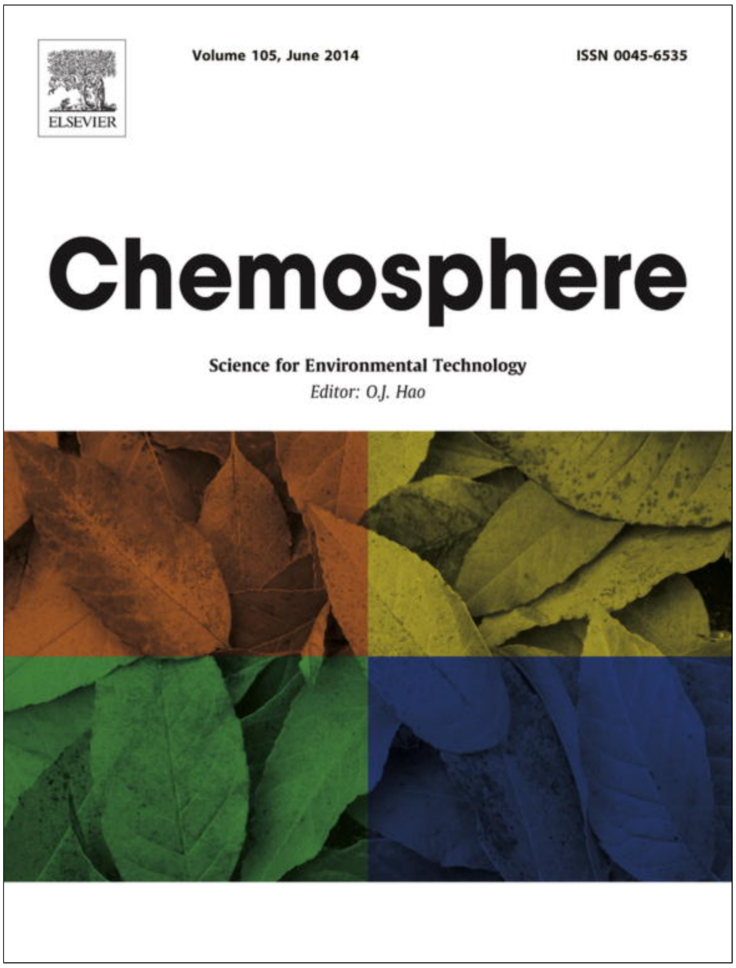
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Technical Note

Effects of temperature and organic loading rate on the performance and microbial community of anaerobic co-digestion of waste activated sludge and food waste

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HIGHLIGHTS

- Co-digestion of WAS and FW was investigated at different temperatures and OLRs.
- The CH_4 yield and VS removal efficiency were decreased as OLR gradually increased.
- The thermophilic system had the highest endurable OLR of 7 g VS L^{-1} d^{-1}.
- The mesophilic system showed the best stability at low OLR (< 5 g VS L^{-1} d^{-1}).
- The microbial community was more affected by temperature than the OLR.

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ABSTRACT

Anaerobic co-digestion of waste activated sludge and food waste was investigated semi-continuously using continuously stirred tank reactors. Results showed that the performance of co-digestion system was distinctly influenced by temperature and organic loading rate (OLR) in terms of gas production rate (GPR), methane yield, volatile solids (VS) removal efficiency and the system stability. The highest GPR at 55 °C was 1.6 and 1.3 times higher than that at 35 and 45 °C with the OLR of 1 g VS L^{-1} d^{-1}, and the corresponding average CH_4 yields were 0.40, 0.26 and 0.30 L CH_4 g^{-1} VS_{added}, respectively. The thermophilic system exhibited the best load bearing capacity at extremely high OLR of 7 g VS L^{-1} d^{-1}, while the mesophilic system showed the best process stability at low OLRs (< 5 g VS L^{-1} d^{-1}). Temperature had a more remarkable effect on the richness and diversity of microbial populations than the OLR.

1. Introduction

Anaerobic digestion (AD) is widely applied for sludge stabilization to reduce the sludge volume, generate methane gas, and yield a nutrient-rich final product (Appels et al., 2011). However, its efficiency is largely limited due to the relatively slow hydrolysis process, as waste activated sludge (WAS) is mainly composed of microbial cells within extracellular polymeric substances, and cell walls are physical barriers that do not permit intracellular organics to be easily biodegraded through digestion (Toreci et al., 2011). In previous studies, pretreatments such as mechanical (Nah et al., 2000), microwave (Toreci et al., 2011), alkaline (Li et al., 2012) and ultrasonic (Xu et al., 2011) were reported to improve the efficiency of AD by disrupting sludge membranes to release the intracellular nutrients, but extra energy or chemicals were greatly consumed simultaneously.

Co-digestion of sludge with other organic-rich residues seems to be an attractive method which has been used to overcome its low digestibility in several studies (Habiba et al., 2009; Silvestre et al., 2011). Meanwhile, proper co-digestion could dilute potential hazardous compounds, promote synergistic effects of microorganisms and enhance biogas yields (Wan et al., 2011). Food waste (FW), with its high organic contents and excellent biodegradability, was regarded as an appropriate substrate that can be treated by AD (Zhang et al., 2007). Furthermore, plenty of attention has been paid due to its huge production from daily life in China. Nevertheless, some literatures pointed out that the digestion of FW alone may lead to the accumulation of abundant volatile fatty acids (VFA)

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especially at high organic loading rate (OLR), which could inhibit the methanogenesis and even destabilize the anaerobic process (El-Mashad et al., 2008; Nagao et al., 2012). These findings led to the investigation of its co-digestion with WAS as an alternative. Moreover, co-digestion of WAS and FW could be a strategic and cross-sectorial solution to deliver beneficial synergies for the water industry and FW management authorities (Iacovidou et al., 2012).

Efficiency and process stability are proved to be the criteria for the performance of AD (Lv et al., 2010). Most recently, impacts of mixing ratios and hydraulic retention time (HRT) on the performance of co-digestion of WAS and FW have been discussed (Heo et al., 2004; Kim et al., 2007; Lee et al., 2009). In fact, the equilibrium and productivity of the fermentation process can also be greatly disturbed by the OLR (Luste and Luostarinen, 2010). The major problem is that with an extremely high OLR, the rate of hydrolysis/acidogenesis could be higher than methanogenesis, and the high concentration of VFA accumulated from hydrolysis/acidogenesis can eventually lead to an irreversible acidification (Nagao et al., 2012). Nevertheless, previous studies mainly focused on the methane production and volatile solids (VS) removal efficiency of co-digestion system in a tolerable range of OLR (Heo et al., 2004; Kim et al., 2004, 2006), the information on maximum feasible loading rate is still lacking. Therefore, it is interesting to investigate the critical value by increasing the OLR stepwise through long-term experience.

Temperature, another important environmental factor, directly affects the dynamic situation of microorganisms. Earlier studies mainly concentrated on improving the efficiency of co-digestion process at a certain operating temperature, such as mesophilic (Dai et al., 2013), thermophilic (Kim et al., 2011), and even under hyperthermophilic condition (Lee et al., 2009). It seems that more data were demanded to compare the co-digestion performances at different temperatures applying the similar OLR, especially using continuously stirred tank reactors (CSTRs) in which all of the reactions (hydrolysis, acidogenesis, and methanogenesis) happened simultaneously. In addition, although the microbial community has been analyzed in the acidogenic fermenter for anaerobic co-digestion of kitchen garbage and sewage sludge (Lee et al., 2009), the information concerning the effects of the gradient temperature and OLR on microbial community structures in CSTRs is currently insufficient.

Consequently, the objective of this study was to evaluate the efficiency and stability of anaerobic co-digestion of WAS and FW under a wide range of OLRs at different temperatures in CSTRs. Meanwhile, the microbial community involved in anaerobic co-digestion process was also investigated by means of 16S rDNA polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE).

2. Methods

2.1. Preparation of feed stocks and inoculums

WAS used in this study was obtained from the secondary clarifier of the second municipal wastewater treatment plant in Changsha, China. Fresh sludge was concentrated by sedimentation for 6 h before being used. FW was collected continuously over 5 consecutive working days from a refectory in Hunan University, Changsha, China. To facilitate the digestion effectively, the FW was crushed to a mean size of 3–5 mm by an electrical food grinder. Samples were stored at 4 °C for no longer than one week.

WAS and FW were mixed with a total solids (TS) ratio of 2:1, which was demonstrated to have the best stability and efficiency in our preliminary experiments, and the value was approximately equal to the optimal mixture suggested by Kim et al. (2007). The detailed characteristics of these substrates are summarized in Table 1.

Table 1
Characteristics of feed stocks and inoculums.

Characteristics	Unit	Inoculums	WAS	FW	Co-substrate
TS	$g\,L^{-1}$	20	25	150	45
VS	% of TS	58	63	90	72
pH	–	7.8	7.2	5.6	6.8
SCOD	$mg\,L^{-1}$	2324	2165	7260	14838
C/N	–	5.8	7.2	34	13
VFA	$mg\,L^{-1}$	860	467	842	748
Alkalinity	$mg\,L^{-1}$ as $CaCO_3$	212	143	453	3662
Ammonium	$mg\,L^{-1}$	221	163	113	160

2.2. Semi-continuous anaerobic co-digestion systems

Three series of lab-scale CSTRs were installed with a working volume of 2.0 L. The temperatures were controlled at 35 ± 2, 45 ± 2 and 55 ± 2 °C by three water baths, and were referred to as R1, R2 and R3, respectively. Mixing was performed intermittently by magnetic stirrers at uniform speed of 200 rpm before and after the new substrate was added. About 60% of digester working volume was filled with inoculums, and the co-substrates were introduced into the reactors as the starting material. The systems were flushed with N_2 for 3 min to create an anaerobic environment before sealing. Then the feeding and withdrawing were conducted once a day according to the needed OLR. Starting-up OLR was 1 $g\,VS\,L^{-1}\,d^{-1}$ and the corresponding HRT was 33 d. The OLR was then increased to next step when the system reached a steady state. Corresponding operation process is shown in Table 2.

2.3. Analytical methods

Biogas volume was daily measured with water displacement, and methane content was analyzed by a gas chromatograph (GC 2010 Shimadzu) equipped with a thermal conductivity detector and a 2 m × 3 mm stainless-steel column packed with Porapak Q (80/100 mesh). Samples from the reactors were immediately centrifuged at 5000 rpm for further analyses. For the analysis of soluble chemical oxygen demand (SCOD) and ammonium, the supernatant was filtrated through a 0.45-μm membrane filter (Whatmann, USA). TS, VS, total VFA (TVFA), alkalinity, total nitrogen, ammonium, and SCOD were determined according to the Standard Methods (APHA, 2005).

2.4. Microbial community analysis

Samples were taken at steady state of each temperature under various OLRs for microbial community analysis. Total genomic DNA was extracted from 0.2 g digestion samples (wet weight) according to the method described by Yang et al. (2007). DNA was dissolved in 200 μL of 50 × TAE (2 M Tris, 1 M Acetate, 0.1 M $Na_2EDTA{\cdot}2H_2O$) buffer and 4 μL of DNA was used for agarose gel electrophoresis.

The PCR amplification was performed on an iCycler IQ5 Thermocycler (Bio-Rad, USA). The primer set GC341F and 534R were used for amplification. DGGE was carried out by using the Dcode Universal Detection System in accordance with the manufacturer's instructions (Bio-Rad, USA). The detailed running procedures for PCR and DGGE were operated as described by Zhang et al. (2011).

Table 2
Operational conditions of semi-continuous anaerobic co-digestion CSTR systems.

Days	1–30	31–60	61–90	91–120	121–150	151–170	171–180	181–188
OLR (g VS L^{-1} d^{-1})	1 ± 0.3	2 ± 0.3	3 ± 0.3	4 ± 0.3	5 ± 0.3	6 ± 0.3	7 ± 0.3	8 ± 0.3
HRT (d)	33.3	16.7	11.1	8.3	6.7	5.6	4.8	4.2
Flow rate (L d^{-1})	0.06	0.12	0.18	0.24	0.30	0.36	0.42	0.48
FW (g)	4.2 ± 0.1	8.4 ± 0.1	12.6 ± 0.1	16.8 ± 0.1	21.0 ± 0.1	25.2 ± 0.1	29.4 ± 0.1	33.6 ± 0.1

3. Results and discussion

3.1. Effect on gas production rate (GPR)

A semi-continuous operation was conducted to verify the performances of R1 (35 °C), R2 (45 °C) and R3 (55 °C) throughout 160, 178 and 188 d, respectively. A comparison of GPRs of the three systems is shown in Fig. 1. It illustrated that GPR apparently increased with increasing OLR before the extreme OLR was reached.

The first 30 d served as a start-up stage for each reactor at an OLR of 1 g VS L^{-1} d^{-1}. As seen from Fig. 1, GPRs in R1, R2 and R3 increased gradually and then became stable around day 7, 14 and 12, with average values of 0.35, 0.43 and 0.55 L L^{-1} d^{-1}, respectively. The relatively low GPR during the first several days was most likely due to the predominant acidogenic activity, and the subsequent steady value indicated that a delicate balance was achieved between the rates of hydrolysis/acidogenesis and methanogenesis.

In mesophilic digestion system, GPR went up to approximately 1.3 L L^{-1} d^{-1} with OLR stepwise increased to 4 g VS L^{-1} d^{-1}. The enhanced GPR was mainly ascribed to the increased biodegradable materials in the feedstock. Thereafter, a significant pH drop to 6.3 was noticed with an OLR of 5 g VS L^{-1} d^{-1} on day 121. In order to maintain a steady state, moderate $NaHCO_3$ (1 M) was added to alleviate pH inhibition. Under that condition, R1 could operate favorably at 5 g VS L^{-1} d^{-1} despite with a relatively low GPR of 1.2 L L^{-1} d^{-1}. As OLR further increased to 6 g VS L^{-1} d^{-1} on day 151, however, GPR decreased drastically close to zero with pH dropping to 4.9, and an irreversible acidification occurred even with a large amount of alkalinity being added into the system. Based on these results, it was possible to predict that the maximum endurable OLR was 5 g VS L^{-1} d^{-1} for mesophilic co-digestion of WAS and FW in this study. For R2, the highest average GPR of 1.6 L L^{-1} d^{-1} was achieved when the reactor operated at an OLR of 5 g VS L^{-1} d^{-1}, while for R3, the corresponding values were 2.1 L L^{-1} d^{-1} at 6 g VS L^{-1} d^{-1}.

Obviously, at the same OLR, average GPR of R3 was about 1.6 and 1.3 times higher than those in R1 and R2, respectively. Despite the same tendency in the case of R2 and R3, the maximum available OLRs were achieved at 6 and 7 g VS L^{-1} d^{-1}. These data indicated that thermophilic methanogens performed more effectively and had a better bearing capacity for high OLR than those at the other two lower temperatures, which was consistent with previous studies that reported the superior performance of the thermophilic processes (Bolzonella et al., 2012; Cavinato et al., 2013).

3.2. Effect on CH_4 yield and VS removal efficiency

Fig. 2 compares the CH_4 yields and VS removal efficiencies obtained from the three systems. As shown in Fig. 2a, it was clear that the CH_4 yield decreased with increasing OLR especially for the thermophilic system, which was reduced by approximately 43% as OLR increased from 1 to 6 g VS L^{-1} d^{-1}. While for R1, only a small decline that varying from 0.26 to 0.23 L CH_4 g^{-1} VS_{added} (1–4 g VS L^{-1} d^{-1}) was found. Although a stable GPR was achieved at the maximum OLR for each system, the CH_4 yield was only around 0.15 L CH_4 g^{-1} VS_{added}.

The decreased CH_4 yield with increasing OLR was also noted in many previous studies (Kim et al., 2006; Linke, 2006). One of the reasons might be that shorter HRT of the system contributed to more active methanogens were washed out during removal of effluent. On the other hand, the average TS concentrations in R1, R2 and R3 gradually increased respectively from 23, 20 and 15 g L^{-1} to 28, 30 and 29 g L^{-1} with OLR increased from 1 g VS L^{-1} d^{-1} to the corresponding maximum values. The increased TS concentration might reduce the mass transfer efficiency in the co-digestion substrate, and finally result in a decrease in methane yield (Nagao et al., 2012).

The relatively similar values ranging from 0.21 to 0.32 L CH_4 g^{-1} VS_{added} at OLRs of 2.0–2.4 g VS L^{-1} d^{-1} were reported in Heo et al. (2004) for anaerobic co-digestion of FW and WAS in

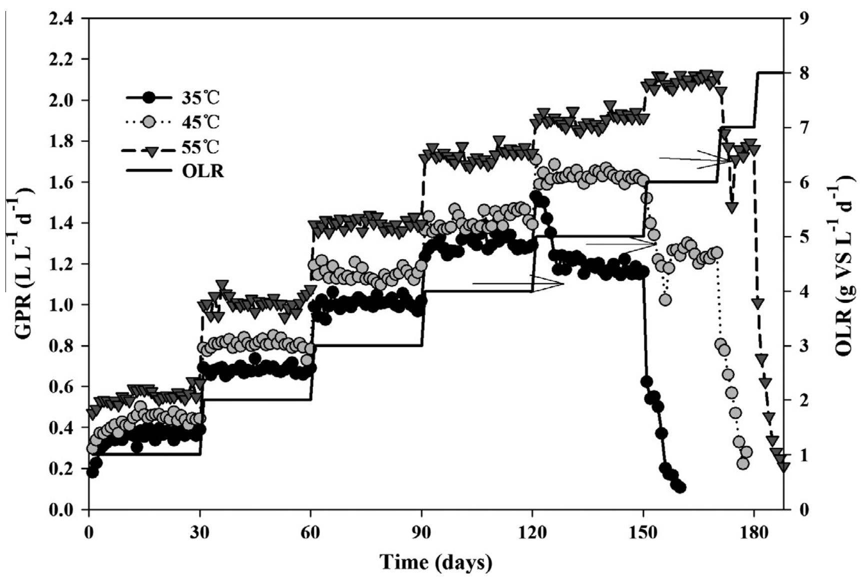

Fig. 1. Variation of GPR according to different temperatures and OLRs. Arrows in this figure mean addition of alkalinity.

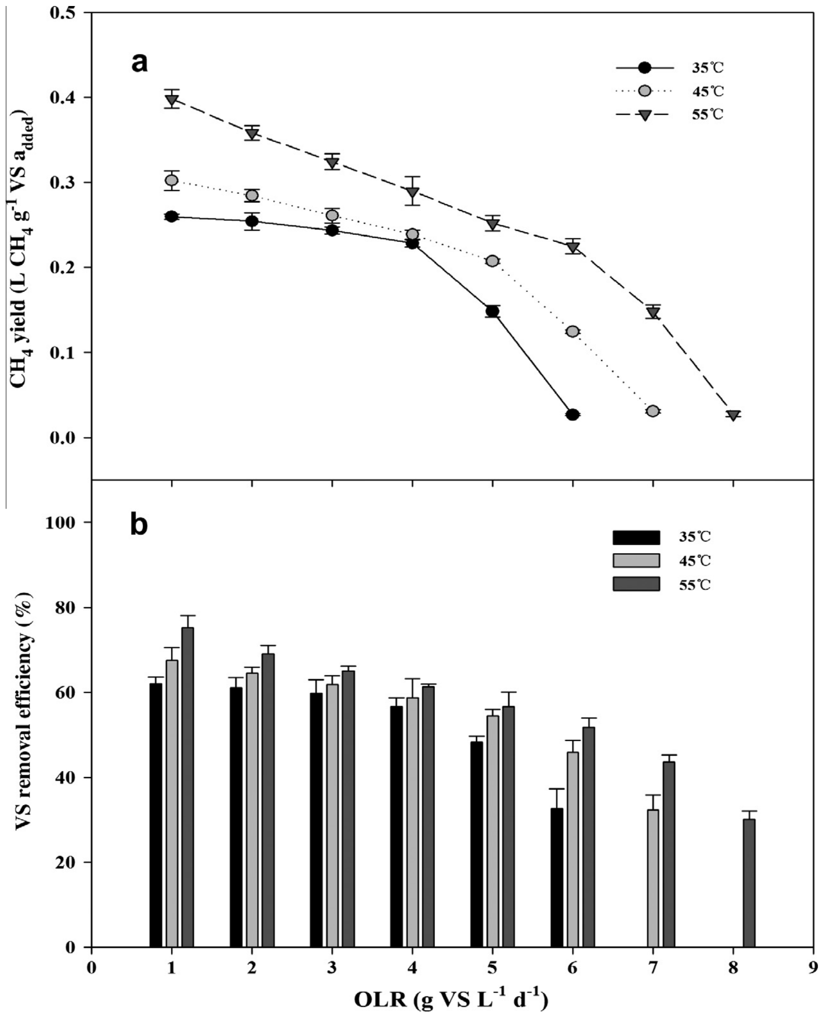

Fig. 2. Comparisons of: (a) CH_4 yield and (b) VS removal efficiency at different temperatures and OLRs.

mesophilic single-stage anaerobic digester. In addition, the highest methane yield of 0.40 L CH_4 g^{-1} VS_{added} in this study was higher than the reported 0.25 L CH_4 g^{-1} VS_{added} by using a temperature-phased anaerobic digester, and the value of 0.23 L CH_4 g^{-1} VS_{added} at 35 °C with the OLR of 4 g VS L^{-1} d^{-1} in present study was also higher than that using a two-stage anaerobic batch reactor (Kim et al., 2011). These data suggest that the methane yield is not a critical performance index for distinguishing the efficiency of different reactor types. While in another study for high-solid mesophilic co-digestion of WAS and FW (Dai et al., 2013), the value of 0.35 L CH_4 g^{-1} VS_{added} at an OLR of 4.6 g VS L^{-1} d^{-1} was considerably higher than 0.20 L CH_4 g^{-1} VS_{added} in this study. This might be attributed to the lower SRT of only around 7 d in current research than that of 30 d, and the methanogens declined steadily when SRT was below 10 d (Lee et al., 2011).

As shown in Fig. 2b, significantly higher VS removal efficiencies were obtained in thermophilic system during the entire operation. The highest average VS removal efficiency of 75% in R3 was achieved at an OLR of 1 g VS L^{-1} d^{-1}, and then gradually decreased to 44% as OLR increased to 7 g VS L^{-1} d^{-1}. For R1, it decreased from 62% to 48% as OLR increased from 1 to 5 g VS L^{-1} d^{-1}, while for R2, the average rate ranged from 68% to 46%. The results proved that at a similar OLR or HRT, the thermophilic system had more advantages in organic conversion efficiency over the other two reactors, which was in agreement with the earlier studies (Lv et al., 2010; Kim et al., 2011) reporting that the thermophilic condition could accelerate biological conversion process even at the same SRT. During the last chaos stage, the VS removal efficiency sharply decreased and reduced by almost a half to 30%, which was mainly due to the excessively high feeding load.

3.3. Effect on the system stability

Variations of TVFA and TVFA/alkalinity during the anaerobic co-digestion period are shown in Fig. 3. It illustrated that the thermophilic system had the best ability to resist the high loadings, while the mesophilic system maintained the best process stability under low OLRs (< 5 g VS L^{-1} d^{-1}).

A noticeable increase of TVFA was reflected in Fig. 3a during the first several days. It reached a peak value of 3550 mg L^{-1} on day 10 for R3, which was especially obvious compared with 1220 mg L^{-1} on day 6 for R1 and 3360 mg L^{-1} on day 18 for R2. The thermophilic condition contributed to the fastest hydrolysis/acidogenesis, inducing the highest amount of TVFA generated in the reactor which was then gradually utilized by methanogen in the following days. Though a slight rise was found as OLR turned to the next step, TVFA levels maintained relatively low from 47 to 318 mg L^{-1} in all systems. When OLR of R1 further increased to 5 g VS L^{-1} d^{-1}, an evident accumulation of VFA was detected that nearly 3-fold value was achieved compared with that at the OLR of 4 g VS L^{-1} d^{-1}. While for R2 and R3, sudden increase of TVFA occurred at OLRs of 6 and 7 g VS L^{-1} d^{-1}, respectively.

Generally, when the TVFA/alkalinity ratio is lower than 0.4, the digester is deemed to be stable (Callaghan et al., 2002). As shown

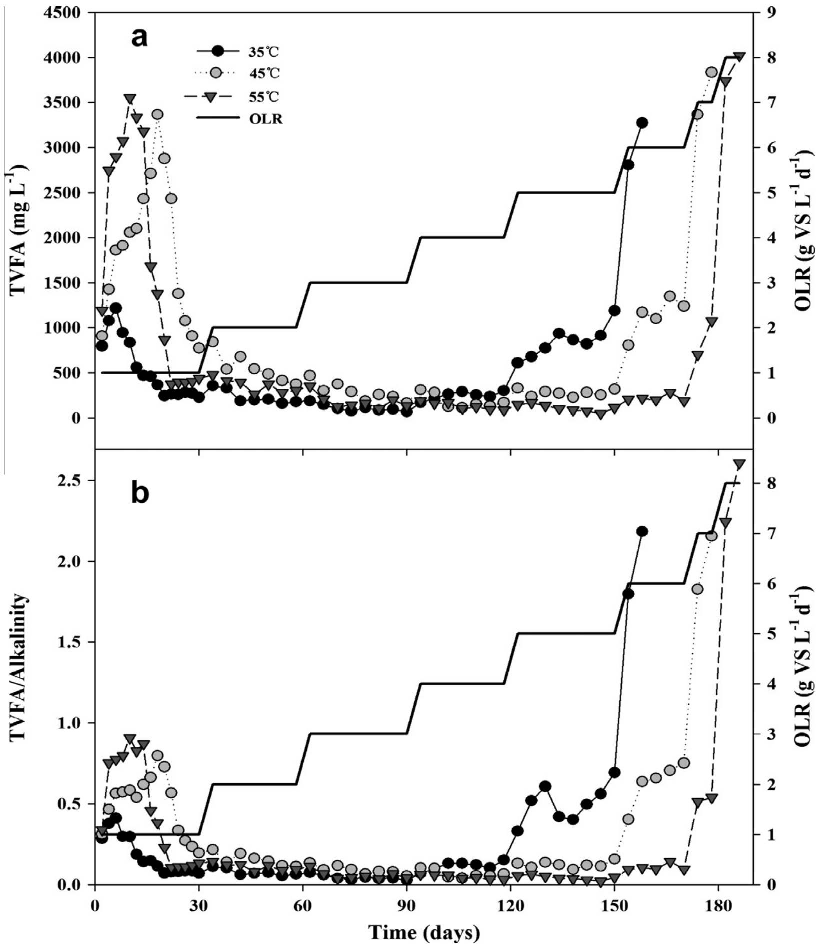

Fig. 3. Variations of: (a) TVFA and (b) TVFA/alkalinity according to different temperatures and OLRs.

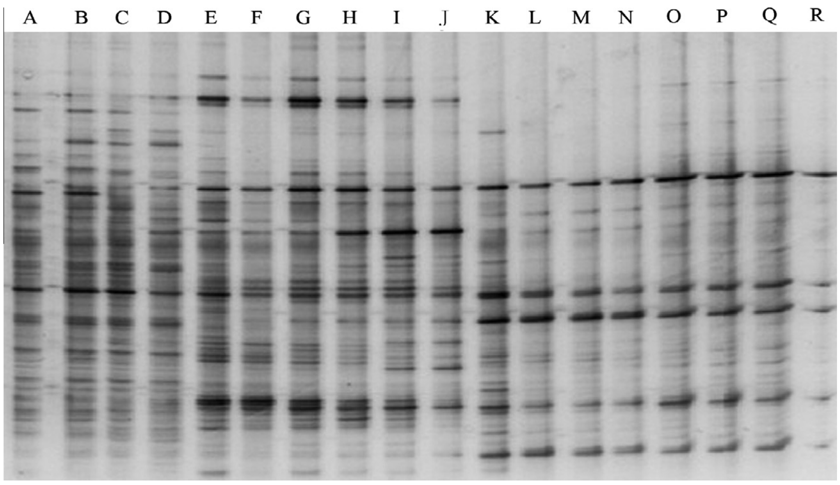

Fig. 4. PCR–DGGE profiles of 16S rDNA fragments for co-digestion samples. A–E: 35 °C from 1 to 5 g VS L^{-1} d^{-1}; F–K: 45 °C from 1 to 6 g VS L^{-1} d^{-1}; L–S: 55 °C from 1 to 7 g VS L^{-1} d^{-1}.

in Fig. 3b, during the start-up stage, TVFA/alkalinity of R2 and R3 rapidly went up to the peak values of 0.8 on day 18 and 0.9 on day 10. The unstable state implied that large amounts of acid had not yet been transformed efficiently. Thereafter, with the reaction proceeding to day 24 and 18 separately for R2 and R3, a satisfying situation was observed. Nevertheless, the ratio for R1 almost maintained below 0.4 during the entire operation until OLR reached 5 g VS L^{-1} d^{-1}. It revealed that under low OLRs (< 5 g VS L^{-1} d^{-1}), the mesophilic digestion system had the best ability to keep stable even with the lowest GPR. The result that AD system at mesophilic range had higher process stability was also demonstrated by Fernández-Rodríguez et al. (2013). As OLR went up to 5 g VS L^{-1} d^{-1}, the value for R1 was nearly 0.7, which meant that poor system stability emerged and the maximum available OLR was 5 g VS L^{-1} d^{-1}. Not surprisingly, similar observations were found for R2 and R3 with OLRs of 6 and 7 g VS L^{-1} d^{-1}, respectively. The maximum OLR further confirmed that the thermophilic system could endure higher feeding load than those at lower temperatures, which also had been substantiated by Bayr and Rintala (2012). When OLR continued to increase, the irreversible acidification led to a sharp pH drop, and the stabilities of all the systems were completely disrupted. The ratios achieved as high as 2.2, 2.1 and 2.6 for R1, R2, and R3 in the end, respectively.

3.4. Effect on microbial community

The DGGE profiles of samples collected at three different systems are shown in Fig. 4. It suggested that temperature has a more remarkable effect on the richness and diversity of microbial populations than the OLR. During the considerably stable stage, the average band numbers were 38, 33, and 29 for R1, R2 and R3, respectively. The evidently higher gene band numbers in methophilic samples might be the reason of its relatively steady CH_4 yield and the VS removal efficiency. Simultaneously, the lack of microorganism diversity for R3 might lead to the rapidly decreased productivity and organics reduction rate.

An abrupt increase in OLR seemed to have little influence on the microbial community. As seen in Fig. 4, the bands were almost at the same level for each temperature, and only with small changes in band numbers as well as the intensity of some microbial species. However, when OLR increased to the maximum value, a reduction of band numbers was obviously detected in R3 with only five species of microorganism remained abundant. It illustrated that thermophilic bacteria were more sensitive to the variation of environment than those at 35 and 45 °C, which, as a result, caused a more rapid decline in CH_4 yield and the VS removal efficiency.

4. Conclusions

Co-digestion of WAS and FW was investigated at three different temperatures within a wide range of OLRs for 188 d. Based on the results, the gradually enhanced GPR was mainly attributed to the increased loading rate of the feedstock. An increase in OLR or a decrease in HRT (SRT) was more destructive to methanogenesis than hydrolysis/acidogenesis, thus leading to the decrease of the methane yield and the VS reduction rate. The maximum endurable OLR for each temperature could be deduced from the sudden decrease of GPR, significantly low CH_4 yield and VS removal efficiency, as well as the undesired accumulation of VFA in system. Differences in temperature had more significant impacts on the microbial community than the increase of OLR. The best process stability was found in the mesophilic system with the most richness of bacteria, while the highest productivity and the best load bearing capacity were observed in the thermophilic system except with the largest investment.

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