

# Anaerobic Co-digestion of Pig Manure with Dried Maize Straw

Xiangyu Song, Keqiang Zhang, Bingya Han, Junfeng Liang, Zhongwei Zhai, and Lianzhu Du\*

The anaerobic co-digestion of pig manure (PM) with dried maize straw (DMS) was studied at 35 °C with a volatile solid (VS) ratio ( $VS_{PM}/VS_{DMS}$ ) of 1:2 in a continuously stirred tank reactor, and the digestions of mono-PM and mono-DMS were evaluated under the same conditions. The organic loading rates (OLRs) of 2, 3, and 4 g VS/L/d were studied and found to correspond to hydraulic retention times (HRTs) of 60, 40, and 30 d, respectively. Under the condition of long HRT and low OLR, PM could be degraded completely. The co-digestion of PM with DMS showed the most stable performance in TAN, whereas TAN in mono-PM increased with the increase of OLR. The specific methane yield (SMY) did not have a linear correlation with OLR, since HRT changed with different OLR. The maximum average SMY in the co-digestion reactor was 272 mL/g VS-fed at an OLR of 3 g VS/L/d and an HRT of 40 d. The SMY in mono-DMS digestion was the lowest and it decreased with the increase of OLR.

*Keywords:* Anaerobic co-digestion; Pig manure; Dried maize straw; Organic loading rates; Specific methane yield

*Contact information:* Agro-Environmental Protection Institute, Ministry of Agriculture, Tianjin 300191, China; \*Corresponding author: [dulianzhu99@163.com](mailto:dulianzhu99@163.com)

## INTRODUCTION

With the development of the livestock industry, pig farming has become one of the most important agricultural industries in China. Pig breeding and pork consumption in China account for about half of the world's total (Qin 2016). Extensive amounts of pig manure (PM) have become a major contributor to soil, water, and air pollution (Windisch 2001; Li and Chen 2005; Song *et al.* 2012). PM largely contains organic matter that is characterized by a suitable ratio of chemical oxygen demand (COD) to total solid (TS) for anaerobic digestion (AD) (Nuchdang and Phalakornkule 2012; Giuliano *et al.* 2013). AD is the most efficient practice for the management of PM because of the renewable biogas energy it provides and because of the suitability of the digested matter as a fertilizer for planting (Walsh *et al.* 2012; Xie *et al.* 2012; Giuliano *et al.* 2013; Muller *et al.* 2013). However, the C/N ratio of PM is within the range of 9.8 to 14.5 (Zhang *et al.* 2014a), which often leads to ammonia inhibition (Krylova *et al.* 1997; Hansen *et al.* 1998; Procházka *et al.* 2012; Wang *et al.* 2012b; Niu *et al.* 2014) because free ammonia molecules passively diffuse into cells, which causes a proton imbalance and/or a potassium deficiency that inhibits the growth of methanogens (Chen *et al.* 2008). To dilute toxic compounds, it is essential to maintain a proper C/N ratio (20 to 30). Some substrates characterized by high C/N ratio are often used to mix with PM to achieve a low ammonia content and proper C/N ratio (Okeh *et al.* 2014; Wang *et al.* 2015). In addition, co-digestion facilitates the

acquisition of a high level of organic matter, as well as provides missing nutrients to the microorganisms, which could result in high biogas yield (Zhang *et al.* 2013).

To date, most research involving the co-digestion of livestock with energy crops has mainly focused on proportion optimization. Lehtomäki *et al.* (2007) observed that 30% of crops in a system showed an increase in methane production by about 16% to 65% compared to digestion of manure alone. However, increasing the proportion of crops further to 40% resulted in a decrease in specific methane yield (SMY) by 4% to 12%. Giuliano *et al.* (2013) found that substitution of 25% energy crop with 25% agro-waste in co-digestion of PM with energy crops achieved the greatest methane production of 0.54 m<sup>3</sup>/kg VS. In addition to these studies, Linke *et al.* (2013) built a model to calculate the maximum methane production that could be achieved at different hydraulic retention time (HRT) by optimizing crop proportion in the co-digestion of cow manure with crops. For instance, it was found that the maximum methane yield of 388 L/kg at HRT of 60 days could be achieved only if choosing the proper crop proportion. The influence of different organic loading rates (OLR) on digester performance has also been studied. Xie *et al.* (2012) studied the OLRs of 1.0, 1.5, 2.0, and 3.0 g VS/L/d in anaerobic co-digestion of PM with dried grass silage, and it was found that tripling the OLR decreased methane production by 38%. Similarly, Lehtomäki *et al.* (2007) found that doubling the OLR from 2 to 4 g VS/L/d decreased the SMY by 16 to 26%. The optimization of the C/N ratio has also been studied. Ye *et al.* (2013) observed that the C/N ratio was 21.7 when the ratio of kitchen waste, PM with rice straw was 0.4:1.6:1, which resulted in the highest biogas yield of 674.4 L/kg VS. Wang *et al.* (2012b) investigated that the performance of pH and ammonia was more stable when C/N ratios were 25:1 and 30:1 than the other circumstances. Wang *et al.* (2015) also found that natural clinoptilolite could be used to control the C/N ratio in AD. However, no particular attention has been paid on the effects of different HRTs and OLRs on methane yield in AD. Dried maize straw (DMS) is a typical agricultural waste with a high C/N ratio (58 to 70) (Ouédraogo *et al.* 2007; Wang *et al.* 2013b). This characteristic makes DMS suitable for co-digestion with pig manure. In China, especially North China, most of the DMS is used as fertilizer or food for animals, and a large part is burned, which leads to a waste of resources and severe air pollution (Wang *et al.* 2011). Several studies have shown that the co-digestion of maize straw (MS) with animal waste is an effective method of managing MS (Dinuccio *et al.* 2010; Wang *et al.* 2012b; Yangin-Gomec and Ozturk 2013; Arici and Koçar 2015; Zou *et al.* 2016). However, the efficiency of integrating maize straw biomass into biogas production is very limited due to the resistance of its complex lignin structure to hydrolysis by bacteria (Jin *et al.* 2014; Khatri *et al.* 2015). Additionally, previous studies have mainly focused on silage MS, which is easier to degrade than DMS (TS, about 10% to 15%). Therefore, it is valuable and important to investigate the anaerobic co-digestion of PM with DMS in balancing OLR and HRT.

In the present study, a comparison of the digestion of mono-PM, mono-DMS, and the co-digestion of PM with DMS under mesophilic conditions (35 °C) was performed. The specific objective of this research was to investigate the effectiveness of DMS that was used to co-digest with PM and to determine the effect of different OLRs and HRTs on the digester stability and performance. The characteristics of methane production, pH, alkalinity, ammonia nitrogen, and volatile fatty acids (VFAs) were also examined in this study.

## EXPERIMENTAL

### Substrates and Inoculum

Both PM and DMS were collected from Yi Lilai Breeding Co. Ltd. (Tianjin, China). The PM was intraday fresh manure that was delivered to the laboratory in a plastic bucket. DMS was crushed to the size of approximately 1 mm and sealed in a plastic bag. Before feeding, PM and smashed DMS were stored in a freezer at  $4 \pm 1$  °C. The inoculum sludge was obtained from a properly functioning pilot continuous stirred-tank reactor (CSTR) in the laboratory that ran the anaerobic digestion of pig manure under mesophilic conditions. The characteristics of the substrates and inoculum sludge are shown in Table 1.

### Experimental Design

The digestion was performed in four CSTRs. The reactor was made of polymethyl methacrylate. The effective diameter, height, and volume of the reactor were 20 cm, 30 cm, and 7 L, respectively. At the top of the reactor was a stirrer device which operated for 2 h by a time relay at the speed of 50 rpm and stopped for 10 min. The gas acquisition and feeding ports were also located at the top of the reactor. The two sample connections were situated on the top and bottom sides of the device, respectively. An outlet was located at the center of the bottom. A double-layer structure maintained the reactor temperature at  $35 \pm 0.5$  °C.

Digesters were seeded with 4 L of inoculum sludge, and tap water was added to a final working volume of 7 L. Prior to loading into the reactors, the sludge was starved for one week and then over the next 20 days acclimated with low concentrations of the substrate. The operation of the four CSTRs is shown in Table 2. PM1 was a contrast that operated the digestion of mono-PM with an OLR of 1 g VS/L/d and a HRT of 80 d. The operational parameters in PM1 remained constant during the entire experiment. The PM, PM+DMS, and DMS were loaded into the reactors of PM2, PM-DMS, and DMS, respectively, and then mixed with an OLR of 2 g VS/L/d and an HRT of 60 d. From the 69<sup>th</sup> day to the 149<sup>th</sup> day, the OLR and HRT of the reactors (PM2, PM-DMS, and DMS) were changed to 3 g VS/L/d and 40 d, and then from the 150<sup>th</sup> day to the 219<sup>th</sup> day they were changed to 4 g VS/L/d and 30 d, respectively. The ratio of  $VS_{PM}/VS_{DMS}$  in PM-DMS was 1:2, which maintained the ratio of C/N at around 26 in the mixed feedstock.

### Analytical Methods

The feedstock was added to the reactors after discharging the digestate from the outlet each day. The digestate was collected every three days to measure pH, ammonia nitrogen ( $NH_4^+-N$ ), total alkalinity, and VFAs (acetate, propionate, isobutyric acid, butyrate, isovaleric acid, and valeric acid). The parameters (pH,  $NH_4^+-N$ , total alkalinity, total Kjeldahl nitrogen (TKN), total organic carbon (TOC)) were analyzed in accordance with the standard methods (APHA 1998). To evaluate the VFAs, the pH of the samples was initially adjusted to 3.0 by using 5%  $H_2SO_4$ , and then centrifuged at 10,000 rpm for 10 min. The supernatants were filtered through 0.45  $\mu m$  cellulose nitrate membrane filters and analyzed in a Thermo trace-1300 gas chromatograph, which was equipped with an FID detector. The temperatures of the injection inlet and detector were 200 °C. The carrier gas (helium) was maintained at a constant flow rate of 8.0 mL/min. The type of capillary column used was M12 (30 m  $\times$  0.53 mm  $\times$  1  $\mu m$ , Thermo). The first stage program temperature rise was at a rate of 5 °C/min from the initial temperature of 110 °C to 150 °C.

**Table 1.** Characteristics of Pig Manure (PM), Dried Maize Straw (DMS), and Inoculum Sludge

AV $\pm$ SD	TS (%)	VS (%)	VS/TS (%)	TKN (mg/g TS)	TOC (mg/g TS)
PM	30.46 $\pm$ 4.66	22.22 $\pm$ 5.17	72.34 $\pm$ 7.39	40.45 $\pm$ 6.72	434.43 $\pm$ 47.09
DMS	88.50 $\pm$ 0.02	80.72 $\pm$ 0.30	91.20 $\pm$ 0.35	11.13 $\pm$ 0.08	667.83 $\pm$ 4.60
Inoculum	4.62 $\pm$ 0.03	3.79 $\pm$ 0.06	82.03 $\pm$ 0.26	-	-

Note: average value (AV), standard deviation (SD)

Biogas was collected in a 20 L aluminum bag, and the gas volume was measured with a wet gas meter each day. The CH<sub>4</sub> and CO<sub>2</sub> content were measured every three days with a Thermo trace-1300 gas chromatograph that was equipped with a thermal conductivity detector, a 2 m  $\times$   $\Phi$  2 mm (diameter) stainless steel chromatographic column (PP-Q packed column), and a stationary phase (PorapakQ polymer beads). Helium was used as carrier gas at a constant pressure of 75 kPa. The temperature of the oven, injection inlet, and detector were 40 °C, 200 °C, and 200 °C, respectively.

**Table 2.** Operation Conditions of Reactors

Reactors	Substrate	Stage	OLR (g VS/L/d)	HRT (d)
PM1	PM	Whole stage	1	80
PM2	PM	I, II, and III	2, 3, and 4	60, 40, and 30
PM-DMS	PM + DMS	I, II, and III	2, 3, and 4	60, 40, and 30
DMS	DMS	I, II, and III	2, 3, and 4	60, 40, and 30

Note: stage I, 1-68 d; stage II, 69-149 d; stage III, 150-219 d

## RESULTS AND DISCUSSION

### Effect of Feedstock and OLR on Methane Production

Table 3 shows the average values of methane production and CH<sub>4</sub> content of PM1. The reactor of PM1 was conducted as a control to see the potential methane production of PM by itself. As the results showed, the average SMY in PM1 (295 mL/g VS<sub>-fed</sub>) was the highest value compared to those of the other reactors at different OLRs, which suggested that long HRT with low OLR could make substrates degrade completely. PM has been demonstrated to be an excellent substrate for AD in various conditions (Xie *et al.* 2011; Zhang *et al.* 2014b). The SMY of mono-PM digestion in this experiment was lower than the reported 354.7 mL/g VS<sub>-fed</sub> of Zhang *et al.* (2014a), but similar to that of 300 mL/g VS<sub>-fed</sub> described by Hansen *et al.* (1998). However, long HRT with low OLR was meant to treat a small quantity of waste over a long period of time, which was regarded as poor efficiency to AD, so the experiment of 2 to 4 g VS/L/d proceeded in PM2.

In PM2, the average SMY at an OLR of 2 g VS/L/d and HRT of 60 d was 203 mL/g VS<sub>-fed</sub>. The SMY increased with the increase of OLR. As time went by, the increase of OLR provided increasing nutrients for the microbe in the reactor, which increased the

activity of the microbe. Meanwhile, the difference between different batches of PM effected the methane production. Besides, when the OLR was increased to 3 g VS/L/d and 4 g VS/L/d, the average SMY increased by only 5% and 8%, respectively, while the SMY in PM2 during the OLR changed from 2 to 4 g. The VS/L/d was 22% to 31% lower than that of PM1, which suggested that the digestion of PM alone did not show a significant response with the increase of OLR.

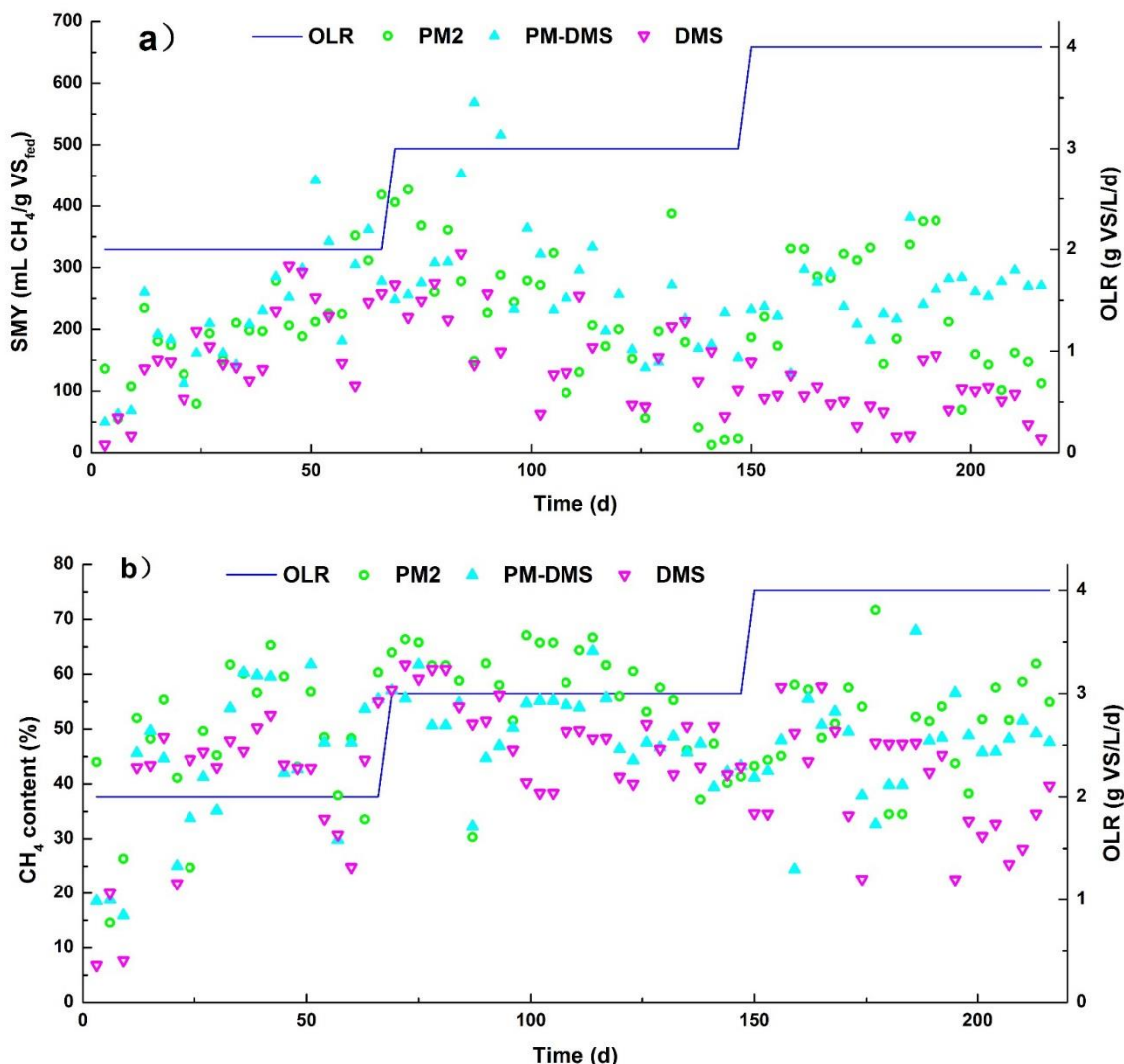


Fig. 1. a) SMY and b) CH<sub>4</sub> content of the reactors

Considering that the C/N ratio of PM is not in the proper range for AD, the co-digestion of PM with DMS was carried out in the reactor denoted as PM-DMS with adjustment of the C/N ratio to 26. Methane production of the co-digestion of PM with DMS was higher than the mono-PM or mono-DMS digestion at the same OLR level. The average SMYs in PM-DMS were 7% to 28% and 34% to 193% higher, respectively, compared to that in PM2 and DMS, thereby showing the advantage of co-digestion over mono-digestion. In a similar experiment conducted by Lehtomäki *et al.* (2007), a 16% to 65% increase in methane production per digester volume was observed in a feedstock that included a 30% proportion of crops when compared to the digestion of manure alone. The

highest SMY values were observed at an OLR of 3 g VS/L/d in PM-DMS, with an average of 272 mL/g VS<sub>-fed</sub>, which were 25% and 7% higher than those of 2 g VS/L/d and 4 g VS/L/d OLR, respectively. The OLR at the first stage was the lowest. The lack of organic matter in the biomass resulted in low methane production, even though HRT was the longest. The increase in SMY achieved by co-digestion in the second stage was attributed to the increase in the biodegradability of DMS by co-digestion or synergism for methane potential. The higher biodegradability in PM-DMS could have been due to the strong microbial activity supported by the readily biodegradable organics in the PM, thereby contributing to a higher hydrolytic capacity of cellulose and hemicellulose in the DMS. Zhang *et al.* (2014b) reported that the co-digestion of PM and dewatered sewage sludge provided balanced amounts of nutrients in the mixed substrates and accelerated bacterial growth and hydrolysis, thereby enhancing methane formation. However, at the third stage, despite the high OLR, the HRT was very short, which was probably limited by the volume of the reactor, which in turn led to insufficient degradation (Lehtomäki *et al.* 2007). These observations indicated that the selection of an appropriate OLR and HRT combination was crucial to increasing methane production. Besides, as a substrate that has poorer degradability than MS silage, DMS in the co-digestion with PM of this experiment did not show any disadvantage, compared with the experiment that did by Arici and Koçar (2015), who observed that the maximum SMY was 215 mL/g VS<sub>-fed</sub> in the co-digestion of cattle manure and maize silage.

In DMS the average SMY values decreased by 8% and 47%, respectively, when the OLR increased from 2 to 3 and 4 g VS/L/d, possibly due to the limited degradation of DMS that resulted from the shielding effect, which was caused by the intense cross-linking of the lignin with cellulose and hemicellulose (Lehtomäki *et al.* 2007). Meanwhile, DMS floated to the up side of the reactor, which made it difficult to stir so that DMS could not contact with the microbe sufficiently.

The CH<sub>4</sub> content of the biogas is shown in Fig. 1b. The CH<sub>4</sub> percentage of PM-DMS was lower than that of PM1 and PM2, but higher than that of DMS. These discrepancies may be attributable to the characteristics of the different substrates. Proteins and fats which are abundant in PM are the main conversion components to methane (Petersen and Henius 1984; Astals *et al.* 2012). However, the poor degradability of lignin in DMS (Lehtomäki *et al.* 2007) decreases the methane potential. The CH<sub>4</sub> percentage in biogas produced by carbohydrates is lower than that produced by protein and fat (Moller *et al.* 2004).

### pH and Ammonia Nitrogen Measurements

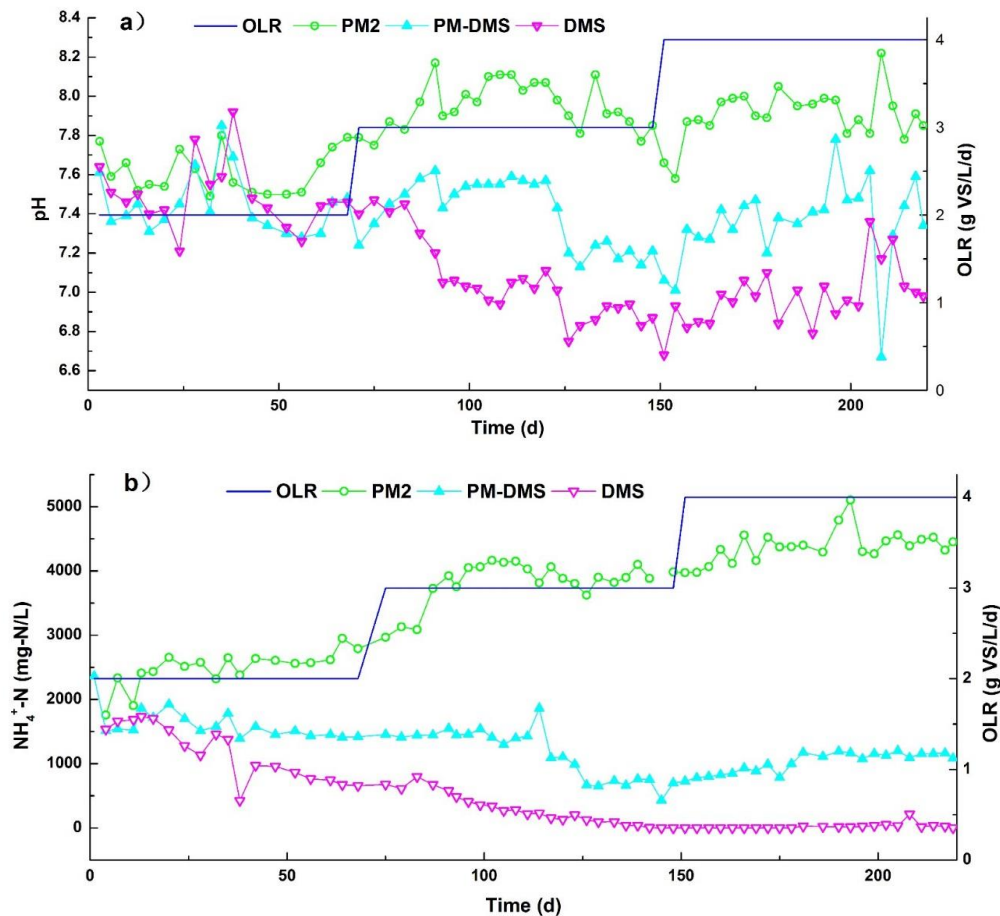
The pH is a key parameter that describes the stability of AD. No significant differences in pH among the four reactors were observed during the first stage (0 to 69 days).

For PM1, with the continuous loading at a low OLR, the pH values were within the range of 7.30 to 8.14. No significant difference in pH was observed between the reactors of PM1 and PM2, for they were both fed with PM alone (Fig. 2a).

The pH value of PM-DMS decreased from 7.48 to 7.24 at the 69<sup>th</sup> day when the OLR was increased to 3 g VS/L/d, which meant that the sudden increase in OLR accelerated the hydrolysis. With the stabilization of OLR, the pH fluctuated at around 7.4, which was consistent with the high buffering capacity and the normal growth of anaerobic microorganisms (Raposo *et al.* 2009). The decrease of pH in PM-DMS at the 69<sup>th</sup> day corresponded with the accumulation of VFA (Fig. 3). Furthermore, with progress in

digestion, a balance between acidogenesis and methanogenesis was achieved, and the performance of the digester gradually stabilized (Wang *et al.* 2013a).

For DMS, the pH rapidly decreased after increasing the OLR from 2 to 3 g VS/L/d and then stabilized at around 7.0 (Table 3). In AD, the observed change in pH was attributed to the degradation and production of VFAs and the conversion of ammonia nitrogen. The decrease of pH in DMS could have been caused by the hydrolysis acidification that resulted in the accumulation of VFA without a high buffering capacity. The increase in total ammonia nitrogen (TAN) could have led to an increase in pH given the chemical equilibrium between ammonium ( $\text{NH}_4^+$ ) and free ammonia ( $\text{NH}_3$ ), which could have been responsible for the high pH that corresponded to the observed elevated levels of ammonia nitrogen (Fig. 2b) in PM1 and PM2.



**Fig. 2.** a) pH values and b)  $\text{NH}_4^+\text{-N}$  concentrations of the reactors during the experiment

The total ammonia nitrogen can also play a critical role in the performance and stability of the anaerobic digestion of nitrogen-rich materials such as animal manure (Massé *et al.* 2014). The increase in TAN concentration provides a better buffering capacity (Walker *et al.* 2011), thereby affecting methane production. However, ammonia inhibition usually happens when the pH is above 7.4 and the TAN is between 1,500 and 3,000 mg/L, whereas inhibition will occur irrespective of pH when the TAN concentration exceeds 3,000 mg/L (Walker *et al.* 2011). According to Procházka *et al.* (2012), the optimal TAN

concentration for methanogens is 2,100 mg/L in the AD of PM, and the highest concentration that methanogens can tolerate is 4,200 mg/L.

In PM1, the TAN was stable at around 2,318 mg/L which was in the safety range of AD and corresponded with the stable performance in PM1 (Table 3).

The average TAN in PM2 gradually increased from 2,482 to 4,384 mg/L, with OLR increasing from 2 to 4 g VS/L/d, for the possible reason that ammonium is readily released during the mono-PM digestion for the relatively low C/N ratio. The average SMY values in PM2 were 203, 213, and 230 mL/g VS<sub>-fed</sub> (Table 3), respectively, at the three OLR levels, which suggested that no inhibition was observed even though the TAN was higher than the reported critical inhibition concentration (4,200 mg/L) when the pH was higher than 7.8. This could be attributed to the acclimation of the inoculum, which weakened the influence of ammonia (Chen *et al.* 2008).

The TAN of PM-DMS that co-digested PM with DMS gradually decreased from 1,572 to 1,154 mg/L, with OLR increasing from 2 to 3 g VS/L/d (Fig. 2b). In contrast, the TAN was stable at around 1,020 mg/L, and it did not decrease when the OLR increased to 4 g VS/L/d in PM-DMS, which showed that the co-digestion of PM with DMS could prevent the accumulation of ammonia.

For DMS, the average TAN gradually decreased, and it was below 300 mg/L after 102 days (Fig. 2b). When the OLR increased to 4 g VS/L/d, the TAN was stable below 50 mg/L. With the increase of OLR, the microbe in the digestion of DMS needed more nitrogen as a nutrient to degrade more substrate, but DMS characterized by the high C/N ratio could not provide enough nitrogen. According to Procházka *et al.* (2012), when the ammonia nitrogen concentration was lower than 500 mg/L, microorganisms were unable to acquire enough ammonia as a nutrient. So the insufficient of nitrogen might be the main reason for the decrease of TAN.

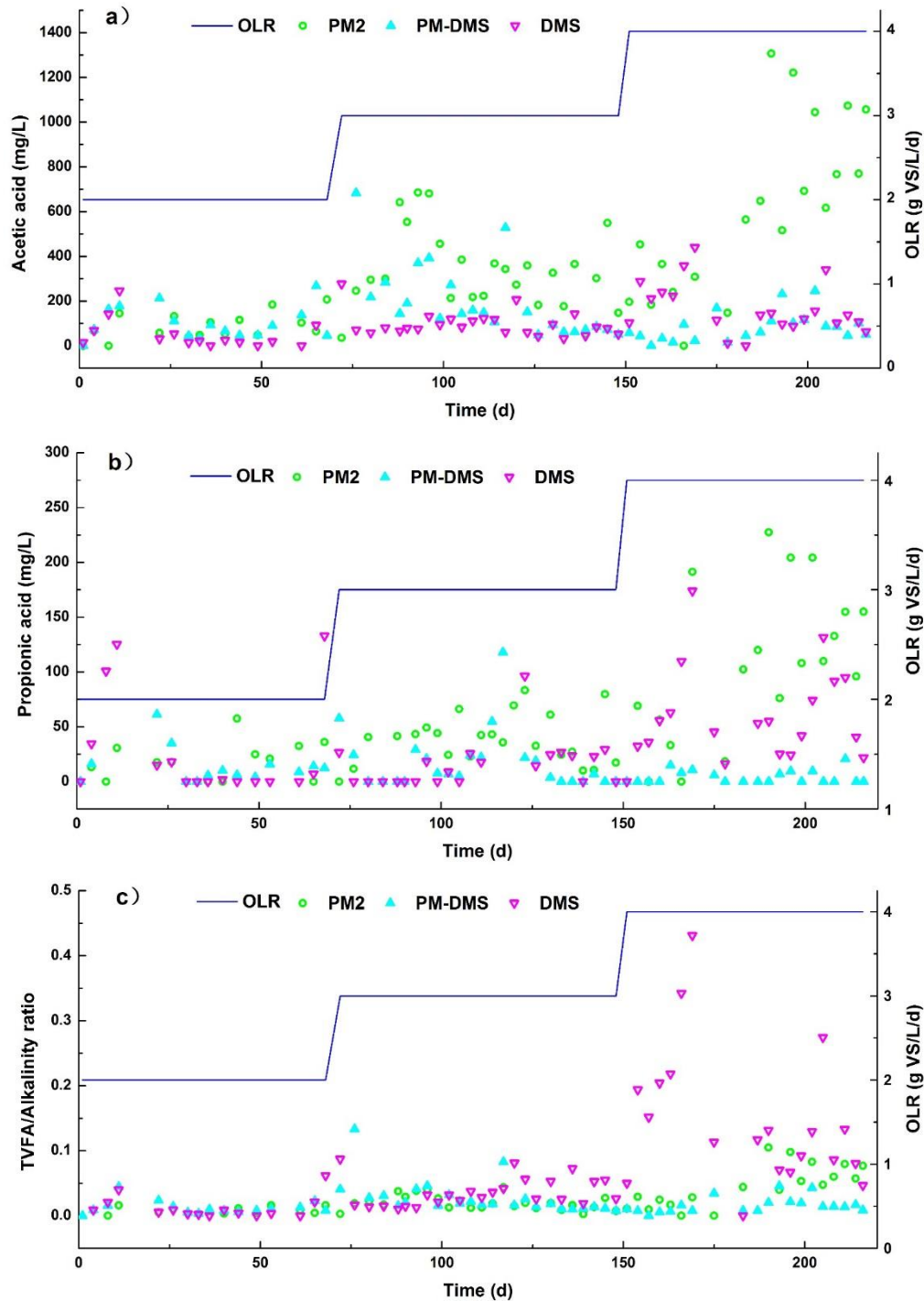
### VFAs and TVFA/Alk

Acetic acid is the main intermediate in AD, and as much as two-thirds of the biologically generated methane is derived from acetate (Liu and Whitman 2008). Propionic acid is the most toxic organic acid and is not easily utilized by methanogens (Procházka *et al.* 2012). The concentrations of propionate, isobutyrate, butyrate, isovalerate, and valerate, together with acetate, were converted into total VFA and used in the calculation of the ratio of TVFA to alkalinity (TVFA/Alk), which is an important index for the evaluation of the stability of the AD process. The profiles of acetic acid, propionic acid, and TVFA/Alk for the four reactors are presented in Fig. 3.

For PM1, the average values of acetic acid and propionic acid were 86.67 mg/L and 28.13 mg/L when the OLR was 1 g VS/L/d, and the TVFA/Alk ratio was below 0.1. No particular imbalance was observed in PM1.

An increase in acetic acid and propionic acid concentration was observed in PM2 when the OLR increased from 2 to 3 g VS/L/d (Fig. 3), which may have been caused by the increase in organic matter in the biomass, leading to an acceleration of acidification. Figure 3c shows that the TVFA/alkalinity ratio in PM2 was below 0.1, which in turn indicated that the high alkalinity was providing a high buffering capacity (Astals *et al.* 2012) to maintain continuous digestion. When the OLR was increased to 4 g VS/L/d, the acetic acid and propionic concentrations in the PM2 increased, but the TVFA/alkalinity ratio remained below 0.1, which meant that the increase in VFA concentration was not sufficient to inhibit the anaerobic process, which in turn was demonstrated by an increase in SMY by 7% compared to that when using an OLR at 3 g VS/L/d.





**Fig. 3.** a) Variations in the levels of acetic acid, b) propionic acid, and c) TVFA/Alk in the four reactors

For PM-DMS, the VFAs (acetate, isobutyrate, butyrate, isovalerate, and valerate) were almost depleted by the methane-producing bacteria during anaerobic digestion. The predominant conjugate base of carboxylic acid was acetate, which comprised more than 60% of the total VFA. Furthermore, isobutyrate and isovalerate, which are considered the best indicators of AD imbalance (Hill and Holmberg 1988; Kalamaras and Kotsopoulos

2014), were almost totally depleted during the anaerobic process. The concentrations of isobutyrate and isovalerate showed a 15- and 10-fold decrease, respectively, compared to that of PM2 (data not shown). Meanwhile, the VFA/Alk ratio was below 0.1 during the experiment, which reflected the fact that the buffer capacity of the AD system was enhanced by the co-digestion of PM with DMS.

For DMS, the concentration of propionic acid was within the range of 50 to 250 mg/L when the OLR was increased to 4 g VS/L/d, which was still within the safe range (<900 mg/L) (Wang *et al.* 2009). The TVFA/alkalinity ratio also had a significant increase, yet remained within the safe range (0.3 to 0.4) according to Wang *et al.* (2012a) and Liu and Whitman (2008), except for a peak value of 0.43 that was observed at the 169<sup>th</sup> day. This could be explained by the poor buffering capacity caused by the extremely low TAN concentrations (Fig. 2). Therefore, higher risks for VFA (propionic acid) accumulation and low methane yield occurred at high OLRs during DMS digestion, which was characterized by the high C/N ratio and was indicated by a decrease in SMY when the OLR increased from 3 to 4 g VS/L/d.

**Table 3.** Characteristics of the Four Reactors Used in this Study

Reactor	OLR (g VS/L/d)	DBP (L/d)	DMP (L/d)	CH <sub>4</sub> (%)	SMY (mL/g VS <sup>-fed</sup> )	NH <sub>4</sub> <sup>+</sup> -N (mg/L)	Alkalinity (mg/L)	pH
PM1	1	4.08 ± 0.20	2.08 ± 0.09	51.7 ± 1.28	295 ± 12	2318 ± 32	12163 ± 180	7.76 ± 0.01
PM2	2	6.22 ± 0.49	2.81 ± 0.24	47.0 ± 2.81	203 ± 18	2482 ± 65	15841 ± 522	7.61 ± 0.03
	3	7.23 ± 0.81	4.27 ± 0.46	56.5 ± 1.92	213 ± 24	3756 ± 96	21427 ± 427	7.95 ± 0.02
	4	11.90 ± 1.10	6.17 ± 0.54	51.1 ± 1.83	230 ± 20	4384 ± 53	18380 ± 287	7.90 ± 0.03
PM-DMS	2	7.16 ± 0.46	3.10 ± 0.30	42.8 ± 3.03	218 ± 22	1572 ± 55	10443 ± 318	7.45 ± 0.04
	3	9.88 ± 0.71	4.87 ± 0.25	50.0 ± 1.33	272 ± 21	1154 ± 80	7706 ± 305	7.41 ± 0.03
	4	14.59 ± 0.39	6.77 ± 0.33	46.7 ± 1.81	254 ± 10	1020 ± 33	5874 ± 318	7.35 ± 0.05
DMS	2	6.08 ± 0.51	2.30 ± 0.24	38.1 ± 2.92	163 ± 17	1175 ± 98	7552 ± 630	7.49 ± 0.04
	3	5.76 ± 0.75	2.95 ± 0.35	48.9 ± 1.39	150 ± 18	284 ± 29	3616 ± 269	7.06 ± 0.04
	4	5.94 ± 0.69	2.26 ± 0.19	39.5 ± 2.15	87 ± 8	22 ± 3	1723 ± 43	6.98 ± 0.03

Note: DBP, daily biogas production; DMP, daily methane production

## CONCLUSIONS

1. The co-digestion of pig manure (PM) with dried maize straw (DMS) showed the advantage of ensuring the stability of anaerobic digestion (AD) and improving methane production by promoting a suitable C/N ratio and buffer capacity compared with the mono-PM digestion.

2. Under the condition of long hydraulic retention times (HRT) and low organic loading rates (OLR), PM could be degraded completely.
3. With the increase of OLR, no linear correlation to specific methane yield (SMY) was observed in this experiment. The maximum average SMYs obtained in mono-PM digestion, co-digestion of PM with DMS and mono-DMS digestion were at different conditions of OLR and HRT.
4. High OLR resulted in an increase in the concentrations of TAN in mono-PM digestion, and it resulted in decrease in TAN in the digestion of mono-DMS, while no significant changes of TAN was observed in the co-digestion of PM with DMS.
5. The DMS was not suitable for digestion alone, but it served effectively as an alternative substrate for co-digestion with PM.

## ACKNOWLEDGMENTS

The National Natural Science Foundation of China (51008163) and the Natural Science Foundation of Tianjin supported this study (16JCYBJC29600).

## REFERENCES CITED

- APHA (1998). *Standard Methods for the Examination of Water and Wastewater*, 20<sup>th</sup> Ed. American Public Health Association, Washington, DC.
- Arici, Ş., and Koçar, G. (2015). "The effect of adding maize silage as a co-substrate for anaerobic animal manure digestion," *Int. J. Green. Energy*. 12(5), 453-460. DOI: 10.1080/15435075.2013.848361
- Astals, S., Nolla-Ardèvol, V., and Mata-Alvarez, J. (2012). "Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: Biogas and digestate," *Bioresource Technol.* 110, 63-70. DOI: 10.1016/j.biortech.2012.01.080
- Chen, Y., Cheng, J. J., and Creamer, K. S. (2008). "Inhibition of anaerobic digestion process: A review," *Bioresource Technol.* 99(10), 4044-4064. DOI: 10.1016/j.biortech.2007.01.057
- Dinuccio, E., Balsari, P., Gioelli, F., and Menardo, S. (2010). "Evaluation of the biogas productivity potential of some Italian agro-industrial biomasses," *Bioresource Technol.* 101(10), 3780-3783. DOI: 10.1016/j.biortech.2009.12.113
- Giuliano, A., Bolzonella, D., Pavan, P., Cavinato, O., and Cecchi, F. (2013). "Co-digestion of livestock effluents, energy crops and agro-waste: Feeding and process optimization in mesophilic and thermophilic conditions," *Bioresource Technol.* 128, 612-618. DOI: 10.1016/j.biortech.2012.11.002
- Hansen, K. H., Angelidaki, I., and Ahring, B. K. (1998). "Anaerobic digestion of swine manure: Inhibition of ammonia," *Water Res.* 32(1), 5-12. DOI: 10.1016/S0043-1354(97)00201-7
- Hill, D. T., and Holmberg, R. D. (1988). "Long chain volatile fatty acid relationships in anaerobic digestion of swine waste," *Biol. Waste* 23(3), 195-214. DOI: 10.1016/0269-7483(88)90034-1

- Jin, W. Y., Xu, X. C., Gao, Y., Yang, F. L., and Wang, G. (2014). "Anaerobic fermentation of biogas liquid pretreated maize straw by rumen microorganisms *in vitro*," *Bioresource Technol.* 153, 8-14. DOI: 10.1016/j.biortech.2013.10.003
- Kalamaras, S. D., and Kotsopoulos, T. A. (2014). "Anaerobic co-digestion of cattle manure and alternative crops for the substitution of maize in South Europe," *Bioresource Technol.* 172, 68-75. DOI: 10.1016/j.biortech.2014.09.005
- Khatri, S., Wu, S. B., Kizito, S., Zhang, W. Q., Li, J. X., and Dong, R. J. (2015). "Synergistic effect of alkaline pretreatment and Fe dosing on batch anaerobic digestion of maize straw," *Appl. Energ.* 158, 55-64. DOI: 10.1016/j.apenergy.2015.08.045
- Krylova, N. I., Khabiboulline, R. E., Naumova, R. P., and Nagel, M. A. (1997). "The influence of ammonium and methods for removal during the anaerobic treatment of poultry manure," *J. Chem. Technol. Biot.* 70(1), 99-105. DOI: 10.1002/(SICI)1097-4660(199709)70:1<99::AID-JCTB684>3.0.CO;2-C
- Lehtomäki, A., Huttunen, S., and Rintala, J. A. (2007). "Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio," *Resour. Conserv. Recy.* 51(3), 591-609. DOI: 10.1016/j.resconrec.2006.11.004
- Li, Y. X., and Chen, T. B. (2005). "Concentrations of additive arsenic in Beijing pig feeds and the residues in pig manure," *Resour. Conserv. Recy.* 45(4), 356-367. DOI: 10.1016/j.resconrec.2005.03.002
- Linke, B., Muha, I., Wittum, G., and Plogsties, V. (2013). "Mesophilic anaerobic co-digestion of cow manure and biogas crops in full scale German biogas plants: A model for calculating the effect of hydraulic retention time and VS crop proportion in the mixture on methane yield from digester and from digestate storage at different temperatures," *Bioresource Technol.* 130, 689-695. DOI: 10.1016/j.biortech.2012.11.137
- Liu, Y. C., and Whitman W. B. (2008). "Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea," *Ann. NY Acad. Sci.* 1125, 171-189. DOI: 10.1196/annals.1419.019
- Liu, X., Gao, X., Wang, W., Zheng, L., Zhou, Y., and Sun, Y. (2012). "Pilot-scale anaerobic co-digestion of municipal biomass waste: Focusing on biogas production and GHG reduction," *Renew. Energ.* 44, 463-468. DOI: 10.1016/j.renene.2012.01.092
- Massé, D. I., Rajagopal, R., and Singh, G. (2014). "Technical and operational feasibility of psychrophilic anaerobic digestion biotechnology for processing ammonia-rich waste," *Appl. Energ.* 120, 49-55. DOI: 10.1016/j.apenergy.2014.01.034
- Møller, H. B., Sommer, S. G., and Ahring, B. K. (2004). "Methane productivity of manure, straw and solid fractions of manure," *Biomass Bioenerg.* 26(5), 485-495. DOI: 10.1016/j.biombioe.2003.08.008
- Muller, C. E., Johansson, M., Salomonsson, A. C., and Albiñ, A. (2013). "Effect of anaerobic digestion residue vs. livestock manure and inorganic fertilizer on the hygienic quality of silage and haylage in bales," *Grass Forage. Sci.* 69(1), 74-89. DOI: 10.1111/gfs.12046
- Niu, Q. G., Hojo, T., Qiao, W., Qiang, H., and Li, Y. Y. (2014). "Characterization of methanogenesis, acidogenesis and hydrolysis in thermophilic methane fermentation of chicken manure," *Chem. Eng. J.* 244, 587-596. DOI: 10.1016/j.cej.2013.11.074

- Nuchdang, S., and Phalakornkule, C. (2012). "Anaerobic digestion of glycerol and co-digestion of glycerol and pig manure," *J. Environ. Manage.* 101, 164-172. DOI: 10.1016/j.jenvman.2012.01.031
- Okeh, O. C., Onwosi, C. O., and Odibo, F. J. C. (2014). "Biogas production from rice husks generated from various rice mills in Ebonyi State, Nigeria," *Renew. Energ.* 62, 204-208. DOI: 10.1016/j.renene.2013.07.006
- Ouédraogo, E., Brussaard, L., and Stroosnijder, L. (2007). "Soil fauna and organic amendment interactions affect soil carbon and crop performance in semi-arid West Africa," *Biol. Fert. Soils.* 44(2), 343-351. DOI: 10.1007/s00374-007-0211-0
- Petersen, G., and Henrius, U. M. (1984). "Biogas production from pig manure, cow manure and hen manure at mesophilic conditions as a function of the ammonia nitrogen level. Exhibitoric effects of ammonia on the conversion of acetic acid, cellulose, protein and fat," in: *Proceedings of Bioenergy 84 1984 Conference*, Goteborg, Sweden, pp. 15-21.
- Procházka, J., Dolejš, P., Máca, J., and Dohányos, M. (2012). "Stability and inhibition of anaerobic processes caused by insufficiency or excess of ammonia nitrogen," *Appl. Microbiol. Biot.* 93(1), 439-447. DOI: 10.1007/s00253-011-3625-4
- Qin, Z. W. (2016). "Slaughter rate of hogs in large scale pig farm in China in 2015," (<http://www.feedtrade.com.cn/livestock/pigs/2016-01-20/2177693.html>), Accessed on 20 January 2016.
- Raposo, F., Borja, R., Martín, M. A., Martín, A., De La Rubia, M. A., and Rincón, B. (2009). "Influence of inoculum-substrate ratio on the anaerobic digestion of sunflower oil cake in batch mode: Process stability and kinetic evaluation," *Chem. Eng. J.* 149(1-3), 70-77. DOI: 10.1016/j.cej.2008.10.001
- Song, K. Y., Li, Y., Ouyang, W., Hao, F. H., and Wei, X. F. (2012). "Manure nutrients of pig excreta relative to the capacity of cropland to assimilate nutrients in China," *Procedia. Environ. Sci.* 13, 1846-1855. DOI: 10.1016/j.proenv.2012.01.178
- Walker, M., Iyer, K., Heaven, S., and Banks, C. (2011). "Ammonia removal in anaerobic digestion by biogas stripping: An evaluation of process alternatives using a first order rate model based on experimental findings," *Chem. Eng. J.* 178, 138-145. DOI: 10.1016/j.biombioe.2009.01.007
- Walsh, J. J., Jones, D. L., Jones, G. E., and Williams, A. P. (2012). "Replacing inorganic fertilizer with anaerobic digestate may maintain agricultural productivity at less environmental cost," *J. Plant. Nutr. Soil. Sc.* 175(6), 840-845. DOI: 10.1002/jpln.201200214
- Wang, L. H., Wang, Q. H., Cai, W. W., and Sun, X. H. (2012a). "Influence of mixing proportion on the solid-state anaerobic co-digestion of distiller's grains and food waste," *Biosystems. Eng.* 112(2), 130-137. DOI: 10.1016/j.biosystemseng.2012.03.006
- Wang, R. F., Zhang, J. W., Dong, S. T., and Liu, P. P. (2011). "Situation of maize straw resource utilization and its effect in main maize production regions of China," *J. Appl. Ecol.* 22(6), 1504-1510. DOI: 1001-9332(2011)22:6<1504:WGYMZC>2.0.TX;2-7
- Wang, X. J., Yang, G. H., Feng, Y. Z., Ren, G. X., and Han, X. H. (2012b). "Optimizing feeding composition and carbon-nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw," *Bioresource Technol.* 120, 78-83. DOI: 10.1016/j.biortech.2012.06.058

- Wang, X. J., Yang, G. H., Li, F., Feng, Y. Z., Ren, G. X., and Han, X. H. (2013a). "Evaluation of two statistical methods for optimizing the feeding composition in anaerobic co-digestion: Mixture design and central composite design," *Bioresource Technol.* 131, 172-178. DOI: 10.1016/j.biortech.2012.12.174
- Wang, Z. C., Gao, M. C., Wang, Z., She, Z. L., Hu, B., Wang, Y. J., and Zhao, C. C. (2013b). "Comparison of physicochemical parameters during the forced-aeration composting of sewage sludge and maize straw at different initial C/N ratios," *J. Air Waste Manage.* 63(10), 1130-1136. DOI: 10.1080/10962247.2013.800616
- Wang, X. W., Zhang, L. Y., Xi, B. D., Sun, W. J., Xia, X. F., Zhu, C. W., He, X. S., Li, M. X., Yang, T. X., Wang, P. F., *et al.* (2015). "Biogas production improvement and C/N control by natural clinoptilolite addition into anaerobic co-digestion of phragmites australis, feces and kitchen waste," *Bioresource Technol.* 180, 192-199. DOI: 10.1016/j.biortech.2014.12.023
- Wang, Y. Y., Zhang, W. L., Wang, J. B., and Meng, L. (2009). "Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria," *Biomass Bioenerg.* 33(5), 848-853. DOI: 10.1016/j.biombioe.2009.01.007
- Windisch, W. (2001). "Pollutants in animal manure: Factors of emission and strategies for reduction," in: *Workshop 4 on Sustainable Animal Production*, Hannover, Germany, pp. 23-25.
- Xie, S., Lawlor, P. G., Frost, J. P., Hu, Z., and Zhan, X. (2011). "Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage," *Bioresource Technol.* 102(10), 5728-5733. DOI: 10.1016/j.biortech.2011.03.009
- Xie, S., Wu, G., Lawlor, P. G., Frost, J. P., and Zhan, X. (2012). "Methane production from anaerobic co-digestion of the separated solid fraction of pig manure with dried grass silage," *Bioresource Technol.* 104, 289-297. DOI: 10.1016/j.biortech.2011.03.009
- Yangin-Gomec, C., and Ozturk, I. (2013). "Effect of maize silage addition on biomethane recovery from mesophilic co-digestion of chicken and cattle manure to suppress ammonia inhibition," *Energ. Convers. Manage.* 71, 92-100. DOI: 10.1016/j.enconman.2013.03.020
- Ye, J. Q., Li, D., Sun, Y. M., Wang, G. H., Yuan, Z. H., Zhen, F., and Wang, Y. (2013). "Improved biogas production from rice straw by co-digestion with kitchen waste and pig manure," *Waste Manage.* 33(12), 2653-2658. DOI: 10.1016/j.wasman.2013.05.014
- Zhang, C., Li, J., Liu, C., Liu, X., Wang, J., Li, S., Fan, G., and Zhang, L. (2013). "Alkaline pretreatment for enhancement of biogas production from banana stem and swine manure by anaerobic codigestion," *Bioresource Technol.* 149, 353-358. DOI: 10.1016/j.biortech.2013.09.070
- Zhang, W. Q., Lang, Q. Q., Wu, S. B., Li, W., Bah, H., and Dong, R. J. (2014a). "Anaerobic digestion characteristics of pig manures depending on various growth stages and initial substrate concentrations in a scaled pig farm in Southern China," *Bioresource Technol.* 156, 63-69. DOI: 10.1016/j.biortech.2014.01.013
- Zhang, W. Q., Wei, Q. Y., Wu, S. B., Qi, D. D., Li, W., Zuo, Z., and Dong, R. J. (2014b). "Batch anaerobic co-digestion of pig manure with dewatered sewage sludge under mesophilic conditions," *Appl. Energ.* 128, 175-183. DOI: 10.1016/j.apenergy.2014.04.071

Zou, S. Z., Wang, X. J., Chen, Y. L., Wan, H. W., and Feng, Y. Z. (2016). "Enhancement of biogas production in anaerobic co-digestion by ultrasonic pretreatment," *Energ. Convers. Manage.* 112, 226-235. DOI: 10.1016/j.enconman.2015.12.087

Article submitted: June 2, 2016; Peer review completed: August 8, 2016; Revised version received: August 26, 2016; Accepted: August 27, 2016; Published: September 6, 2016.  
DOI: 10.15376/biores.11.4.8914-8928