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Effects of shearing on biogas production and microbial community structure during anaerobic digestion with recuperative thickening

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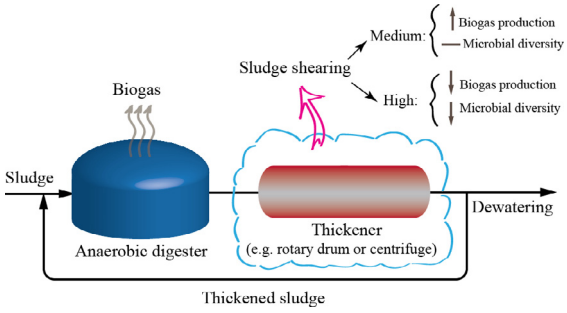
HIGHLIGHTS

- Medium shearing during thickening for recuperative AD improved biogas production.
- Medium shearing increased the evenness and diversity of the microbial community.
- High level shearing negatively affected AD operation.
- High level shearing decreased microbial diversity in the digester.
- Hydrolysis and acetogenesis bacterial order abundance decreased at high shearing.

GRAPHICAL ABSTRACT



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ABSTRACT

Recuperative thickening can intensify anaerobic digestion to produce more biogas and potentially reduce biosolids odour. This study elucidates the effects of sludge shearing during the thickening process on the microbial community structure and its effect on biogas production. Medium shearing resulted in approximately 15% increase in biogas production. By contrast, excessive or high shearing led to a marked decrease in biogas production, possibly due to sludge disintegration and cell lysis. Microbial analysis using 16S rRNA gene amplicon sequencing showed that medium shearing increased the evenness and diversity of the microbial community in the anaerobic digester, which is consistent with the observed improved biogas production. By contrast, microbial diversity decreased under either excessive shearing or high shearing condition. In good agreement with the observed decrease in biogas production, the abundance of *Bacteroidales* and *Syntrophobaterales* (which are responsible for hydrolysis and acetogenesis) decreased due to high shearing during recuperative thickening.

1. Introduction

Anaerobic digestion is widely used to stabilise sewage sludge in wastewater treatment plants (WWTPs) prior to land application or other forms of disposal (Sawatdeenarunat et al., 2016; Shen et al., 2015). Importantly, in the anaerobic digestion process, organic matter in sewage sludge is converted to biogas for subsequent bioenergy production (Ratanatamskul and Saleart, 2016; Sawatdeenarunat et al., 2016; Tuyet et al., 2016). Indeed, under an optimal condition, WWTPs can potentially achieve up to about 70% energy self-sufficiency through the production of biogas from wastewater sludge (Jenicek et al., 2012; Nghiem et al., 2017).

A relatively low cost technique to achieve a high conversion of the chemical energy content in sludge (in the form of chemical oxygen demand) to biogas is recuperative thickening. Recuperative thickening was first introduced in 1967 (Torpey and Melbinger, 1967) but has only been intensively explored in full scale WWTPs

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in recent years (Bharambe et al., 2015; Yang et al., 2015) to establish whether it can both increase biogas production and minimise biosolids odour, thus, improving biosolids quality. Recuperative thickening can decouple the sludge retention time (SRT) from the hydraulic retention time (HRT) by obtaining and thickening a proportion of the sludge and returning the thickened sludge to the digester (Bharambe et al., 2015; Reynolds et al., 2001; Torpey and Melbinger, 1967; Yang et al., 2015). Thus, with recuperative thickening, SRT can be increased independently of the HRT value. In other words, recuperative thickening can allow for about 25% increase in anaerobic digestion capacity without any expansion of the digester volume (Reynolds et al., 2001). In addition, the high conversion of organic content from sludge to biogas could also reduce malodour from the produced biosolids (Bharambe et al., 2015).

As noted above, recuperative thickening could allow for an increase in treatment digestion capacity without the need for significant additional space and excessive capital expenditure. In recent years, urbanization and population growth have exerted an event greater treatment capacity demand on existing WWTPs and waste management facilities. Capacity expansion via the construction of new digesters is a major capital investment hurdle consideration for water utilities. In addition, in many cases, due to space limitation, expansion of the physical footprint of the treatment plant is not always possible.

SRT extension can improve the conversion of organics to methane and increase the volatile solid (VS) reduction (Sieger et al., 2004; Yang et al., 2015). There have been several reports of successful full scale recuperative thickening applications in the US. Using conventional dissolved air flotation (DAFT) for sludge thickening, Reynolds et al., (2001) observed improvement in biogas production by up to 110% and volatile solid (VS) reduction by 15–30% due to recuperative thickening. Similarly, Cobbledick et al., (2016) conducted a lab-scale demonstration of recuperative thickening anaerobic digestion using a gravity thickening process (no shearing) and observed on average 10% increase in biogas yield compared to the control system (no recuperative thickening).

DAFT and gravity thickening do not impart any shearing to the sludge, however, they are less efficient and require much more physical footprint compared to other thickening technologies such as rotary drum and centrifuge. On the other hand, during the thickening process by a centrifuge or rotary drum, the sludge is subjected to shearing. There is some evidence that sludge shearing may also affect the microbial structure and thus methanogenic activities during recuperative thickening. Sludge thickening by centrifugation has also been reported but the increase in biogas production due to recuperative thickening was considerably lower than values obtained without any shearing (e.g. via DAFT or gravity thickening). For example, thickening centrifuge treatment increased the biogas production by 11–31% (Jenicek et al., 2013) and 15–26% (Zabranska et al., 2006). Deveci (2002) conducted a series of lab-scale batch tests and suggested that shear forces induced by four-blade impeller (in a speed range of 2.0–3.4 m/s; shear rate of $G = 2000–3400\ s^{-1}$ in a 1 mm tip field normally encountered in medium shearing thickening devices such as rotary drum) might cause the loss in the viability of bacterial population but only at an excessively high solids content (>10%). This finding is in agreement with a full-scale observation (Batstone et al., 2015) where high speed centrifuges ($G > 5000\ s^{-1}$ in a 1 mm tip field) led to 20–90% decrease of viability of methanogens.

In anaerobic digestion, the conversion of organics to biogas is accomplished by a dynamic consortium of several groups of micro-organisms. Thus, the stability and efficiency of anaerobic digestion rely on the syntrophic relationship among microbial population including hydrolysing and fermenting bacteria, specialized acidogenic and acetogenic syntrophs, and methanogenic archaea (Guo et al., 2015; Vanwonterghem et al., 2014). Anaerobic cellulolytic bacteria hydrolyse cellulose to soluble sugars, which can be utilized by acidogenic bacteria. Acetogenic and/or acidogenic bacteria produce acetate and/or ($H_2 + CO_2$), which is converted to methane by methanogens (Li et al., 2009). Cellulose hydrolysers include the order *Halanaerobium* (Guo et al., 2015), *Clostridiales* and *Bacteroidales* (Vanwonterghem et al., 2014) and the genus *Acetivibrio* (Li et al., 2009). The *Clostridia* class and the *Bacteroidaceae* family (Guo et al., 2015) performed in the acidogenic process; and genus *Clostridium*, *Treponema*, *Eubacterium*, *Thermoanaerobacter*, *Moorella* (Guo et al., 2015), *Methanosaeta* (Riviere et al., 2009) and *Porphyromonadaceas* (Li et al., 2009) were the dominant acetogenic bacteria.

Based on a comprehensive survey of nine anaerobic digestion plant, Werner et al. (2011) showed that the microbial community structure in good performing full-scale digesters were also stable and resilient. Werner et al. (2011) also demonstrated that ecological dynamics of syntrophic populations were highly selective along environmental gradients and that communities with greater evenness had a higher methanogenic activity. Operational factors (*e.g.* operating conditions, process configurations, and substrate characteristics) could lead to the variability in structure and function of microbial population, hence the performance of anaerobic digester system (Zhang et al., 2016). Indeed, microbial diversity such as community evenness could be an indicator for stability and robustness of the community function (Vanwonterghem et al., 2014; Werner et al., 2011).

Shearing can also influence the microbial structure thus playing a significant role in biogas production or methane production activity. Nevertheless, to date, there have been very few studies focusing on the influence of shear force on the microbial community structure. Kundu et al. (2014) applied hydrodynamic shear (upflow velocities from 4 up to 10 m/h) to a mesophilic hybrid anaerobic reactor. They observed 60 and 40% reduction in methane production and COD removal respectively under high upflow velocity (>8 m/h) which can be inferred to high shearing. The abundance and diversity of archaea and bacteria were also reduced (Kundu et al., 2014). Microbial community was also affected by the shear in the continuously stirred anaerobic digester (Hoffmann et al., 2008; Jiang et al., 2016). Hoffmann et al. (2008) found that different mixing intensities ranging from 250 to 1500 rpm influenced the competition between the acetoclastic methanogens, *M. concilii* and *Methanosarcina* spp. *Methanosarcina* spp. became more abundant in the intensely mixed digester. Compared to recuperative thickening operation by rotary drum or centrifuge, in an upflow or continuously mixed anaerobic digester, shearing takes place continuously but usually at the much lower intensity. Thus, while these previous studies suggest the potential impact of shearing on the microbial community structure, it is not possible to directly apply these results to recuperative thickening operation.

Recuperative thickening has been successfully applied by Sydney Water (Sydney, Australia) to reduce biosolids malodour and increase biogas production from primary sludge at the Bondi WWTP. An examination of the literature presented above and our initial work (Bharambe et al., 2015; Yang et al., 2015) have established several knowledge gaps in implementing recuperative thickening in a wider context. In particular, little is known about the effects of different levels of shearing of the thickened sludge on biogas production and the microbial community. Thus, this study aims to quantify the effects of shearing during recuperative thickening on biogas production as well as COD and VS removal by anaerobic digestion. The microbial community structure of the digested sludge is also systematically examined to elucidate possible dynamic changes in microbial community in response to shearing during the thickening in rotary drums or centrifuges prior to putting the thickened sludge back in the anaerobic digester.

2. Methods

2.1. Operational protocol of anaerobic digesters

2.1.1. Laboratory anaerobic digesters set-up

Three identical 28 L lab-scale anaerobic digesters were used in parallel. One digester was used as the control (i.e. without any shearing) while medium and high shearing were applied to the recuperative sludge of the other two digesters (as will be described in a later section). Each digester consisted of a 28 L conical shape stainless steel reactor (Core Brewing Concepts, Victoria, Australia), a peristaltic hose pump (DULCO®flex from ProMinent Fluid Controls, Australia), a temperature control unit (Neslab RTE 7), a thermal couple with temperature gauge, an online gas meter and a gas trap for biogas sampling. The digesters were heated by hot water in plastic tubes which were wrapped around the digester. The hot water in the plastic tube was regulated by the temperature control unit to keep the digester at 35.0 ± 0.5 °C. Volumes of biogas production from each digester was monitored using the online gas meter. Once a week, biogas was captured in the gas trap for composition analysis. Anaerobically digested sludge from the Wollongong WWTP (New South Wales, Australia) was used to seed all three digesters simultaneously at the beginning of the study.

Primary sludge was also collected from the Wollongong WWTP every fortnight and used as the feed. This primary sludge has an average TS content of 24.5 ± 2.1 g/L (average ± standard deviation of 20 samples), and was stored at 4 °C in the dark.

2.1.2. Experimental protocol

All three digesters were operated with recuperative thickening to achieve an SRT of 30 days while maintaining an HRT value of 20 day. The digester was mixed by sludge circulation at 60 L/h. Each day, 2 L of sludge was extracted from the digester and a high molecular weight cationic thickening polymer (Zetag 8169, BASF) was added at a dose of 7.5 g/kg dry sludge. The sludge was allowed to settle for 10 min. After settling, 0.67 L of the supernatant was wasted and the remaining (1.33 L of thickened sludge and supernatant) was subjected to specified shearing levels. This procedure is to ensure consistent thickening regardless of the shearing condition. Then, 1 L of the thickened and sheared sludge was returned to the digester together with the daily feed (i.e. 1 L of primary sludge). The excess thickened sludge (0.33 L) was discarded.

Digester D1 was the control system with gravity thickening (designated as no shearing) during the thickening process. Shearing was applied to thickened sludge from digesters D2 and D3 (Table 1). An agitator (Servodyne mixer head, model 50003-25, Boronia, Australia) with a 2-blade bending paddle impeller (5 cm × 10 cm) was used to provide medium shearing at 300 rpm ($G = 3140\ s^{-1}$ comparable to the shearing level of a typical rotary drum) and high shearing at 600 rpm ($G = 6280\ s^{-1}$ comparable to the shearing level of a typical high speed centrifuge) to the thickened sludge from D2 and D3, respectively. A food blender (Sunbeam, model PB9500, Australia) was also used to simulate excessive shearing to the thickened sludge from digester D3 (Table 1). In all cases, the shearing process lasted 5 min.

2.1.3. Regeneration of the anaerobic digester

Due to the deteriorated digester performance caused by excessive or high shearing, digester D3 was regenerated by renewing part of the biomass content with 5 L of digestate from a full scale WWTP at the beginning of the third experimental period. At the beginning of the 4th experimental period, another 5 L of the biomass in digester D3 was replaced by freshly collected anaerobically digested sludge from the full scale WWTP. During period 3 and 4, digester D3 remained at SRT of 30 d and HRT of 20 d with recuperative thickening, meanwhile, no shearing was applied to the thickening process (Table 1). The aim of this experimental component is to determine if the digester can be recovered after being negatively affected by excessive shearing.

2.2. Anaerobic digestion parameter analysis

2.2.1. Biogas production and composition analysis

Biogas production was monitored using an online gas counter (Yang et al., 2016). Biogas composition was analysed weekly using a portable gas analyser (GA5000 gas analyser, Geotechnical Instruments (UK) Ltd, England) as previously described elsewhere (Nghiem et al., 2014). Methane production activity ($L\text{-}CH_4/g\ COD_{removed}$) was calculated based on the methane composition in biogas and the biogas production rate.

2.2.2. Sludge characteristics

Basic characteristic parameters of the feed sludge to the anaerobic digesters were analysed on a weekly basis. These parameters include TS, VS, total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), pH, and alkalinity. pH was measured by a pH and conductivity meter (Orion 4 Star, Thermo Scientific, Australia). The procedures to measure TS, VS, and alkalinity have been described elsewhere (Semblante et al., 2015). COD was measured following the US-EPA Method 8000 using high range COD vials (HACH, USA). The supernatant used for measurement of sCOD was obtained by centrifuging sludge sample at 3200g for 10 min (Allegra X-12R centrifuge, Beckman Coulter, Australia), and then filtering through 1 μm glass filter paper (Filtech, Australia).

2.3. Microbial community structure analysis

2.3.1. DNA extraction and 16S rRNA gene amplicon sequencing

Duplicate digested sludge samples were collected from all three anaerobic digesters at the end of the 1st (Day 55) and 2nd (Day 110) experimental period (Table 1). DNA extraction was conducted

Table 1
Operational regime of the three anaerobic digesters.

Operational parameters	Period 1 (Day 1–55)			Period 2 (Day 56–114)			Period 3 & 4 (Day 115–142)		
	D1	D2	D3	D1	D2	D3	D1	D2	D3
Shearing (rpm)	0	300	600[#]	0	300	600	0	300	0
Recuperative thickening	Yes			Yes			Yes		
HRT (d)	20			20			20		
SRT (d)	30			30			30		
Withdrew sludge (L/d)	2			2			2		
Wasted sludge (L/d)	0.67								
Thicken ratio	1.33								
Primary sludge feed (L/d)	1								

[#] A food blender was used to simulate excessive shearing.

immediately using the FastDNA spin kit for soil (MP Biomedical, NSW, Australia). DNA quality was assessed using 1% agarose gel electrophoresis and Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Detailed description of this DNA extraction procedure is available elsewhere (Luo et al., 2016).

Extracted genomic DNA was submitted to the Australian Genome Research Facility (Brisbane, QLD, Australia) for amplicon sequencing on the Illumina MiSeq platform, utilizing Illumina's Nextera XT Index's and Paired End sequencing chemistry. V3-V4 variable regions of microbial 16S rRNA gene were targeted using primer pairs: 341F (5′–CTAYGGGRBGCASCAG–3′) and 806R (5′–GGACTACNNGGGTATCTAAT–3′).

2.3.2. Sequence analyses

Amplicon sequences were processed using the QIIME (version 1.9.1) (Caporaso et al., 2010b) and USEARCH (version 8.1.1861) (Edgar, 2013) software packages. Paired-end reads were merged using fastq-join method with minimum overlap of 100 bp. Primers were trimmed using QIIME script. The Fastq file of trimmed sequences was processed following UPARSE pipeline: quality filtering (maximum error rate of 0.5; sequences were trimmed to 240 bases and any with less than 240 bases excluded), discarding full length duplicates, abundance sorting, disposing singletons and chimera filtering. Sequences were clustered into operational taxonomic units (OTUs) and reads were then mapped back to OTUs with a minimum identity of 97%. Taxonomy was assigned by *uclust* (Edgar, 2010) using Greengenes database (version 13_8, Aug 2013) in QIIME. Representative sequences were aligned using PyNAST (Caporaso et al., 2010a). Aligned sequences were filtered the gaps and then used to generate phylogeny tree by method FastTree (Price et al., 2010).

After quality filtering, removing chimeric and singletons, 1,959,000 paired-end reads were obtained for total samples with sequence statistics of 110,000/269,000/139,000/163,000/47,000 (min/max/median/mean/std, respectively). A total of 3051 operational taxonomic units (OTUs) at 97% sequence similarity were assigned. For summary of microbial composition, OTU with an abundance of less than 0.05% was removed.

For α and β-diversity analysis, to eliminate the heterogeneity caused by having different numbers of sequences among the samples, equivalent numbers of sequences were subsampled by rarefaction (10 iterations) to the lowest number of sequences (110000 sequences) found among the samples. Specifically, α-diversity comparisons were determined using observed species, phylogenetic diversity (PD_whole_tree) and Simpson index. Good's coverage was calculated to assess the completeness of sampling and the possibility that an amplicon sequence selected randomly has already been sequenced. For β-diversity comparison, a weighted UniFrac distance metric (Lozupone et al., 2007) was constructed and then visualized via PCoA (Principal Coordinate Analysis). All analyses were implemented in QIIME. All sequencing data in this study are available at the Sequence Read Archive (accession number: SRP074867) in the National Centre for Biotechnology Information.

3. Result and discussion

3.1. Digesters performance under different levels of shearing

There were some discernible effects of shearing on biogas production during recuperative thickening (Fig. 1). Compared to the control digester (D1), digester D2 produced approximately 15% more biogas throughout the experiment periods (Fig. 1), which is comparable to the 10% increase in biogas yield observed by Cobbledick et al., (2016) when they conducted recuperative thickening experiment without any shearing. In our laboratory scale study, the thickened sludge that was circulated back to digester D2 was also subjected to medium shearing (equivalent to that from a rotary drum). On the other hand, excessive shearing (by a food blender) was detrimental to biogas production. Biogas production from digester D3 was approximately 30% lower than that of the control digester (D1) in the 1st experimental phase. The level of shearing applied to the thickened sludge of digester D3 was induced by a mixer at 600 rpm (equivalent to that from a high speed centrifuge) rather than the food blender in period 2; however, improvement in biogas production could not be observed (Fig. 1). Similar trends were observed when examining the methane production activity (Table 2). Methane production activity of D2 (approximately 0.5 L CH_4/g $COD_{removed}$) was similar to that of the control system D1 throughout experiment periods 1 and 2, and gradually increased to approximately 0.73 L CH_4/g $COD_{removed}$ at the end of the experiment (period 4). By contrast, excessive or high shearing led to a low methane production activity of D3 (0.24–0.26 L CH_4/g $COD_{removed}$) in period 1 and 2. In contrast to previous results by Jiang et al., (2016) who reported a decrease in methane content in biogas due to shearing, in this study, biogas composition was not affected by the shearing. Indeed, all biogas samples were composed of approximately 60% methane and 40% carbon dioxide. Our results suggest that the impact of shearing was mostly on hydrolysis, acidogenesis, and acetogenesis rather than methanogenesis since this last step (methanogenesis) was responsible for the conversion of intermediate products (e.g. organic acids) to methane and carbon dioxide.

Following the experimental period 2, regeneration of D3 was conducted in period 3 and 4, respectively, by renewing 25% of the working volume each time (Table 1). The regeneration led to a notable recovery of biogas production (Fig. 1) and methane production activity (Table 2), resulting in similar level of control system (digester D1) at the end of period 4. These results reaffirm that excessive or high level of shearing could negatively affect the methanogenic activity.

Due to the temporal variation in TS and VS content of the thickened primary sludge between wet and dry weather conditions, the removals of TS and VS by all three digesters were highly variable. There were similar variations in tCOD (from 19,000 to 39,000 mg/L) and sCOD (from 1200 to 2300 mg/L) in the primary sludge. Nevertheless, the effects of digestate shearing during recuperative thickening on both tCOD and sCOD removals by all three anaerobic digesters could be observed (Fig. 2). Compared to the control digester (D1), digester D2 with medium shearing showed similar tCOD and sCOD removal efficiencies during the entire experimental periods (from 1 to 4). This observation is consistent with the methane production activity of D1 and D2 (Table 2). On the other hand, digester D3 with excessive and high shearing showed higher tCOD removal but lower sCOD removal during period 1 when excessive shearing was applied. In period 2, tCOD removal decreased to a similar of control digester (D1) when shearing was changed from excessive (using the food blender) to high (i.e. G = 6280 s^{-1}) as can be seen in Fig. 2a. These results indicate that excessive shearing could solubilise some solid COD and the benefit from an increase in the soluble COD fraction in the substrate may offset any negative impact from cell rupture and exposure to oxygen during the recuperative thickening process. On the other hand, excessive or high level of shearing (digester D3) resulted in a significant increase in the sCOD fraction (Hoffmann et al., 2008), thus, causing an increase in tCOD removal (Fig. 2a) but a notable decrease in sCOD removal (Fig. 2b).

It is noteworthy that the alkalinity and pH value of each digester were stable throughout the experiment. The digestate pH of all three digesters was ranging from 7.01 to 7.72, which was typical for normal anaerobic digestion. Alkalinity of all digesters was also

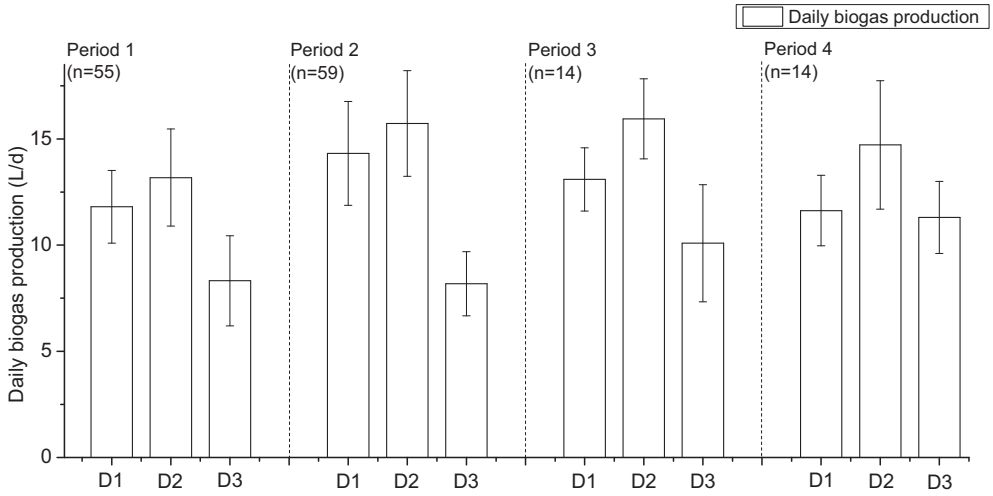

Fig. 1. Daily biogas production from each individual digester. In the 3rd and 4th experimental period, the biomass in D3 was regenerated as described in Section 2.1.3 while operation of D1 and 2 was the same as in period 2 (error bars show the standard deviation from eight measurements in period 1 and 2; and four measurements in period 3 and 4; n = number of days of operation).

Table 2
Methane production activity and biogas composition during the experiment.

		D1	D2	D3
Period 1	Methane production acitivity (L CH_4/g COD $_{removed}$)	0.51	0.49	0.24
	CH_4/CO_2 (%/%)	60.4/38.1	59.8/38.5	58.1/39.0
Period 2	Methane production acitivity (L CH_4/g COD $_{removed}$)	0.49	0.52	0.26
	CH_4/CO_2 (%/%)	59.7/38.6	60.6/38.2	59.0/39.0
Period 3	Methane production acitivity (L CH_4/g COD $_{removed}$)	0.62	0.73	0.35
	CH_4/CO_2 (%/%)	59.6/39.5	59.6/37.9	60.2/39.1
Period 4	Methane production acitivity (L CH_4/g COD $_{removed}$)	0.49	0.66	0.56
	CH_4/CO_2 (%/%)	60.2/38.4	61.1/38.7	59.2/39.4

stable, ranging from 2700 to 3600 mg$CaCO_3$/L. Over all, all three digesters were in good condition throughout the current study. There was no indication of volatile fatty acid or ammonia accumulation in the digesters.

3.2. Impact of shear stress on microbial community dynamics

3.2.1. Microbial diversity

Duplicated microbial community samples were taken at the end of period 1 (day 55) and 2 (day 110), respectively for each digester. Overall, 25 bacterial and one archaeal phyla were assigned for all samples and only very small number of sequences (1.7 ± 1.5%, n = 6) were not classified at this level. Major bacterial phyla were *Bacteroidetes* (31.9 ± 9.5%, n = 6), *Firmicutes* (17.5 ± 8.5%, n = 6), *Proteobacteria* (13.8 ± 3.6%, n = 6) and *Spirochaetes* (10.1 ± 9.7%, n = 6). Other bacterial phyla (*Acidobacteria, Actinobacteria, Caldiserica, Chloroflexi, Elusimicrobia, Fibrobacteres, OP8, Planctomycetes, SAR406, Synergistetes, Theromotogaes, Verrucomicrobia* and *WWE1*) can present up to 10% of the sequences. The rare phyla (<0.5%) were grouped into 'minor groups', including *Chlorobi, Cyanobacteria, Fusobacteria, Lentisphaerae, NKB19, OP3, OP9,* and *WPS-2*. The sequence distribution among bacterial and archaeal phylogenetic groups in this study was consistent with the core of microorganisms involved in anaerobic digestion systems (Riviere et al., 2009).

The rarefaction curves (at 97% sequence similarity) from all samples were showed in Fig. 3. Consistent with the observed increase in biogas production, digested sludge of D2 (medium shearing) also exhibited the highest microbial diversity in terms of observed_species and phylogenetic diversity. On the other hand, excessive shearing applied to D3 (sample D3_d55) led to the lowest microbial diversity. It is also notable that an increase in the microbial diversity (Observed_species and Phylogenetic diversity) at the end of period 2 (D3_d110) when D3 condition was changed from excessive shearing to high shearing. Based on Simpson index, sludge samples from D2 and D1 were more evenly distributed than those of D3. Similarly, Rochex et al., (2008) reported a decrease of biofilm diversity under high shear stress (0.238 Pa) in biofilm formation system. The lower Simpson index of sample D3_d110 than that of sample D3_d55 probably indicated that the D3 may have not reached steady state after 55 d at high shearing level. Good_coverage showed >99% coverage for each sample, indicating that only less than 1 additional OTU would be found if 100 additional sequences were provided.

The weighted UniFrac distance metric, which based on the relative abundances of all phylotypes in a sample, was interpreted via PCoA (Fig. 4). The close clustering within locations indicates that samples were more similar to each other in phylogenetic structure than they were to samples from other locations. As expected, all duplicate samples were plotted either very closely or overlapped with each other. As can be seen from Fig. 4, samples from D2 and D3 were clustered in three groups along the PC1 vector (accounted for 59% variation) in corresponding to the applied shearing force, namely, excessive shearing (D3_d55 of digester 3), high shearing (D3_d110 of D3) and medium shearing (all sample of D2). This result indicated the impact of shearing on microbial community structure.

3.2.2. Dynamics of microbial communities

Taxonomic classification at order level was systematically examined to verify the dynamics of microbial communities. Overall, 50 microbial orders were identified and only small proportion (1.7–6.6%) of reads was unclassified at this level. Of which, 16

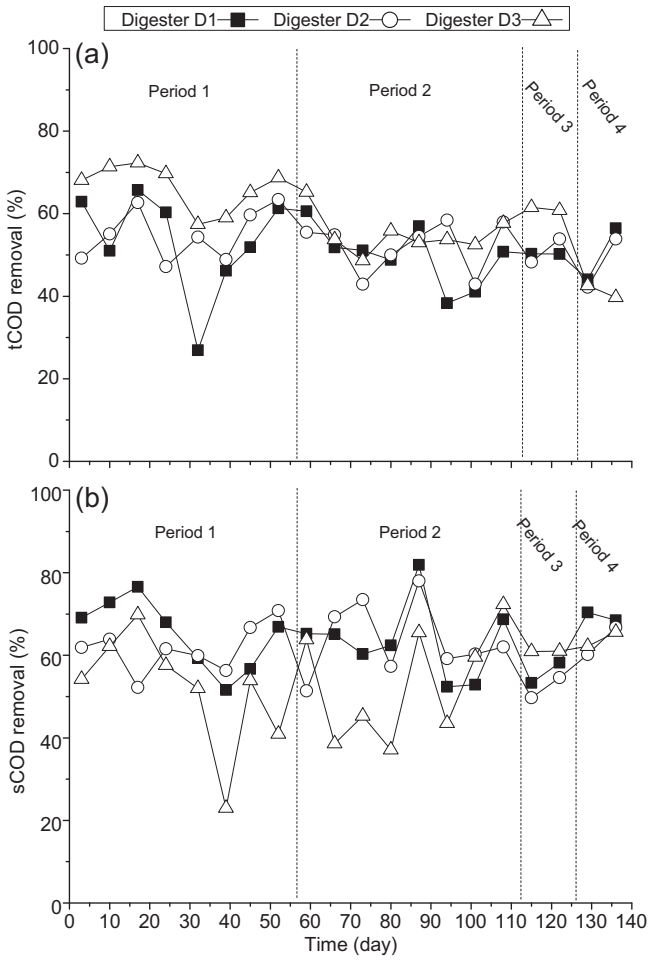

Fig. 2. Removals of (a) tCOD and (b) sCOD by the three anaerobic digesters with recuperative thickening and different levels of shearing.

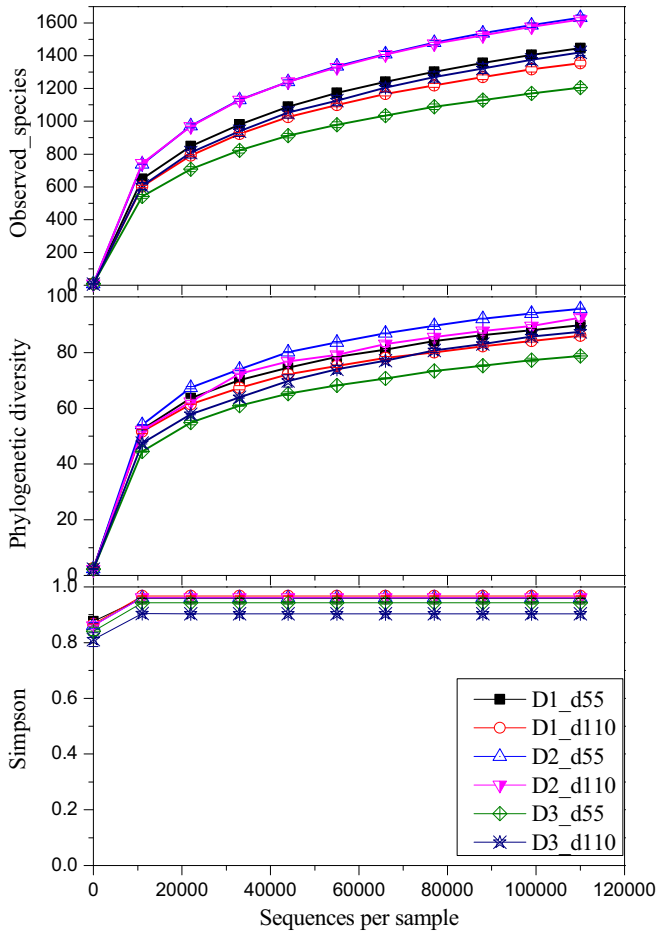

Fig. 3. Rarefaction curves (at 97% sequence similarity) for Observed_species, Phylogenetic diversity and Simpson were analysed at event sequencing depth of 110,000 sequences per sample (lowest sequence reads noted among samples). Error bars indicate standard deviation of duplicate samples collected from at day 55 and day 110 of experimental period for three anaerobic digesters: digester 1 (D1_d55 and D1_d110), digester 2 (D2_d55 and D2_d110) and digester 3(D3_d55 and D3_d110).

orders were accounted for > 80% of the population abundance (Fig. 5). *Bacteroidales* (31.6 ± 9.4%, n = 6) was the most abundant order, following by *Clostridiales* (17.1 ± 8.6%, n = 6), *Spirochaetales* (8.7 ± 9.8%, n = 6), *Cloacamonales* (5.1 ± 3.6%, n = 6) and *Syntrophobacterales* (5.0 ± 2.2%, n = 6). The most abundant archaeal population belonged to the order *Methanomicrobiales* (1.4 ± 0.4%, n = 6).

In terms of relative abundance, significant shearing impact was observed with four bacterial orders namely *Bacteroidales*, *Clostridiales*, *Syntrophobacterales* and *Spirochaetales*. These are well known orders of anaerobic microbes in anaerobic digestion of wastewater sludge. *Bacteroidales* was the most abundance in D2 (medium shearing) (42.3 ± 2.3%, n = 2), following by D1 (control) (28.4 ± 2.9%, n = 2). This order was lowest in D3 when excessive shearing applied (18.4%), but it was significantly increased to 30% when switching to high level shearing for 55 days during period 2. The distribution of *Clostridiales* was quite stable in D1 and D2 (11.2–15.6%). However, their abundance in D3 was increased significantly from 15.6% to 34.4% when shearing was decreased from excessive to high level. *Bacteroidales* and *Clostridiales* are well known for their role in hydrolysis and fermentation (Jaenicke et al., 2011; Nolla-Ardevol et al., 2015; Regueiro et al., 2012; Schlüter et al., 2008). Werner et al., (2011) proposed that these bacterial groups rely more on redundancy to maintain the overall community function. The abundance of syntrophic division *Syntrophobacterales* was highest in D2 (from 5.8% in day 55 to 8.0% in day 110), following by D1 (from 5.3% in day 55 to 6.0% in day 110) and then lowest in D3 (from 2.1% in day 55 to 2.7% in day 110). *Syntrophobacterales* was a specialized group for metabolic function of short-chain fatty acid oxidation (Ariesyady et al., 2007; McInerney et al., 2009). *Syntrophobacterales* population was found to be the most sensitive to perturbation during anaerobic digestion processes. Results reported here suggest that this bacterial group was able rebound after perturbation rather than being replaced by other groups with similar function and that the level of perturbation by medium shearing was not detrimental to anaerobic digestion. On the other hand, *Spirochetales* (mainly genus *Treponema*) was particularly the most abundant order (28.5%) in D3 when excessive shearing applied, and it was significantly decreased to 5.2% when shearing level reduced in D3 for 55 days. The presence of *Spirochaetales* in D1 and D2 was low and slightly decreased from 6.8 % and 4.2% (day 55) to 5.0% and 2.3% (day 110), respectively. The function of *Treponema* in anaerobic digestion was poorly understood. It may play a role on acetate production at the acetogenesis step (Guo et al., 2015) or relate to utilization of glucose (Ariesyady et al., 2007).

Dynamic changes in bacterial community were also observed for other orders including *Burkholderiales*, *Rhodocyclales* (belonging to *β-Proteobacteria*) and *Synergistales*. These bacterial orders was reported to involve in utilization of fatty acids (propionate, butyrate or acetate) (Ariesyady et al., 2007). Overall, the trend of

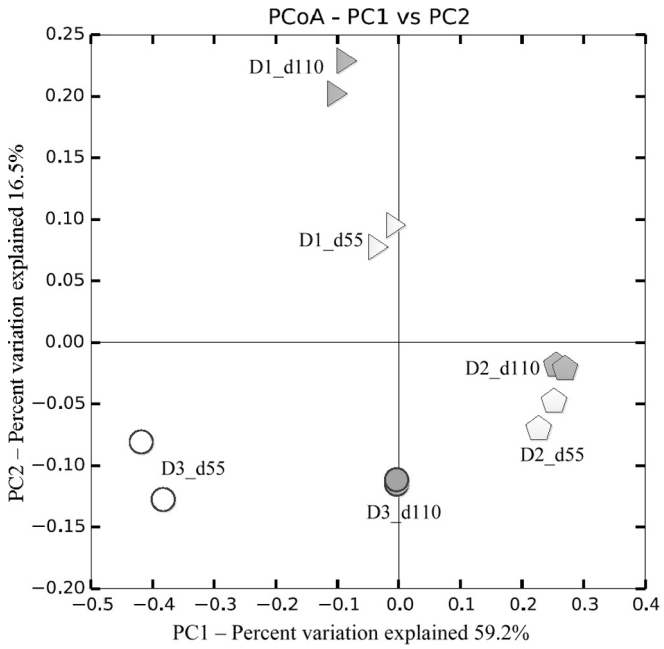

Fig. 4. Phylogenetic distances between samples determined via weighted UniFrac principal coordinates analysis (distance matrix calculated at even sequencing depth of 110,000 sequences per sample). Duplicate samples collected from three anaerobic digesters at day 55 and day 110 of experimental period: digester 1 (D1_d55 and D1_d110), digester 2 (D2_d55 and D2_d110) and Digester 3 (D3_d55 and D3_d110).

microbial communities observed in D1 and D2 showed the increase of even distribution of the bacterial phylotypes from day 55 to day 110, the decrease of abundant phylotypes as well as increase of minor groups (Fig. 5). A greater evenness of community was considered as an indicator of better performance of anaerobic digestion process (Werner et al., 2011).

Archaeal population was present at low abundance in all samples with only one phylum *Euryarchaeota* (1.2–2.5%). No significant variation between samples was observed for this population (Fig. 5). The most abundant order was *Methanomicrobiales* (0.8–2.0%), following by *Methanosarcinales* (0.1–0.4%), E2 (belonging to *Thermoplasmata*, <0.4%) and *Methanobacteriales* (<0.2%). Syntrophic association between *Clostridiales* (mainly genus *Clostridium*) populations and hydrogenotrophic methanogens (*Methanomicrobiales*) has been reported in the literature (Jaenicke et al., 2011; Schlüter et al., 2008). Such syntrophic association can explain for the prevalence of *Methanomicrobiales* compared to other archaea as observed here. It is noted that the primer pairs 341F/806R applied in this experiment was not specialized to target archaeal, so it probably led to underestimate the archaeal population. However, Hanreich et al., (2013) observed that methanogenic population represented less than 4% of the community, but protein of archaeal origin accounted for 20–30% of the identified protein, suggesting a disproportional active of methanogens.

3.2.3. Correlation between digester performance and microbial community structure

A good correlation between microbial diversity and reactor performance was observed in this study. D2 with medium shearing sustained the development of microbial communities with higher diversity and evenness (Fig. 3) that was well correlated with a better biogas production (Fig. 1). These results highlighted the importance of microbial diversity and evenness of anaerobic digestion communities. The results in this study suggest that diversity and evenness of microbial community and their dynamic over time are important ecological parameters to maintain functional stability and robustness of anaerobic digesters. Anaerobic digestion communities with greater evenness and phylogenetic variability could function more efficiently. Taxonomic classification demonstrated the dynamic of microbial community over time. It also indicated the impact of shear force on important functional bacterial groups. The abundance and stable of *Bacteroidales* and *Clostridiales*,

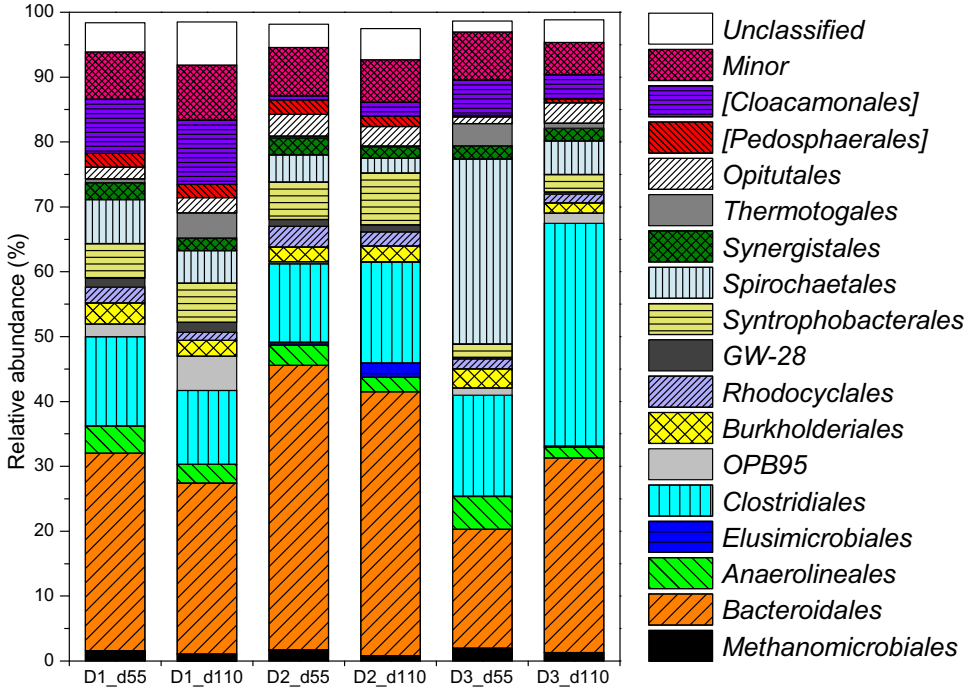

Fig. 5. Relative abundance of microbial community at order level. Plotted values are mean of duplicate samples collected from three anaerobic digesters at day 55 and day 110 of experimental period: digester 1 (D1_d55 and D1_d110), digester 2 (D2_d55 and D2_d110) and digester 3 (D3_d55 and D3_d110). Microbial orders less than 1.5% in relative abundance were grouped in Minor. The sum did not reach 100% since operational taxonomic units (OTUs) less than 0.05% was filtered from OTU table.

important hydrolytic and fermentative bacteria, in digester D2 resulted in higher capacity to use redundant functional pathways to maintain the efficiency of the system. The resilient abundance of *Syntrophobacterales* increased over time, particularly in digester D2, which emphasized on their specialized function in short-chain fatty acid oxidation (Vanwonterghem et al., 2014). It is also indicated that excessive or high level of shearing in digester D3 did not favour the *Bacteroidales* and *Syntrophobaterales*, which worked as hydrolyzer and acetogens, respectively, in the anaerobic digestion process, and led to reduced biogas production for digester D3 (Vanwonterghem et al., 2014). Despite the lack of specific Archaeal target primers, the syntrophic association between *Clostridiales* (mainly genus *Clostridium*) populations and hydrogenotrophic methanogens (*Methanomicrobiales*) was demonstrated. Excessive shearing created the condition that highly favoured the development of *Spirochaetales* (mainly *Treponema*). Probably, the high available sCOD/organic matters released during excessive shearing process in digester D3 explained for this high abundant of *Treponema*.

4. Conclusions

This study elucidates the effect of shearing (comparable to rotary drum and high speed centrifuge) due to sludge thickening on microbial community structure and anaerobic digestion performance during recuperative thickening operation. Medium shearing improved biogas production and tCOD removal, while high or excessive shearing negatively affected the digester performance. Shearing had a noticeable effect on the microbial population. Medium shearing improved the diversity and evenness of microbial community, resulting in an improved digestion performance in terms of biogas production and tCOD removal, while high shearing was not beneficial to hydrolyzer and acetogens of anaerobic digestion, leading to deteriorating digestion performance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.03.051.

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