taken on a weekly basis during the varied mixing period, which lasted for 98 days. Genomic DNA was extracted in duplicates from the samples using the Power Soil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions and pooled to reduce sample variability. The quality (A260/A280) and quantity (A260) of the extracted genomic DNA was determined using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Triplicate PCR reactions were performed for each sample in a 25 µL reaction volume using the HotStar TaqPlus Master Mix (Qiagen, Valencia, CA), 0.5 µM of each primer, and 100-200 ng of template DNA. The V3-V4 hyper variable region of 16S rRNA genes were amplified using auniversal primer set for prokaryotes: Pro 341F (5'-Illumina adapter-Barcode-Linker-CCTACGGGNBGCASCAG-3') and Pro 805R (5'-5'-Illumina adapter-Linker -GACTACNVGGGTATCTAATCC-3') (Takahashi *et al.*, 2014). PCR was performed using life technologies veritus thermocycler with the following PCR conditions: initial denaturation at 94^{o}C for 3 min, followed by 28 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 53^{o}C for 40 seconds, extension at 72^{o}C for 1 min and a final extension at 72^{o}C for 5 min.

Following PCR, all amplicon products from the different samples were mixed in equal concentrations, purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA), and sequenced on the Illumina TruSeq technology (San Diego, CA) according to manufacturer's instructions. The 16S rRNA sequences were processed using the Quantitative Insights Into Microbial Ecology (QIIME v 1.9.0) pipeline (Caporaso *et al.* 2010 a). Raw reads

were first demultiplexed, trimmed and filtered for quality. The minimum acceptable length was set to 200bp (Caporaso *et al.* 2010 a). Sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using the uclust algorithm (Edgar 2010). A representative sequence from each OTU was aligned using PyNAST (Caporaso *et al.* 2010 b), and these were phylogenetically assigned to a taxonomic identity (phylum, class and genus level) using the RDP Naive Bayesian rRNA classifier at a confidence threshold of 80% (Wang *et al.* 2007). Chimeric sequences were identified and removed from the aligned sequences using chimera Slayer as implemented in QIIME.

3. Results and discussion

3.1. Waste characteristics

The waste was a combination of discarded produce from a local fruit and vegetable market and leftovers from a fast food restaurant. The waste was characterized for various parameters as summarized in Table 1. It exhibited a substrate C:N ratio of 13.3 comparable to those reported by Shen *et al.* (2013), and a low pH of 5.1 similar to that of Lindmark *et al.* (2014a), though a high bicarbonate alkalinity at 2.3 g $CaCO_3$/l, which can maintain a stable pH in the digester.

Table 1
Characteristics of the prepared waste before dilution with deionized water

Parameter		Value
pH		5.1
Total Solids (TS) (%)		33.1
Volatile Solids (VS) (%)		31.2
Total nitrogen (%, dry basis)		3.5
Total carbon (%, dry basis)		46.1
Carbon to Nitrogen (C:N) ratio		13.3
Total Organic Carbon (%, wet basis)		41.9
Phosphorus ($mg.kg^{-1}$, wet basis)		930.0
Chemical Oxygen Demand (COD)	Total ($g.l^{-1}$)	564.4
	Soluble ($g.l^{-1}$)	110.7
Alkalinity	Total (g $CaCO_3.l^{-1}$)	2.3
	Partial (g $CaCO_3.l^{-1}$)	0
Total Volatile Fatty Acids (VFA) ($g.l^{-1}$)		7.3

3.2. Startup phase

Upon seeding, methane generation started at a high rate, possibly due to the degradation of the organics in the seed, and dropped upon initiation of the wasting/feeding process. During the rest of the startup period (weeks 5 to 12) methane generation stabilized at an average of508ml.gVS^{-1} in A and465ml.gVS^{-1}in B (Figure 1). Similarly, total and soluble COD were comparable in both digesters with averages of 11.5-11.9 $g.l^{-1}$ and 3.0 $g.l^{-1}$, respectively. This similarity is expected considering identical inoculation, feeding and operating conditions.

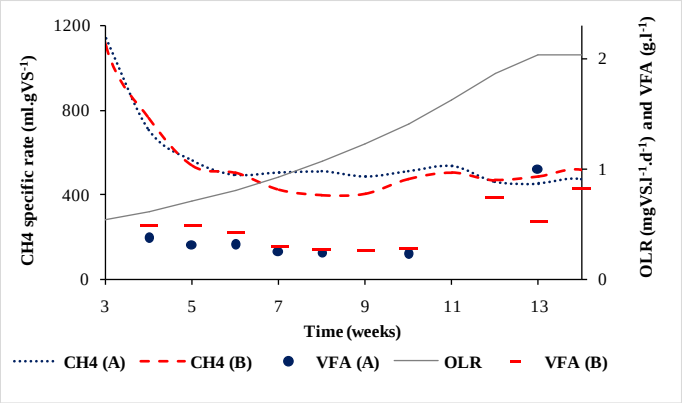


Figure 1. Organic loading rate (OLR), volatile fatty acids (VFA), and CH_4 generation during startup

The concentrations of VFA and alkalinity, and the resulting ratios of VFA-to-alkalinity (VFA/TA) and intermediate-to-partial alkalinity (IA/PA), were relied upon in examining the stability of the system. In both digesters, the average total alkalinity was 5.2 $g.l^{-1}$; and partial

alkalinity (PA, defined as alkalinity due to HCO_3^-species) and intermediate alkalinity (IA, defined as alkalinity due to VFAs) were 4.2 and 1.0 $g.l^{-1}$, respectively. The average intermediate-to-total alkalinity ratio (IA/TA) was 0.20, and the average intermediate-to-partial alkalinity ratio (IA/PA) was 0.25, both below the reported thresholds of 0.5 for IA/TA and 0.9 for IA/PA to avoid failure of thermophilic digesters during transient conditions (Ferrer *et al.* 2010). In addition, VFA/TA varied from 0.05 upon seeding to 0.10-0.13 upon reaching the design OLR of 2.0 g $VS.l^{-1}.d^{-1}$. While the VFA/TA should reportedly remain below 0.7 (Velmurugan and Ramanujam, 2011), systems with VFA/TA< 0.3-0.4 are considered stable and higher values may indicate digester upset with a risk of acidification (Schoen *et al.* 2009). Accordingly, the startup was considered successful with stabilized digesters prior to initiating different mixing intensities to avoid the impact of shock loading.

3.3. Varied mixing period

3.3.1. Digester A

At 80 rpm (weeks 13 to 17), VFA concentrations reached 1.6 $g.l^{-1}$, remaining below the threshold level of 2 $g.l^{-1}$ considered inhibitory to methane formation (Jayalakshmi *et al.*, 2009). The average specific methane yield was 405 $ml.gVS^{-1}$, which is close to reported ranges of 332-392 $ml.gVS^{-1}$ for similar systems (Ghanimeh *et al.*, 2013). The digester was stable with an average IA/PA ratio of 0.34and VFA/TA of 0.35 (Figure 2.a and b). For stable operation, it is desirable to keep IA/PA below the critical value of 0.4 (Astlas *et al.*, 2012), or VFA/TA below 0.3-0.4 (Schoen *et al.* 2009).Also, the system's efficiency exhibited high removal rates of 85% TCOD, 77% SCOD and 86% VS (Figure 2.c and d).

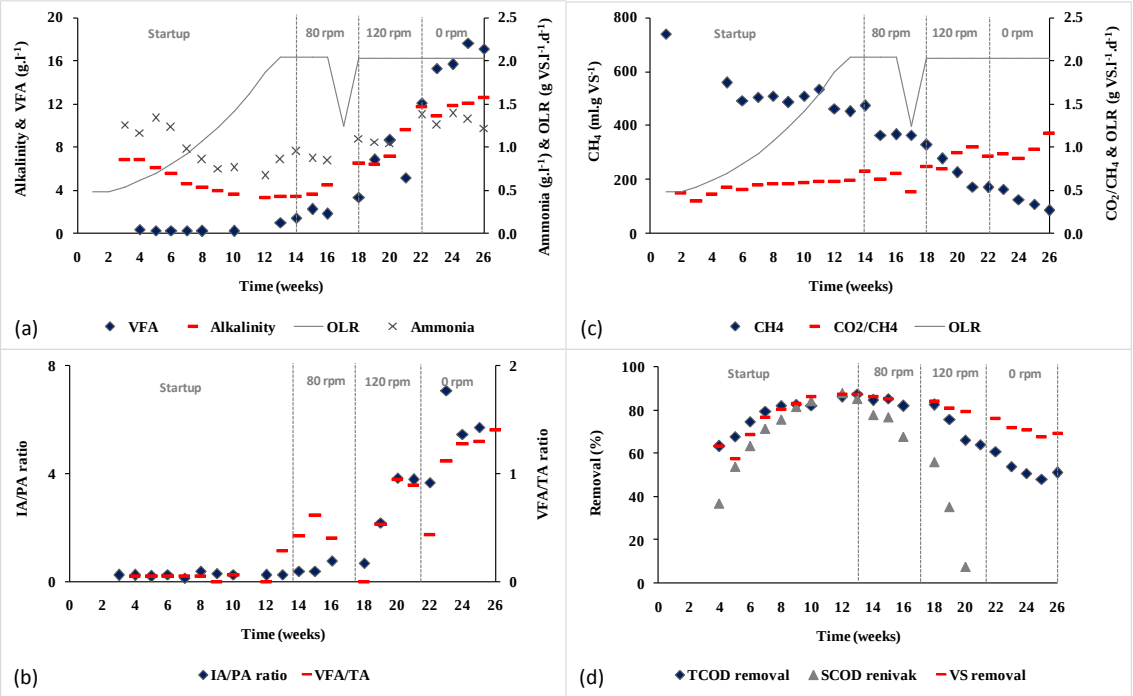


Figure 2. Performance and stability indicators of digester A*

**Feeding was suspended for two days during week 17 for technical reasons.*

Upon increasing the stirring speed to 120 rpm, the VFA concentration increased significantly to an average of 6.0g.l^{-1} and IA/PA and VFA/TA ratios rose to 2.6 and 0.6, reflecting a potential loss of stability (Figure 2.a and b). Concomitantly, the ratio of CO_2-to-CH_4 increased by 38% of its average value at 80rpm, leading to a decrease in methane yield to 251ml.gVS^{-1}, which is below reported values for similar systems (Ghanimeh *et al.*, 2012). A deterioration in performance was evident with a decrease in removal rates to 72% TCOD and 81% VS. The accumulation of acids affected mostly the removal rate of SCOD, which dropped to 20% (Figure 2.c and d).

Next, mixing was interrupted in an attempt to recover the system's performance following a high-intensity mixing phase. While successful attempts for the recovery of mesophilic AD were reported (Stroot *et al.* 2001), interrupted mixing in digester A led to further loss of stability and efficiency. It caused stratification of the liquor, accumulation of grit on the bottom of the digester and formation of a thin layer of scum on top. The VFA concentration reached as high as 17.6 g.l^{-1} (average = 15.6 g.l^{-1}) with excessively high IA/PA

= 6.4 (16 times higher than the 0.4 critical value for stable digesters) and VFA/TA = 1.1 (compared to 0.3-0.4 in stable systems). Also, the CO_2-to-CH_4 ratio increased to as high as ~1.0 and methane generation decreased to as low as 132ml.gVS^{-1}. The removal rate of soluble COD dropped to below zero, indicating the accumulation of soluble compounds and the inhibition of methanogenic activity. Similar observations were reported for irreversible acidification of thermophilic digesters treating SS-OFMSW (Ghanimeh *et al.*, 2012). The results imply that mixing is mandatory, especially when the VFA level increases (which is the case after the 120 rpm stage) necessitating efficient dispersion to avoid localized inhibiting environments.

3.3.2. Digester B

Similar to digester A, digester B exhibited a stable and highly performing process at 80 rpm, with low VFA concentration (0.7 g.l^{-1}), IA/PA ratio (0.3) and VFA/TA ratio (0.17) (Figure 3.a and b), and high removal rates of TCOD (85%), SCOD (83%) and VS (86%) (Figure 3.d). The average specific methane generation rate was 456ml.gVS^{-1}, considered within reported ranges for thermophilic AD of food waste (336 to 557 ml.gVS-1 at OLR = 2.0 and 1.5 gVS.l^{-1}.d^{-1}; respectively, Guo *et al.*, 2014).Upon reducing the stirring velocity to 50 rpm, methane production increased by 26% to reach 573ml.gVS^{-1}, considered at the high-end of reported values (Guo *et al.*, 2014) (Figure 3.c). The average VS and TCOD removal rates remained almost the same (86% and 83%, respectively) and within reported ranges in digesters treating fruit and vegetable waste (Ganesh *et al.*, 2014); yet soluble COD removal dropped slightly (from 83% to 75%) (Figure 3.d). The increase in VFA concentrations to 2.0 g.l^{-1}, when coupled with the low IA/PA ratio of 0.3, can still be considered acceptable for AD of SS-OFMSW (Martin-Gonzalez *et al.*, 2013).

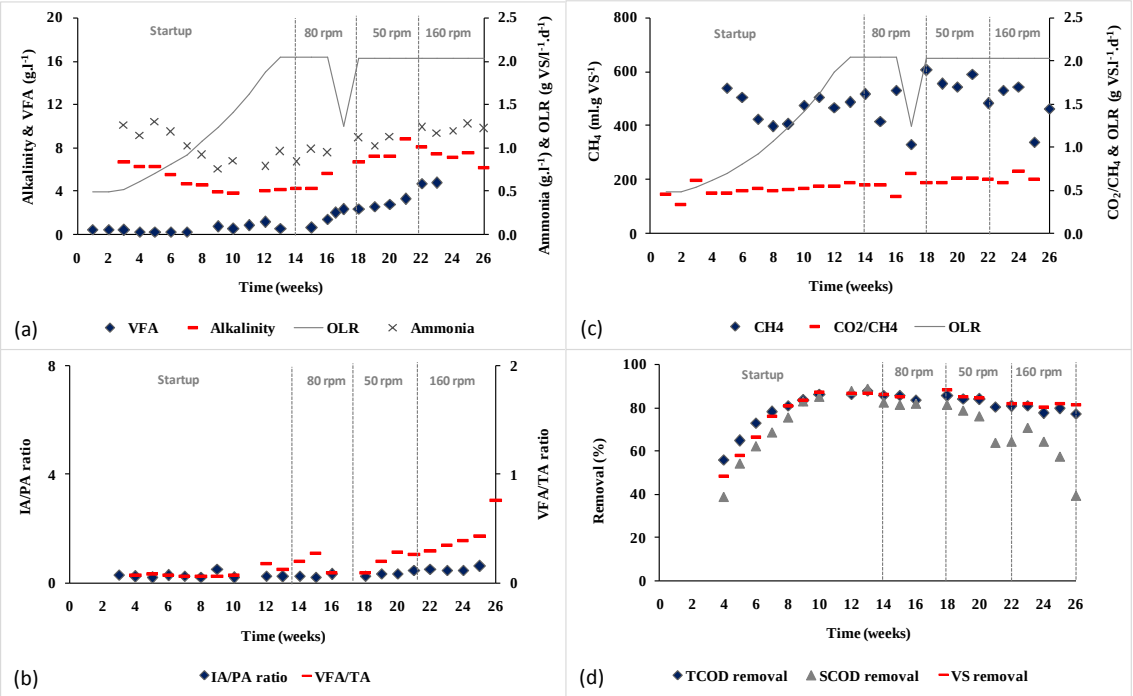


Figure 3. Performance and stability indicators of digester B

In contrast, the vigorous mixing (160 rpm) in digester B affected the process stability by increasing the IA/PA level to 0.45 and VFA concentration to 3.6 $g.l^{-1}$ (Figure 3a and b). This was accompanied by an increase in ammonia concentration to 1.2 $g.l^{-1}$ – above the 1.0 $g.l^{-1}$threshold at which inhibition is initiated in thermophilic AD of OFMSW (Yenigün and Demirel, 2013). As a result, the soluble components accumulated leading to SCOD concentration of 8.8 $g.l^{-1}$, corresponding to a low removal rate of 59% (Figure 3.d). In addition, methane generation decreased by 18% (from 573 to 471 $ml.gVS^{-1}$), but can still be considered high for thermophilic AD of food waste (Figure 3.c). These observations suggest that slow mixing, (50 rpm) is more beneficial for thermophilic AD of food waste than vigorous (160 rpm) mixing.

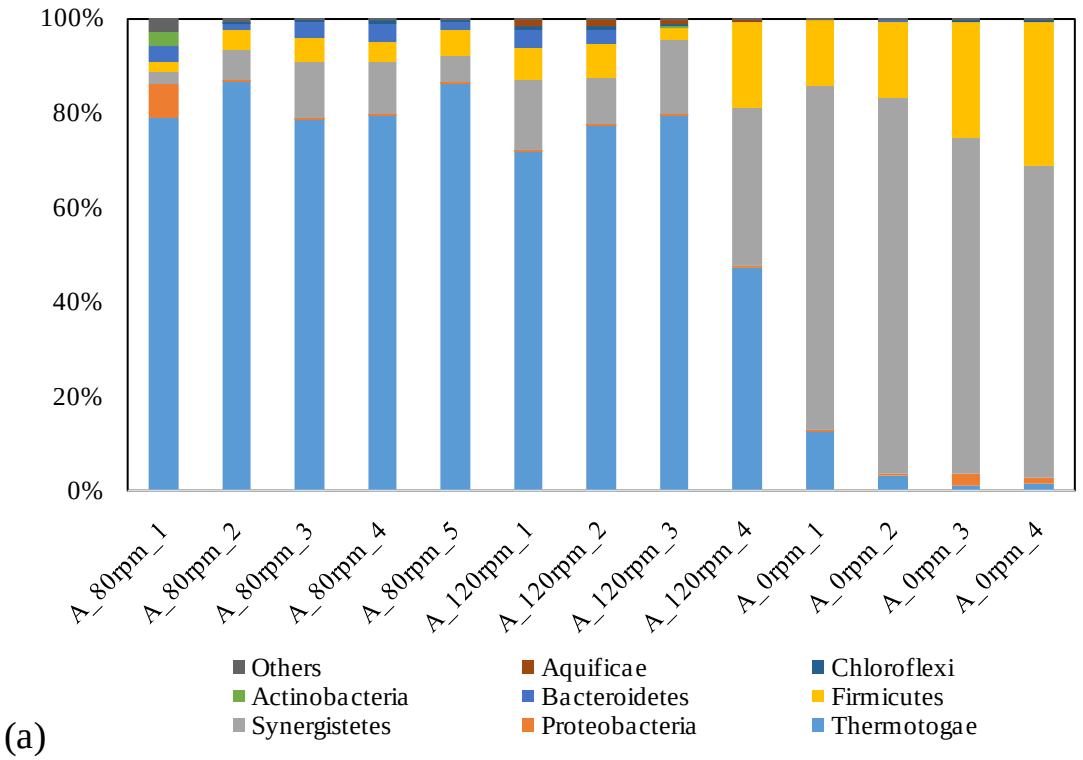
3.3.3. Microbial analysis

The domain *Bacteria* in digester A was dominated (82%), at 80 rpm, by *Thermotogae* phylum, which was gradually replaced by *Synergistetes*. Under no-mixing conditions,

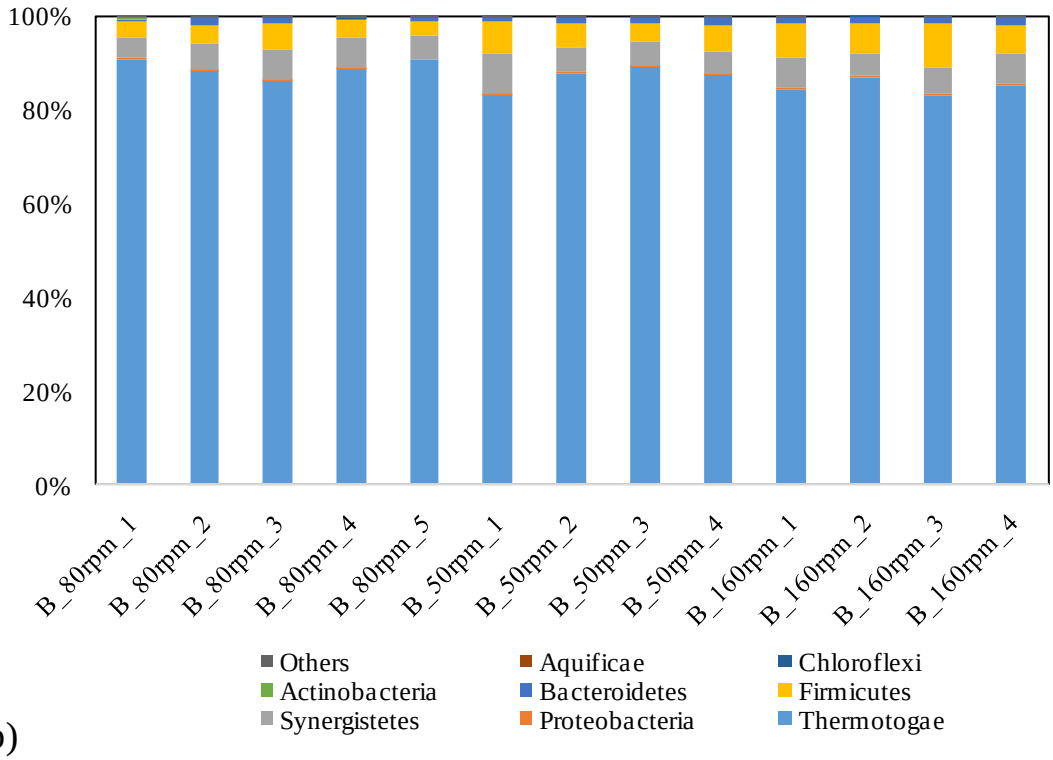
Thermotogae abundance was reduced to 5% with dominance of *Synergistetes* (72%) and *Firmicutes* (22%) (Figure 4.a). Apparently, with time, the environmental conditions in digester A became more favorable for the growth of *Synergistetes*. In comparison, digester B was continuously dominated by the phylum *Thermotogae* (89% at 80 rpm, 87% at 50 rpm and 85% at 160 rpm), followed by *Synergistetes* (average of 6%) and *Firmicutes* (average of 5%) (Figure 4.b). Similar *Thermotogae* dominance has been reported in thermophilic digesters treating organic solid waste, food waste, food wastewater and sugar beet tailings (Jang *et al.*, 2016; Guo *et al.*, 2014; Tian *et al.*, 2013; Sasaki *et al.*, 2011). The reported *Thermotogae* phyla were often dominated by *Petrotoga*, *Defluviitoga* or *Fervidobacterium* genera (Jang *et al.*, 2016; Guo *et al.*, 2014). For further identification of the bacteria residing in the digesters, genus level affiliations were determined with the results showing that the *Thermotogae* phylum consisted mainly of one genus: *Petrotoga*, with a negligible presence (< 1%) of the *Thermopallium* genus. Similarly, the *Synergistetes*phylum consisted of the *Anaerobaculum* genus, with < 1% *Synergistes*genus. Also, *Firmicutes* consisted mainly of the *Bacillus* genus (Figures 5 and 6).

The *Petrotoga* genus (of phylum *Thermotogae*) seems to proliferate under mixing conditions only (digesters A and B, at the exception of zero-mixing, Figures 5 and 6). A similar observation was reported by Tian *et al.* (2013) where *Petrotoga* dominated in thermophilic agitated reactors but was absent in non-agitated ones. *Petrotoga* is known for its high production of H_2through sugar fermentation (Balk *et al.*, 2002). High concentrations of H_2 can affect the degradation of VFAs. Yet, the low VFA concentrations in the digesters during high *Petrotoga* presence suggests that a syntrophic interaction is taking place with hydrogenotrophic methanogens to reduce H_2 concentration and maintain thermodynamically favorable conditions for VFA degradation. In fact, *Thermotogalettinga* (a *Petrotoga* relative) was reported to grow in syntrophic relation with the hydrogenotrophic methanogen *Methanothermobacter* (Balk *et al.*, 2002). In addition, concomitant dominance of *Thermotogae* and hydrogdgenotrophic

methanogens was observed by Guo *et al.* (2014) and Sasaki *et al.* (2011). Indeed, the 16S rRNA gene sequencing results in this study showed that both reactors were dominated by two methanogens that can use H_2 for methane generation: *Methanosarcina* (order *Methanosarcinales*) and *Methanothermobacter* (order *Methanobacteriales*); at the exception of the interrupted mixing case where both genera were absent (Figure 7). Also, it is worth noting that *Petrotoga* dominance was observed in the absence of hydrogenotrophic species (Tian *et al.*, 2013).



(a)



(b)

Figure 4. Taxonomic compositions of bacterial communities at phylum level in digesters A (a) and B (b)

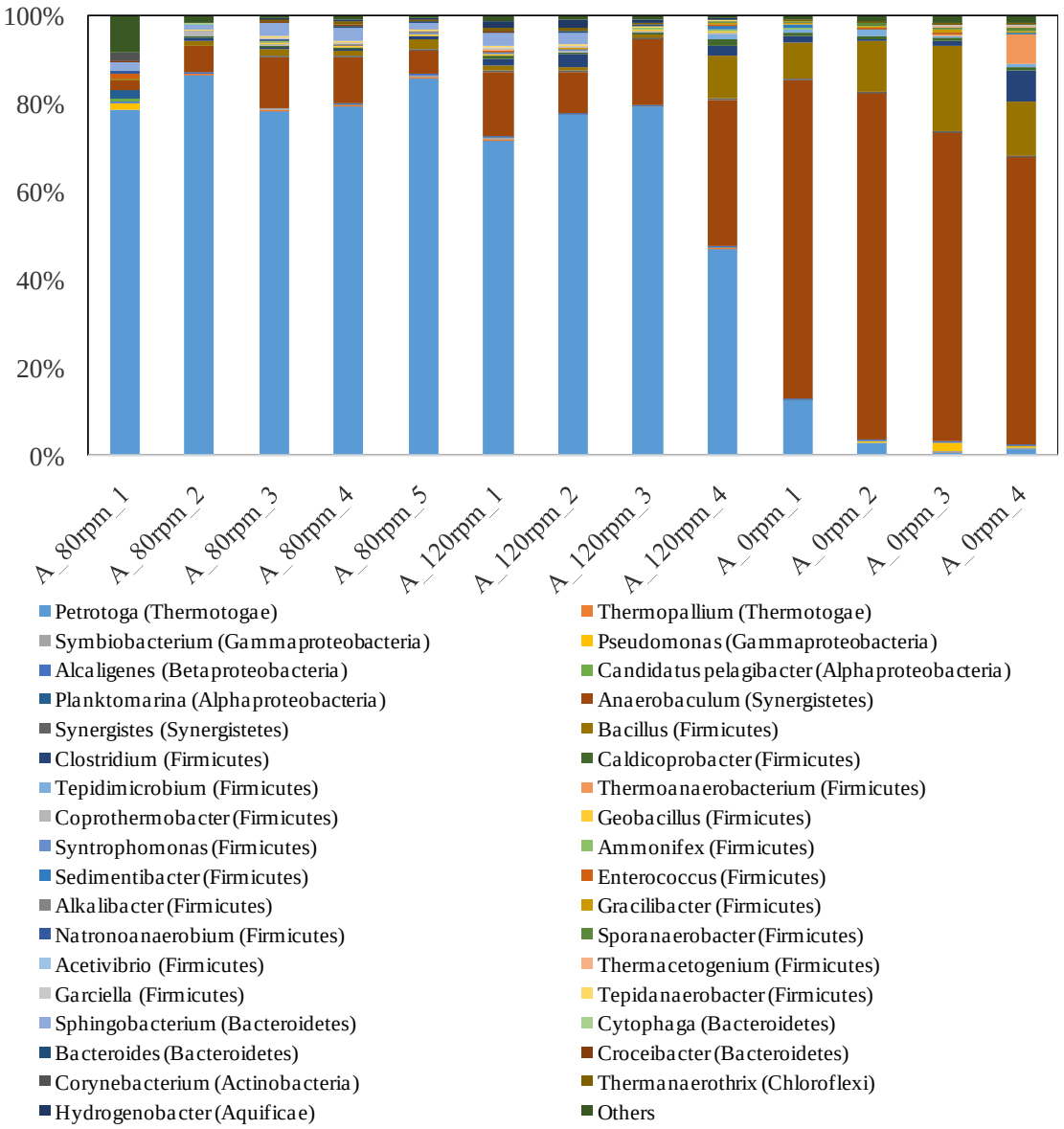


Figure 5. Taxonomic compositions of bacterial communities at the genus level in digester A

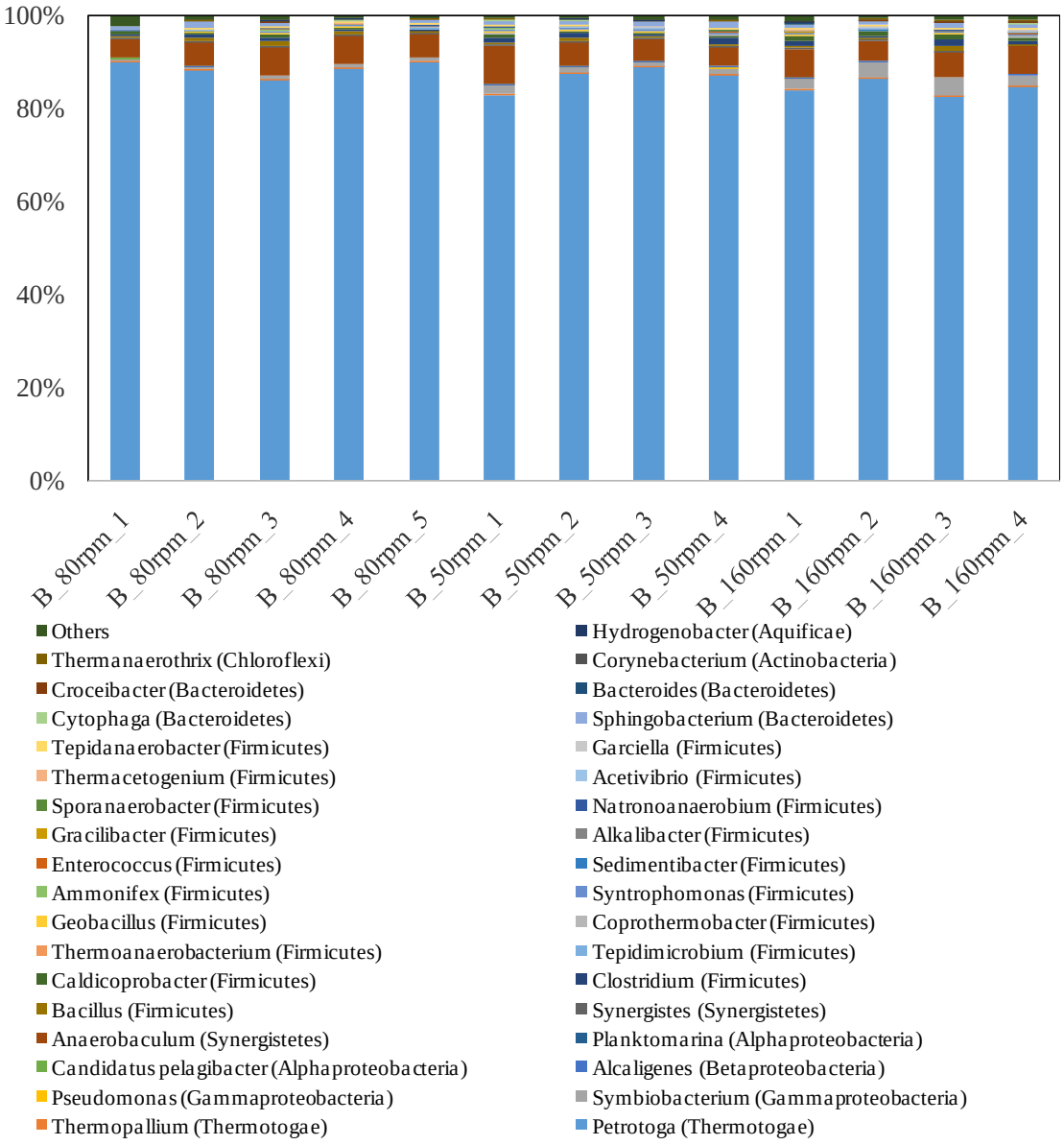


Figure 6. Taxonomic compositions of bacterial communities at the genus level in digester B

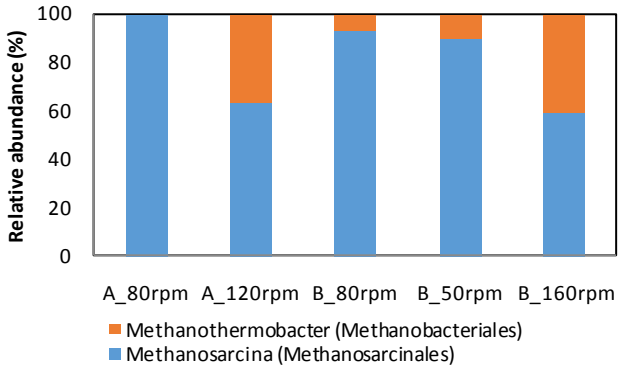


Figure 7. Taxonomic compositions of methanogenic communities at the genus level.

3.3.4. Correlation between bacterial dominance and system performance

The key performance parameters were calculated and correlated with the relative abundance of *Petrotoga* (phylum *Thermotogae*) and *Anaerobaculum* (phylum *Synergistetes*) (Figure 7). An inversely proportional relationship was discerned between the abundance of *Petrotoga* and *Anaerobaculum* (phylum *Synergistetes*) (R^2=0.976, Figure7a). The observed pattern of genera *Petrotoga* and *Anaerobaculum* on one hand, and the deterioration of process performance during interrupted mixing on the other, suggests a strong relationship between *Petrotoga* and process indicators. In fact,a significant correlation was observed between the relative abundance of *Petrotoga* and: VFA (R^2=0.873), IA/PA (R^2=0.789), TCOD removal (R^2=0.860), VS removal (R^2=0.794), and CH_4 specific generation rate (R^2=0.708) (Figure 8b to f).These correlations imply that the genus *Petrotoga* had an important role in the degradation of organic matter.

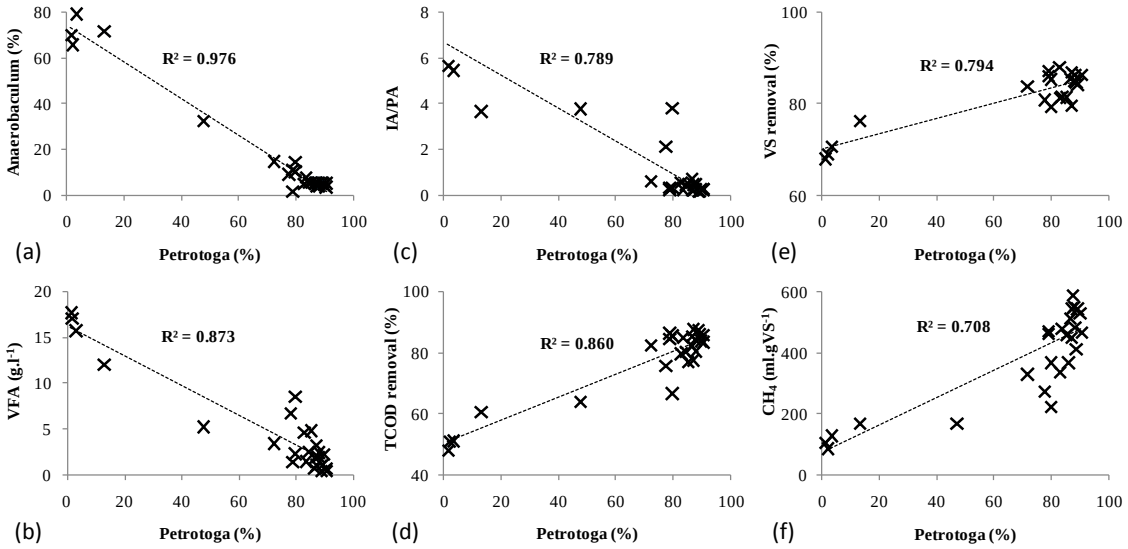


Figure 8. Correlations between *Petrotoga* and performance indicators

3.3.5. Impact of mixing intensity

The periods of 80 and 50 rpm corresponded to the highest process stability in both digesters, with a VFA concentration below 2 $g.l^{-1}$ and IA/PA and VFA/TA ratios below the 0.4 critical value. As a result, both digesters exhibited good removal of organic matter of 83 to

85% TCOD and 75 to 83% SCOD. Yet, the highest methane generation was achieved at 50 rpm mixing at 573 ml.gVS^{-1} which is 26% to 41% higher than the specific methane generation rate at 80 rpm (Table 2). The digesters were dominated (82-89%) by the genus *Petrotoga* (phylym *Thermotogae*), which seems to contribute significantly to process performance.

Table 2.Performance of the digesters at different mixing velocities
Average values (standard deviation)

Mixing velocity	**VFA *(g.l^{-1})***	**IA/PA**	**VFA/TA**	**CH_4 *(ml.gVS^{-1})***	**NH_3 *(g.l^{-1})***	**TCOD *(g.l^{-1})***	**SCOD *(g.l^{-1})***	**SCOD removal *(%)***
Digester A								
80 rpm	1.6(0.5)	0.4(0.2)	0.35(0.23)	405(54)	0.88(0.05)	16.6(2.5)	5.0(1.6)	77.0(7.2)
120 rpm	6.0(2.2)	2.6(1.5)	0.59(0.44)	251(67)	1.07(0.03)	30.5(9.4)	17.3(7.2)	19.8(33.2)
0 rpm	15.6(2.2)	6.4(2.4)	1.11(0.39)	132(37)	1.31(0.07)	51.7(5.3)	32.8(2.7)	< 0
Digester B								
80 rpm	0.7(0.3)	0.3(0.0)	0.17(0.07)	456(83)	0.93(0.06)	15.9(1.9)	3.6(0.7)	83.5(3.4)
50 rpm	2.0(0.5)	0.3(0.1)	0.21(0.08)	573(30)	1.09(0.06)	18.3(2.3)	5.4(1.7)	75.0(7.9)
160 rpm	3.6(1.0)	0.5(0.1)	0.45(0.18)	471(81)	1.22(0.04)	23.0(1.9)	8.8(2.6)	59.1(12.0)

In comparison, vigorous mixing (120-160 rpm) resulted in reduced stability and efficiency in both digesters, reflected in high VFA, IA/PA and VFA/TA, as well as reduced removal efficiencies, leading to high TCOD and SCOD of 30.5 and 17.3 g.l^{-1} in digester A and 23.0 and 8.8 g.l^{-1} in digester B. The SCOD reached under vigorous mixing was 250% and 60% higher than that under mild mixing in digesters A and B, respectively. Similarly, the VFA was 3.8 and 1.8 times higher and methane generation was 38% and 18% lower in digesters A and B, respectively, compared to mild mixing conditions. The increase in VFA and drop in SCOD removal rates at 120 and 160 rpm supports reported observations on the damage to syntrophic microbial flocks under vigorous mixing, leading to VFA accumulation and reduced removal efficiencies (Suwannoppadol *et al.*, 2011).

At interrupted mixing, souring occurred with excessively high VFA (15.6 g.l^{-1}) and IA/PA and VFA/TA ratios, indicative of process failure, with a concomitant dominance of the

bacterial genus *Anaerobaculum* (pylum *Synergistetes*). The soluble compounds accumulated, resulting in negative SCOD removal and low methane generation (132 $ml.gVS^{-1}$). This observation is consistent with previous findings on the necessity of mixing in thermophilic AD of SS-OFMSW (Ghanimeh *et al.* 2012).

In the presence of mixing, the H_2 generating *Petrotoga* was the dominating genera. Apparently, high production of H_2 was not a limiting factor during low intensity mixing (50 and 80 rpm) due to the presence of H_2 consuming methanogens: *Methanosarcina* (order *Methanosarcinales*) and *Methanothermobacter* (order *Methanobacteriales*). However, high intensity mixing (120 and 160 rpm) seems to break microbial flocks and hinder syntrophic interactions. As a result, methanogenesis is slowed down and methane generation rates dropped.

4. Conclusion

Two digesters, that underwent identical inoculation, were run under similar operating and feeding conditions with various mixing intensities. The digesters performed well under moderate mixing speeds (50 and 80 rpm), with the highest efficiency and stability accomplished at 50 rpm. Both mixing speeds resulted in a stable operation with an IA/PA ratio below 0.4, VFA concentrations below 2 $g.l^{-1}$, TCOD and SCOD removal rates of 83-85% and 75-83%, respectively. In contrast, vigorous mixing (120 and 160 rpm) was harmful to the digestion process. VFA accumulation occurred with a loss of stability, drop in removal efficiencies and methane generation. Also, interrupted mixing led to near-failure conditions; the conversion of soluble matter ceased resulting in overall negative SCOD removal and inhibited methane generation. Both digesters were dominated by one bacterial genus (*Petrotoga*; phylum *Thermotogae*) at all mixing velocities except at 0 rpm, where the community was dominated by another bacterial genus (*Anaerobaculum*; pylum *Synergistetes*).

Significant correlations were drawn between the abundance of *Petrotoga* and performance indicators.

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