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The effect of organic loading rate on VFA/COD ratio for methane production from an EGSB reactor

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Abstract

The present study evaluated the effect of organic loading rate (OLR) on VFA/COD ratio for continuous production of methane using an expanded granular sludge bed(EGSB) reactor for 200 d. Reactor performances were studied in treating high OLRs ranging from 4.91±0.54 to 16.79±1.62g-COD $l^{-1}d^{-1}$ of glucose-based synthetic wastewater in a mesophilic condition. Results showed that performance of anaerobic fermentation system was distinctly influenced by OLR in terms of organic removal efficiency, VFA yield, methane production rate and system stability.Acetic and propionic acids accounted for 80-90% of total VFA, and presented highest VFA concentration and composition of VFA showed minor changes with OLR variation. Moreover, an increase in OLR increased VFA/COD ratio in the whole operation period and high VFA/COD ratio could inhibit methanogenesis at high OLR (16.79±1.62g-COD $l^{-1}d^{-1}$).

Key words

Anaerobic fermentation, organic loading rate, Methane, VFA/COD ratio

Introduction

Nowadays, anaerobic digestion is attracting much attention as treatment method for organic waste due to economic advantages of its energy production in the form of biogas (Cui *et al*., 2011; Rajagopal *et al*., 2013; Xu *et al*., 2012). Anaerobic digestion is a complex process in which organic matter is transformed to methane through various steps: hydrolysis, acidification, acetogenesis and methanogenesis (Gujer *et al*., 1983; Liu *et al*., 2008). However, this cost-effective and environmentally friendly process is not a well-established technology, and it is put at risk given the fluctuations in the quantity and quality of waste organics to be processed and the presence of inhibitory compounds. Considering the metabolic pathway of anaerobic processes, symbiotic relationship among acetogens and methanogens is vital to achieve process stability, and consequently higher treatment performances.

During anaerobic fermentation reactor, volatile fatty acids (VFA) are also an important mid-production.Their concentrations can be considered as good indicators of anaerobic reactor performances, specifically in the activity of acetogenic bacteria and methanogens. Moreover, instability of anaerobic reactors treating high strength wastewaters is usually associated with an uneven production and consumption of VFA. Among all VFA, acetic acid and butyric acid are most favorable for methane formation, while contribution of acetic acid is more than 70% (Chang *et al*., 2010; Khanal, 2008). Owing to the importance of VFA, many studies have been carried out and related to environmental condition(Komemoto *et al*., 2009; Wang *et al*., 2014), substrate (Liu *et al*., 2008; Shen *et al*., 2014), pretreatment (Yu *et al*., 2014; Zhou *et al*., 2014) or a combination of all (Liu *et al*., 2012; Subramanian *et al*., 2014).

On the other hand, most of methane produced in conventional anaerobic digesters is derived from VFA such as acetic acid(HAc) and butyric acid (HBu).The conversion rates of VFA to methane vary in the order of HAc>HBu>propionic acid (HPr), (Ren *et al.,* 2003). Before getting degraded to methane, all VFA are first degraded to HAc, and their conversion rates vary in the order of HBu>HPr. Therefore, accumulation of HPr always results in failure of anaerobic digesters (Ren *et al*., 2005).

The performance of reactor system is influenced by a variation in environmental parameters such as temperature, pH, hydraulic retention time (HRT) and OLR (Mohan *et al.,* 2007).

Among these parameters, OLR is a critical operation parameter for anaerobicfermentation process (Liu *et al*., 2012; Reungsang *et al*., 2013). It is still considered as a feed parameter. On the premise of system stability, higher OLR means higher waste treatment capacity and biogas production, so suitable operating OLR is always a hot topic in research of anaerobic fermentation. Previous studies have reported the effects of OLR in the production of VFA from organic waste and performance ofreactor (Jiang *et al*., 2013; Wijekoon *et al*., 2011). However, relationship between VFA/COD ratio and OLR is not clear. Accordingly, the main objective of this study was to identify the effects of organic loading rate on VFA production and composition, especially the proportion of VFA to COD in an EGSB reactor.

Materials and Methods

Sources of inoculum sludges and feed characteristics: Inoculum was anaerobic granular sludge from a full-scale UASB reactor treating wastewater from beer plant (750m^3) in Xi'an, China.It was collected when the process was being operated at mesophilic conditions(about 26ºC). pH,alkalinity, total solid (TS) and volatile suspended solids (VSS) concentration of inoculums were 7.4, 4.63 g $CaCO_3$ l^{-1}, 32.0 and 21.3 g l^{-1}, respectively.

A synthetic wastewater mainly made up of glucose, which is readily degradable as a sole carbon and energy source was used for bioreactor. Nutrients (nitrogen and phosphorus as NH_4Cl and KH_2PO_4, respectively) were added to nutrient balance in feed solution according to the C:N:P ratio of 200:5:1. Synthetic wastewater was buffered with $NaHCO_3$ and with macro- and micro-nutrients. Detailed composition of synthetic wastewater is given in Table 1.

Table 1 : Synthetic wastewater composition

	Component	Concentration (mg l^{-1})
1.	Carbon source	
	$C_6H_{12}O_6$	1200¯4600
2.	Growth medium	
	KH_2PO_4	9.5
	$Na_2HPO_4{\cdot}7H_2O$	25.3
	$MgSO_4{\cdot}7H_2O$	8.7
	NH_4Cl	38
	Cysteine	46.5
3.	Mineral	
	$FeCl_2{\cdot}4H_2O$	180
	$CoCl_2{\cdot}6H_2O$	2000
	$MnCl_2{\cdot}4H_2O$	50
	$ZnSO_4{\cdot}7\,H_2O$	80
	$ZnCl_2$	50
	$AlCl_3{\cdot}6H_2O$	90
	$NiCl_2{\cdot}6H_2O$	92
	$Na_2SeO{\cdot}5H_2O$	162
	EDTA	1000
	H_3BO_3	60
	$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$	100

Reactor setup and operations : Lab-scale expanded granular sludge bed(EGSB) was set up in 4.63 l (900mm high by 60mm ID) plexiglass with an active volume of 3.87 l. Influent was pumped by peristaltic pumps from the storage tank into reactor (influent volume flow:0.49-0.63l h^{-1}). A recirculation pump (flow:1.89 l h^{-1}) was used for mixing and setting up the upflow velocity to about 0.22 m h^{-1}.In the upper part of reactor, three-phase separator split gas, water and solid-phase and retained the biomass in the reactor. Lab-scale reactor was placed in water bath. Temperature inside the reactor was adjusted to 35±1 ºC by an external, thermostatically controlled water bath.1.5 l of seeding source was inoculated, and the reactor was purged with N_2 for 5 min in order to provide anaerobic conditions. For Lab-scale EGSB, the reactor was operated with solid retention time(SRT) of 22 days.

Reactor was continuously operated for 200 days after inoculating the inoculums mentioned above. Hydraulic retention time(HRT) gradually decreased from 9.53 to 7.31h after pre-adaptation.Glucose was only carbon source in the synthetic glucose medium. To provide a buffer capacity, $NaHCO_3$ was added to feedstock between 4–6 gl^{-1} with OLR increased. Different influent organic concentrations in terms of chemical oxygen demand (COD) – 1200, 2200, 3000, 3500,and 4300 mg l^{-1} in theory. The organic loading rate(OLR) was step-increased in reactor with COD concentrations increasing until overloading. The entire experiment in the present study lasted approximately for 210 days at different loading conditions, as described in Table 2: phase I from day 1 to day 40, phase II from day 41 to 81, phase III from day 82 to 122, phase IV from day 123 to 163 and phase V from day 164 to 204, respectively.

Analytical methods : Samples were collected from reactor 2-3 times per week, depending on reactor performance. Biogas production from reactor was recorded using gas meter (Chang Chun, China) and analyzed by gas chromatograph (GC, 6890N,Agilent Technologies, USA) equipped with an HP-5 capillary column and a thermal conductivity detector. Temperature of the injection port, oven and detector were 250,60 and 250ºC respectively. Helium was used as carrier gas with a flow-rate of 10 ml min^{-1}. The pH of samples was measured using PHG-1101 pH meter (Thermoscientific, USA). The concentrations of chemical oxygen demand(COD),partial alkalinity, total solids (TS), volatile solids (VS), and volatile suspended solids (VSS) were determined according to Standard Methods American Public Health Association (APHA, 2005). Analysis of VFA composition was determined by GC-FID (GC7890A, Agilent, USA) equipped with HP-INNOWAX capillary column (Agilent, USA). Nitrogen was used as carrier gas. Aqueous effluents were centrifuged (10,000rpm, 5min), and supernatants were filtered using a syringe filter (0.45μm) to determine the concentration of VFA. VFA concentration was

Table 2 : Operational characteristic of expanded granular sludge bed reactor

Parameter	Time period				
	phase I	phase II	phase III	phase IV	phase V
Duration (days)	1-40	41-81	82-122	123-163	164-204
HRT (h)	9.53	7.31	7.31	7.31	7.31
Flux ($l\ m^{-2}h^{-1}$)	190	222	222	222	222
MLSS ($g\ l^{-1}$)	49.2±0.36	33.87±1.21	38.17±0.45	40.9±0.36	36.3±0.56
Feed strength ($mgCOD\ l^{-1}$)	1256.51±137.41	2217.66±210.39	2985.23±117.2	3512.6±298.49	4296.68±415.91
OLR ($g\text{-}CODl^{-1}d^{-1}$)	4.91±0.54	8.67±0.82	11.66±0.45	13.73±1.16	16.79±1.62

converted to COD concentration by using the following conversion factors:1.07 gCOD g^{-1} acetic acid,1.51 gCOD g^{-1} propionic acid,1.82 gCOD g^{-1} butyric and isobutyric acid,2.04 gCOD g^{-1} valeric and isovaleric acid. VFA production was expressed as COD equivalent (g COD).

Errors in measurements : Data were expressed as mean value ± SD of the number of experiments (n ≥ 8) using Microsoft® Excel 2007.

Results and Discussion

The first 20 days served as a start-up period for bioreactor (Fig.1), COD influent concentration was 1256.51±137.41mg l^{-1}. During the startup period, EGSB reactor performed as an enhanced settler as COD removal started directly since the onset of the reactor operation, and COD removal efficiency of EGSB increased gradually in phase I and reached an efficiency greater than 70%.After synthetic wastewater was adapted by biomass, stepwise increase in COD concentration was done by increasing OLR.

Influent COD increased to around 2217.66±210.39 mg l^{-1}in the second phase did not improve the overall removal degree, producing a higher effluent COD, probably as a result of reduced biodegradability due to sharp increase in OLR from 4.91±0.54 to 8.67±0.82g-COD l^{-1} d^{-1}. The COD removal efficiency dropped from 73-87% in first phase to 69-79% in the second phase(Fig.1).With OLR further increasing from 11.66±0.45 to 13.73±1.16 g-COD l^{-1} d^{-1} in the third and fourth phase, the overall COD removal efficiency of the reactor was around 80%.To carry out the present study, synthetic wastewater was used to create an increasing organic load until the reactor capability was reached. After that, instability in COD removal efficiency occurred in the fifth phase. In phase V, starting from day 163, the influent COD value was as high as 4296.68±415.91mg l^{-1}. The COD removal efficiency of EGSB dropped from approximately 78% to 64% and exhibited unstable behavior with regard to this parameter (Fig.1). High OLR perhaps resulted in poor contact between microorganism cell and substrate, thus minimumizing degradation of incoming COD. It was found that COD removal efficiency obviously decreased and fluctuated in days 150-210.Therefore, to keep a certain COD removal efficiency (≥ 80% COD removal) and stability, the maximum OLR of bioreactor was below 16.79±1.62g-COD l^{-1} d^{-1}.

VFA concentration is an important indicator in anaerobic reactor, specifically for acetogenic bacteria and methanogens activities. Changes in VFA indicate stability of anaerobic reactor.

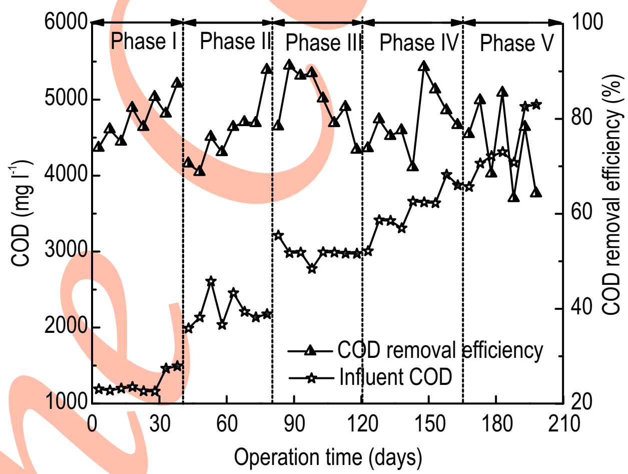

Fig. 1 : Influent COD and removal efficiency during treatment of wastewater using EGSB

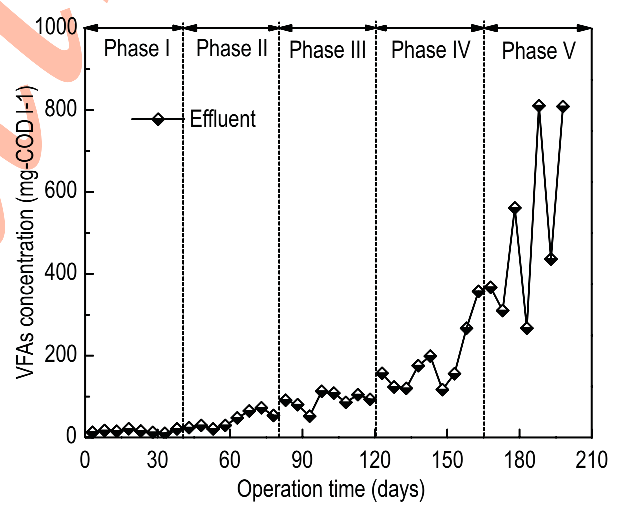

Fig. 2 : VFA profile of effluent during wastewater treatment in EGSB reactor

Therefore, VFA was also measured in order to evaluate organic overloading of the process, as shown in Fig. 2, which illustrates variations in VFA with changing OLR.

VFA production was very low in the experimental reactor feed with organic substrate. During the entire period of first 30 days, the feed strength was set to 1256.51±137.41 mg COD l^{-1}. Influent COD value was low, while VFA levels in the reactor were lowest in the overall system, revealing that VFA did not accumulate in phase I. Average VFA concentration in effluent was 15.75 mg-CODl^{-1}. VFA value in phase II were slight higher than phase I which indicates better utilization of VFA by microorganism in lower OLR (8.67±0.82 g-COD $l^{-1}d^{-1}$). Consequently, a rapid increase in VFA value was maintained coupling with OLR from 11.66±0.45 (phase III) to 13.73±1.16 (phase IV) g-COD $l^{-1}d^{-1}$. Meanwhile, COD removal efficiency of the reactor showed a drastic drop (Fig. 1 and 2). This could be attributed to two reasons, including rapid increase in loading rate and bearing capacity of biomass being close to saturation. In phase V (days 164-204), VFA value showed a remarkable increase along with increasing OLR. The maximum VFA concentrations were 357 to 809 mg-COD l^{-1} for OLR 16.79±1.62 g-COD $l^{-1}d^{-1}$,which indicated VFA accumulation in the reactor. VFA is also an important intermediate product of acidification phase, and VFA concentration changed substantially during the reaction period with increasing OLR. Comparing Fig. 1 with Fig. 2, the changes in OLR could be noticed corresponding to VFA concentration, so changing of OLR was the major influencing factor of VFA concentration which also means that an appropriate VFA concentration was crucial for anaerobic fermentation system.

The produced VFA composition is important as it can provide useful information regarding degree of hydrolysis and fermentation. In the present study, VFA reached a plateau in each phase of EGSB reactor. No significant composition difference was observed during the whole operation period. Furthermore, predominant VFA were HAc and HPr, accounting for 80–90% of total VFA. Moreover, i-HBu, HBu and i-HVa were also detected but in smaller percentages (<10.0%). Variation in HAc and HPr with OLR change of experiment period is given in Fig.3. Because the OLR in phase I was lowest in the present study, very little HAc and/or HPr were found with effluent. And the concentration of HAc was slight by higher than HPr. With higher OLR in phase II-IV, the concentration of HAc and HPr increased. It was noticed that the concentration of HPr was higher than HAc. This observed change in composition of VFA at higher OLR may be due to substrate HAc being used by methanogens directly. However, thermo-dynamically, the reaction of HPr degradation to HAc is unfavorable and can only proceed if hydrogen partial pressures are kept low by coupling with H_2-consuming methanogenesis (Hao *et al.,* 2011;Schink *et al.,* 1997). Increase in VFA was higher than OLR compared phase V with phase IV. Highest OLR applied of 16.79±1.62g-COD $l^{-1}d^{-1}$ (phase V) was accompanied with accumulation of VFA. As these value were higher, VFA accumulated to a certain degree. As a result, fluctuation in HAc-HPr concentration in each phase was due to OLR changes. Although fluctuation in HAc-HPr concentration was observed, there were only minor changes in the VFA composition. The results of the present study is in confirmation with the previous studies (Wijekoon *et al.,* 2011;Yuan *et al.,* 2011). The above results show that an increase in OLR did not affect the composition of VFA produced in anaerobic fermentation.

As compared with methane production, the ratio of VFA to COD closely reflects the role of acidogenic phase and VFA productivity all stages. The goal of anaerobic fermentation is to produce methane. Fig. 4 shows VFA/COD ratio and methane production rate during the whole operation time. Continuous methane production was observed during the entire experimental

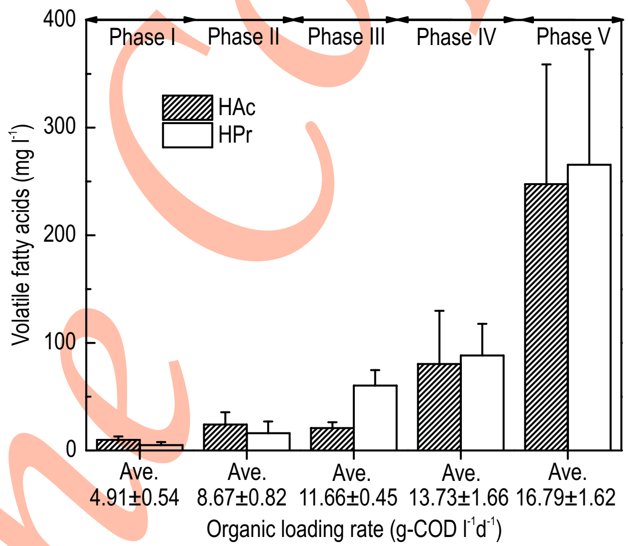

Fig. 3 : Values of acetic acid and propionic acid in each phase

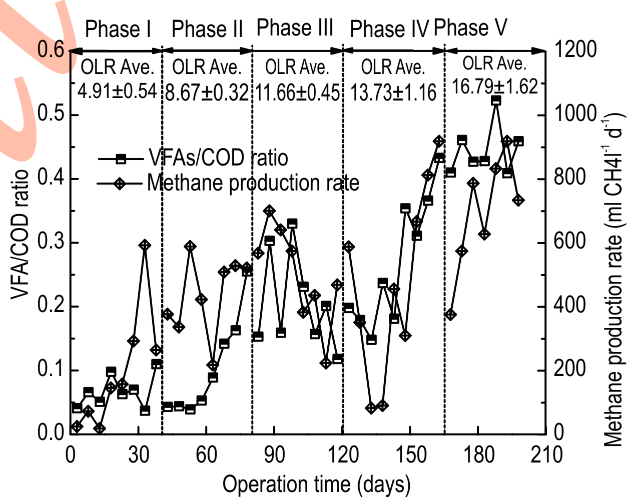

Fig. 4 : Variation of methane production rate according to different VFA/COD ratios and OLR (g-CO D $l^{-1}d^{-1}$)

period. Different OLR, that were evaluated, showed strong influence on methane production and yield. Methane production and yield increased with increasing OLR between 4.91±0.54 g-COD l^{-1} d^{-1} and 16.79±1.62 g-COD l^{-1} d^{-1}(phase I-V). In the first 20 days(start-up period), methane yield was low. After that, methane yield was higher than 200 ml l^{-1} d^{-1}. Higher methane yield exhibited in phase IV-V. Nevertheless, OLR in phase V (16.79±1.62 g-COD$l^{-1}d^{-1}$) was higher in phase IV (13.73±1.16 g-COD$l^{-1}d^{-1}$) which denotes that methane production rate probably did not correspond with OLR increase. An increase in methane production rate was observed during each OLR shock. The production and yield of methane as a function of OLR were considered to increase in certain OLR ranges. Further increase in OLR (phase V) caused a decline in these rates. Therefore, the extent of influence was determined by OLR shocks and tolerance of the microorganism in sludge liquid, which is in agreement with the findings of El-Mashad *et al*. (2004). Decreased CH_4 yield with increasing OLR was also noted in previous studies(Kim *et al*., 2006; Linke 2006).

VFA to COD ratio was significantly used in this study ,as it showed the amount of substances converted into VFA (Jiang *et al*.,2013) . The ratio represents the degree of acidification or the amount of COD which was converted into acids. VFA/COD ratio in glucose-based synthetic feed wastewater was identified as 0.05-0.1 in phase I, which was relatively low. During whole period, OLR (4.91±0.54 g-COD $l^{-1}d^{-1}$) was lowest in this phase. This indicates that system was capable of removing most acid matter. From day 60 to 100, this ratio was reduced to 0.05 and 0.34 in the second and third loading rates, respectively. This indicates that the proportion of soluble compounds was further converted into VFA. In phase III-IV, the ratio represents increased increments. VFA/COD ratio was more than 0.4, with a further increase in OLR during phase V. Combined with methane production rate, it indicated that strong acidic conditions inhibited the activity of acetate-utilizing methanogens on increasing VFA to COD ratio. This result suggests that microbial activity was inhibited at this high OLR (16.79±1.62 g-COD $l^{-1}d^{-1}$). It was also confirmed how critical methanogenic process between OLR and VFA/COD ratio. Furthermore, the present investigation suggests that a corresponding increase in VFA/COD ratio with increase in OLR during the entire experimental period.

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References

Chang, H.N., N.J. Kim, J. Kang and C.M. Jeong: Biomass-derived volatile fatty acids platform for fuels and chemicals. *Biotechnol. Bioproc. Eng*., **15**, 1-10(2010).

Cui, F., S. Lee and M. Kim: Removal of organics and nutrients from food wastewater using combined thermophilic two-phase anaerobic digestion and shortcut biological nitrogen removal. *Water Res*., **45,** 5279-5286 (2011).

El-Mashad, H.M., G. Zeeman, W.K.P. van Loon, G.P.A. Bot and G. Lettinga: Effect of temperature and temperature fluctuation on thermophilic anaerobic digestion of cattle manure. *Biores. Technol*., **95,** 191-201(2004).

Gujer, W. and A.J.B. Zehnder: Conversion processes in anaerobic digestion. *Water Sci. Technol.*, **15**, 127-167(1983).

Hao, L.P., F.LÜ, P.J. He, L. Li and L.M. Shao: Predominant contribution of syntrophic acetate oxidation to thermophilic methane formation at high acetate concentrations. *Environ. Sci. Technol.*, **45,** 508-513 (2011).

Jiang, J.G., Y.J. Zhang, K.M. Li, Q. Wang, C.X. Gong and M.L. Li: Volatile fatty acids production from food waste: Effects of pH, temperature, and organic loading rate. *Biores. Technol*., **143,** 525-530(2013).

Khanal, S.K.: Anaerobic biotechnology for bioenergy production: Principles and applications. Jhon Willey & Sons, USA, 1st Edn., ISBN : 978-0-8138-2346- (2008).

Kim, H.W., S.K. Han and H.S. Shin: Simultaneous treatment of sewage sludge and food waste by the unified high-rate anaerobic digestion system. *Water Sci. Technol.*, **53**, 29-35 (2006).

Komemoto K., Y.G. Lim, N. Nagao, Y. Onoue, C.Niwa and T. Toda: Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste. *Waste Manage.*, **29**, 2950-2955 (2009).

Linke, B.: Kinetic study of thermophilic anaerobic digestion of solid wastes from potato processing. *Biomass Bioenery*, **30**, 892-896 (2006).

Liu, X., W. Wang, Y.C. Shi, L. Zheng, X.B. Gao, W. Qiao and Y.J. Zhou: Pilot-scale anaerobic co-digestion of municipal biomass waste and waste activated sludge in China: Effect of organic loading rate. *Waste Manage*., **32,** 2056-2060 (2012).

Liu, X.L., H. Liu,Y.Y. Chen, G.C. Du and J. Chen: Effects of organic matter and initial carbon-nitrogen ratio on the bioconversion of volatile fatty acids from sewage sludge. *J. Chem. Technol. Biotechnol*., **83,** 1049-1055 (2008).

Liu, Y. and W.B. Whitman: Metabolic, phylogenetic and ecological diversity of the methanogenic archaea. *Ann. N.Y. Acad. Sci.,* **1125**, 171-189(2008).

Mohan, S.V., V.L. Babu and P.N. Sarma: Anaerobic biohydrogen production from dairy wastewater treatment in sequencing batch reactor (AnSBR): Effect of organic loading rate. *Enzyme Microb. Technol.*, **41,** 506-515(2007).

Rajagopal, R., J.W. Lim,Y. Mao, C.L. Chen and J.Y. Wang: Anaerobic co-digestion of source segregated brown water (feces-without-urine) and food waste: For Singapore context. *Sci. Total Environ.*, **443,** 877-886(2013).

Ren, N.Q, A.J. Wang and F. Ma: Acid-producing fermentative microbe physiological ecology. 1st Edn; Science Press, Beijing, China (2005).

Ren, N.Q., M. Liu, A.J. Wang, J. Ding and H.M. Li: Organic acids conversion in methanogenic – phase reactor of the two phase anaerobic process. *Environ. Sci. (Huanjing Kexue)*, **24**, 89-93 (2003).

Reungsang, A., S. Sittijunda and S. O-thong: Bio-hydrogen production from glycerol by immobilized *Enterobacteraerogenes* ATCC 13048 on heat-treated UASB granules as affected by organic loading rate. *Int. J. Hydrogen Energy*, **38**, 6970-6979 (2013).

Schink, B.: Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol. Mol. Biol. Rev.*, **61**,262-280(1997).

Shen, L., H.Y. Hu, H.F. Ji, J.Y. Cai, N.He, Q.B. Li and Y.P. Wang: Production of poly(hydroxybutyrate-hydroxyvalerate) from waste organics by the two-stage process: Focus on the intermediate volatile fatty acids. *Biores. Technol*., **166**,194-200(2014).

Subramanian B. and K.R. Pagilla: Anaerobic digester foaming in full-scale cylindrical digesters – Effect of organic loading rate, feed characteristics and mixing. *Biores. Technol*., **159,182**-192 (2014).

Wang K., J. Yin, D.S. Shen and N. Li: Anaerobic digestion of food waste for volatile fatty acids(VFAs) production with different types of inoculum: Effect of pH. *Biores. Technol*., **161,** 395-401(2014).

Wijekoon, K.C., C. Visvanathan and A. Abeynayaka: Effect of organic loading rate on VFA production, organic matter removal and microbial activity of a two-stage thermophilic anaerobic membrane bioreactor. *Biores. Technol*., **102**,5353-5360(2011).

Xu, S.Y., O.P. Karthikeyan, A. Selvam and J.W.C. Wong: Effect of inoculum to substrate ratio on the hydrolysis and acidification of food waste in leach bed reactor. *Biores. Technol*., **126,** 425-430 (2012).

Yu, L., M. Bule, J. Ma, Q.B. Zhao, C. Frear and S.L. Chen: Enhancing volatile fatty acid(VFA) and bio-methane production from lawn grass with pretreatment. *Biores. Technol*., **162**, 243-249 (2014).

Yuan, Q., R.Sparling and J.A. Oleszkiewicz: VFA generation from waste activated sludge: Effect of temperature and mixing. *Chemosphere*, **82**, 603-607 (2011).

Zhou, A.J., J.W. Du, C.Varrone, Y.Z. Wang and A.J. Wang: VFAs bioproduction from waste activated sludge by coupling pretreatments with *Agaricusbisporus* substrates conditioning. *Process Biochem.*, **49,283**-289(2014).