

# Dry co-digestion of sewage sludge and rice straw under mesophilic and thermophilic anaerobic conditions

Xiangqian Chu<sup>1,4</sup> · Guangxue Wu<sup>2</sup> · Jiaquan Wang<sup>3</sup> · Zhen-Hu Hu<sup>1</sup>

Received: 5 May 2015 / Accepted: 13 July 2015 / Published online: 25 August 2015  
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**Abstract** Dry anaerobic digestion of sewage sludge can recover biogas as energy; however, its low C/N ratio limits it as a single substrate in the anaerobic digestion. Rice straw is an abundant agricultural residue in China, which is rich in carbon and can be used as carbon source. In the present study, the performance of dry co-digestion of sewage sludge and rice straw was investigated under mesophilic (35 °C) and thermophilic (55 °C) conditions. The operational factors impacting dry co-digestion of sewage sludge and rice straw such as C/N ratio, moisture content, and initial pH were explored under mesophilic conditions. The results show that low C/N ratios resulted in a higher biogas production rate, but a lower specific biogas yield; low moisture content of 65 % resulted in the instability of the digestion system and a low specific biogas yield. Initial pH ranging 7.0–9.0 did not affect the performance of the anaerobic digestion. The C/N ratio of 26–29:1, moisture content of 70–80 %, and pH 7.0–9.0 resulted in good performance in the dry mesophilic co-digestion of sewage sludge and rice straw. As compared with mesophilic digestion,

thermophilic co-digestion of sewage sludge and rice straw significantly enhanced the degradation efficiency of the substrates and the specific biogas yield ( $p < 0.05$ ) at the conditions of C/N ratio 26:1, moisture content 80 %, and natural initial pH. Although high concentrations of ammonia–nitrogen ( $\text{NH}_4\text{-N}$ , 1500 mg/kg wet weight) were formed during thermophilic digestion, there was no obvious inhibition occurred. The results indicated that rice straw can be used as carbon source for the dry co-digestion of sewage sludge under mesophilic and thermophilic conditions.

**Keywords** Biogas · Co-digestion · Dry anaerobic digestion · Rice straw · Sewage sludge

## Introduction

According to the statistics of the Ministry of Housing and Urban-Rural Development of the People's Republic of China (MOHURD), until the end of March 2014, a total of 3622 wastewater treatment plants (WWTPs) have been constructed in China and the treatment capacity is 153 million  $\text{m}^3/\text{day}$ . The sewage sludge generated in these WWTPs is nearly 30 million tons per year (wet weight) ([http://www.mohurd.gov.cn/zxydt/201405/t20140519\\_217947.html](http://www.mohurd.gov.cn/zxydt/201405/t20140519_217947.html)). The disposal of sewage sludge is a challenge for these WWTPs. Rice straw is a very abundant agricultural waste in China and its disposal is also a challenge (Huang et al. 2013).

Conventionally, sewage sludge is disposed of via incineration, landfill, and ocean disposal or is reused as soil conditioner in agriculture through land application. However, due to the contamination, the sludge dumping to the sea has been banned. With the concern to groundwater contamination, sludge landfilling has been gradually reduced (Kelessidis and Stasinakis 2012). Because sewage sludge contains

Responsible editor: Gerald Thouand

✉ Jiaquan Wang  
jiaquan.wang@163.com

✉ Zhen-Hu Hu  
zhhu@hfut.edu.cn

<sup>1</sup> School of Civil Engineering, Hefei University of Technology, Hefei 230009, China

<sup>2</sup> Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, Guangdong, People's Republic of China

<sup>3</sup> School of Resources and Environmental Engineering, Hefei University of Technology, Hefei 230009, China

<sup>4</sup> School of Mechanical and Automotive Engineering, Hefei University of Technology, Hefei 230009, China

organic matters, it has been recognized as resources for reutilization (Karthikeyan and Visvanathan 2013). Energy recovery from sewage sludge can be achieved through anaerobic digestion, incineration, gasification, and pyrolysis (Tyagi and Lo 2013). Among them, anaerobic digestion is the most popular sludge stabilization technology currently used in the market, recovering biogas as energy and recycling nutrients as organic fertilizer (Zhang et al. 2013). The combustion of methane for power generation can offset the energy consumed during wastewater treatment in WWTPs (Bennett 2007; Rojas and Zhelev 2012), and the use of residue from anaerobic digestion of sewage sludge as fertilizing nutrients in agriculture can also save energy associated industrial fertilizer production (Chen and Chen 2013).

According to the moisture content of the feedstock, anaerobic digestion can be categorized into wet anaerobic digestion if the moisture content is more than 85 % and dry anaerobic digestion if the moisture content is less than 85 % (Kothari et al. 2014). Dry anaerobic digestion can even handle about 30–35 % of dry solid content. Dry anaerobic digestion has many advantages over wet digestion, such as a smaller reactor volume, less liquid digestate arising, higher biogas production rate, and easy handling of digested residues (Zeshan and Visvanathan 2012). Dry anaerobic digestion is usually operated under mesophilic (35–40 °C) or thermophilic (50–55 °C) conditions (Cavinato et al. 2013). Until the beginning of the 1990s, all plants were operated under mesophilic conditions. After then, the advantages of thermophilic operation were investigated and reported, such as offering higher biomass conversion efficiency and more net energy (Fdez-Gueelfo et al. 2010). The number of plants using thermophilic operation was then increased remarkably during the past two decades.

The performance and stability of dry anaerobic digestion are affected by many factors, and the characteristic of feedstock is one of vital factors (Rajagopal et al. 2013). Sewage sludge is composed of organic matter and inorganic matter, and organic matter contains high contents of protein, so sewage sludge has a low C/N ratio (Murray et al. 2008). Low C/N ratio results in the release and accumulation of  $\text{NH}_4\text{-N}$  during anaerobic digestion. High concentrations of  $\text{NH}_4\text{-N}$  have been reported as the primary cause of digester failure because of its direct inhibition on microbial activities, especially on methanogens' activity (Rajagopal et al. 2013). The optional C/N ratio of the feedstock for anaerobic digestion is around 25–40 (Zeshan and Visvanathan 2012). Therefore, organic matter rich in carbohydrates needs to be added to adjust the low C/N ratio of sewage sludge. Rice straw is rich in cellulose, hemicellulose, and lignin and has a high ash content (Huang et al. 2013). The dry anaerobic digestion of composting rice straw has been reported recently (Yan et al. 2015). Sasaki et al. (2011, 2010) have reported that carbon fiber textiles (CFT) can mediate the toxicity of  $\text{NH}_4\text{-N}$  to microorganisms in

wet anaerobic digestion systems. Their results showed that there was stable removal of organic compounds and biogas production under ammonia–nitrogen ( $\text{NH}_4\text{-N}$ ) concentration of 3000 mg/L in the reactors containing CFT. However, the removal of organic compounds and biogas production was inhibited under  $\text{NH}_4\text{-N}$  concentration of 1500 mg/L in the reactors without CFT. The moisture content affected the digestibility and digestion time for the dry anaerobic digestion (Li et al. 2014). Therefore, it can be supposed that the fiber of rice straw not only supplies carbon source for microorganisms, but also mediates the ammonia inhibition in the dry digestion system. Up to date, the performance of dry co-digestion of sewage sludge and rice straw has not been reported.

Therefore, in this study, the feasibility using rice straw as carbon source to adjust the C/N ratio of sewage sludge was investigated. The performance of the co-digestion of sewage sludge and rice straw under mesophilic and thermophilic conditions was explored and compared.

## Materials and method

### Characteristics of sewage sludge and rice straw

The sewage sludge used in this study was collected from the Chengbai Wastewater Treatment Plant, located in Lu'an City, Anhui Province, China, which uses the oxidation ditch technology with a treatment capacity of 80,000 m<sup>3</sup>/day. After thickened in the thickening tank, the excess sludge in the plant was dewatered by a belt press filter and the moisture content of the dewatered sludge was around 78–85 %. The fresh dewatered sludge was collected, and the dry matter content of the sludge used in this study was 15.6±1.0 %. Dry rice straw, collected from the suburb of Hefei City, Anhui Province, China, was grounded to <1 cm small pieces. The characteristics of sewage sludge and rice straw are listed in Table 1.

**Table 1** Characteristics of sewage sludge, rice straw and inoculums

Items	Sewage sludge	Rice straw	Inoculums
Total solids (TS, %)	15.6±0.3	91.1±0.8	11.5±0.2
Volatile solids (VS, %)	45.6±0.9	83.8±0.7	44.8±0.6
pH	7.2±0.2	–	8.1±0.2
Total nitrogen (TN, g/kg)	48.42±2.34	9.80±0.42	13.73±0.76
C/N ratio	5.2:1	47.0:1	18.0:1
Ammonia-nitrogen ( $\text{NH}_4\text{-N}$ , g/kg)	0.26±0.03	–	6.36±0.24
Total phosphorus (TP, g/kg)	20.02±0.42	–	13.92±0.29
Soluble phosphorus (g/kg)	0.17±0.03	–	2.83±0.11

The C/N ratios of the sewage sludge and rice straw were 5.2 and 47.0, respectively.

**Inoculums of mesophilic and thermophilic anaerobic digestion**

The original inoculums were collected from the mesophilic and thermophilic anaerobic reactors treating sewage sludge in the laboratory. Two kinds of acclimated anaerobic inoculums were used in this experiment. One was the mesophilic inoculums, which was acclimated at 35±3 °C for 2 months with sewage sludge and rice straw as substrate. The other was the thermophilic inoculums, which was acclimated at 55±3 °C for 2 months with the same substrate as the mesophilic conditions. After the acclimation, both the mesophilic and thermophilic inoculums showed stable methanogenic activities. The characteristics of inoculums are listed in Table 1.

**Experimental setup of dry co-digestion**

Dry co-digestion was carried out in batch reactors made of glass with internal diameter of 8.0 cm and height of 25.0 cm. The working capacity of the reactors was 1.2 L. The reactors were placed in two incubators in the laboratory where the temperatures were controlled at 35±3 and 55±3 °C respectively. The volume of biogas generated was collected using 10.0 L sampling bags.

**Experimental design**

This experiment consisted of three parts. In the first part, the anaerobic digestion of sewage sludge, sewage sludge, and inoculums was separately investigated under mesophilic and thermophilic conditions as the control, as listed in Table 2. The aim of assays 1, 2, and 3 was to investigate the potential of biogas production and degradation efficiency of the inoculums, sewage sludge, and rice straw under mesophilic and thermophilic conditions. For

**Table 2** Anaerobic digestion of the original substrates under mesophilic (35±3 °C) and thermophilic (55±3 °C) conditions

Composition	Assay number		
	1	2	3
Sewage sludge (g)	0	400.5±4.2	0
Rice straw (g)	0	0	200.6±2.7
Inoculums sludge (g)	150.4±1.9	150.6±1.8	150.3±1.9
VS (g)	7.7±0.2	36.2±0.4	160.4±2.8
C/N ratio	18.0:1	6.22:1	43.7:1
Moisture content (%)	88.2±2.2	85.5±2.3	80.3±1.8

the digestion of sewage sludge and inoculums, the moisture content was not adjusted, while for the rice straw, the moisture content was adjusted to 80 %. The incubation time lasted 75 days for the mesophilic digestion and 25 days for the thermophilic digestion.

In the second part, the influence of C/N ratio, initial pH, and moisture content on the performance of dry co-digestion was investigated under mesophilic condition (35±3 °C). Four levels of C/N ratios, moisture content, and initial pH values were studied respectively. The experimental design and the composition of the feedstocks and the addition of inoculums are listed in Table 3.

In the third part, based on the determined C/N ratio, moisture content, and initial pH obtained in the second part, the performance of dry co-digestion of sewage sludge and rice straw under mesophilic (35±3 °C) and thermophilic (55±3 °C) conditions was investigated and compared. The composition of the substrates used in the third part is listed in Table 4.

**Analysis**

The solid samples collected during digestion were diluted with deionized water at a ratio 1:10, and the mixture was centrifuged at 10,000 rpm for 10 min (Centrifuge 5810R, Eppendorf). The supernatant was collected for the analysis of volatile fatty acids (VFAs), NH<sub>4</sub>-N, soluble phosphorus, and pH. Dry matter, total solids (TS), volatile solids (VS), NH<sub>4</sub>-N, and soluble phosphorus were analyzed according to the Standard Methods (APHA 1998). Biogas was collected by 10-L gas sampling bag during the digestion (Safelab Ltd., China). The volume of the collected biogas was measured using water displacement method everyday (Hu et al. 2012; Hu and Yu 2005). The composition of biogas was determined using a gas chromatography with a TCD detector (SP-6800, Lulan Co., China). VFAs were determined with a gas chromatography using a FID detector (GC-6890N, Agilent Inc., USA). The total kjeldahl nitrogen (TKN) was analyzed by acid digestion and distillation procedure using a semi-automatic nitrogen analyzer.

**Statistical analysis**

All the assays were carried out in triplicate and means were reported here. The differences of degradation efficiency and biogas yield of co-digestion of sewage sludge and rice straw between means were analyzed by one-way analysis of variance (ANOVA), followed by Duncan’s multiple range test at a significance level of 0.05 using SPSS 17.0 Statistical Software Program (SPSS Incorporated, Chicago).

**Table 3** Experimental design and composition of the substrates used in the second part of the experiment under mesophilic condition (35±3 °C)

	C/N ration				Moisture content (%)				pH			
	18:1	23:1	26:1	29:1	65	70	80	85	7.0	8.0	9.0	10.0
Sewage sludge (g)	400.3±3.4	399.6±3.9	399.7±4.2	399.3±4.1	400.6±2.9	399.8±3.2	400.4±3.7	400.3±3.2	399.9±3.7	400.4±3.3	400.9±4.2	399.7±3.4
Rice straw (g)	50.7±0.8	100.3±1.8	150.8±1.9	200.6±2.1	199.7±1.9	200.2±2.1	199.6±1.7	200.3±1.8	200.7±2.1	199.7±1.6	200.5±2.3	200.2±1.9
Inoculums sludge (g)	60.9±0.4	90.2±0.8	119.8±0.7	149.6±1.8	150.2±1.4	150.2±1.6	150.6±1.7	149.7±1.8	149.9±1.5	150.4±1.2	149.3±1.8	150.5±1.6
VS (g)	74.3±0.7	112.5±0.9	150.7±1.4	188.6±1.7	188.9±1.8	188.3±1.4	188.5±1.6	188.4±1.7	188.2±1.9	188.8±1.8	188.4±1.6	188.9±1.7
Water (mL)	60.4	235.6	410.4	520.5	0	93.2	520.4	970.8	520.3	520.4	520.6	520.2
Reactor volume (mL)	600±20	800±40	1000±40	1205±50	1180±40	1190±50	1220±60	1210±50	1210±60	1200±50	1230±60	1210±50
C/N ratio					29:1	29:1	29:1	29:1	29:1	29:1	29:1	29:1
Moisture content (%)	79.8±2.2	80.2±2.4	80.6±1.9	80.3±2.1					80.8±1.5	80.4±1.8	80.2±1.6	80.6±1.9
pH	7.2±0.3	7.2±0.2	7.1±0.2	7.1±0.2	7.1±0.2	7.2±0.3	7.1±0.3	7.1±0.2	7.1±0.3	8.2±0.2	9.1±0.4	10.2±0.4

**Table 4** Composition of the substrates used in the third part of the experiment

Treatment temperature (°C)	35±3	55±3
Sewage sludge (g)	399.6±3.7	400.8±4.4
Rice straw (g)	150.3±1.4	149.8±1.6
Inoculums (g)	150.8±1.6	150.4±1.5
VS (g)	136.0±4.2	136.4±4.2
Water (mL)	310	310
Reactor volume (mL)	960±30	950±30
C/N ratio	26.0:1	26.1:1
Moisture content (%)	80.2±2.2	80.7±1.8
Volume (mL)	1060	1060
pH	7.2±0.2	7.1±0.2

## Results and discussion

### Anaerobic digestion of the substrates

The anaerobic digestion of sewage sludge, rice straw, or inoculums was carried out for 75 days. Table 5 lists the result of degradation efficiency and biogas yield of the substrates. It can be found that the degradation efficiency was enhanced by thermophilic conditions as compared with mesophilic conditions. However, the degradation efficiency varied from 28.5 to 41.3 %, which was relatively low as compared with previous report (Karthikeyan and Visvanathan 2013). Anaerobic digestion of sewage sludge resulted in the accumulation of NH<sub>4</sub>-N (Rajagopal et al. 2013), which might inhibit the methanogenic activities. While for rice straw, the very high C/N ratio limited the microbial growth and inhibited the methanogenic activities, resulting in low degradation efficiency. Therefore, the co-digestion of sewage sludge and rice straw might improve the degradation efficiency and biogas yield.

**Table 5** Degradation efficiency and biogas yield of only sewage sludge, rice straw, or inoculums

Treatment temperature (°C)	Substrate	Degradation efficiency of VS (%)	Biogas yield <sup>a</sup> (mL/g VS)
35±3	Inoculums	31.5±2.7	263±13
	Sewage sludge	37.5±3.4	308±16
	Rice straw	28.5±3.1	222±8
55±3	Inoculums	33.6±4.2	279±10
	Sewage sludge	41.3±2.6	344±19
	Rice straw	31.7±3.6	248±13

<sup>a</sup> Biogas yield per initial gram of VS

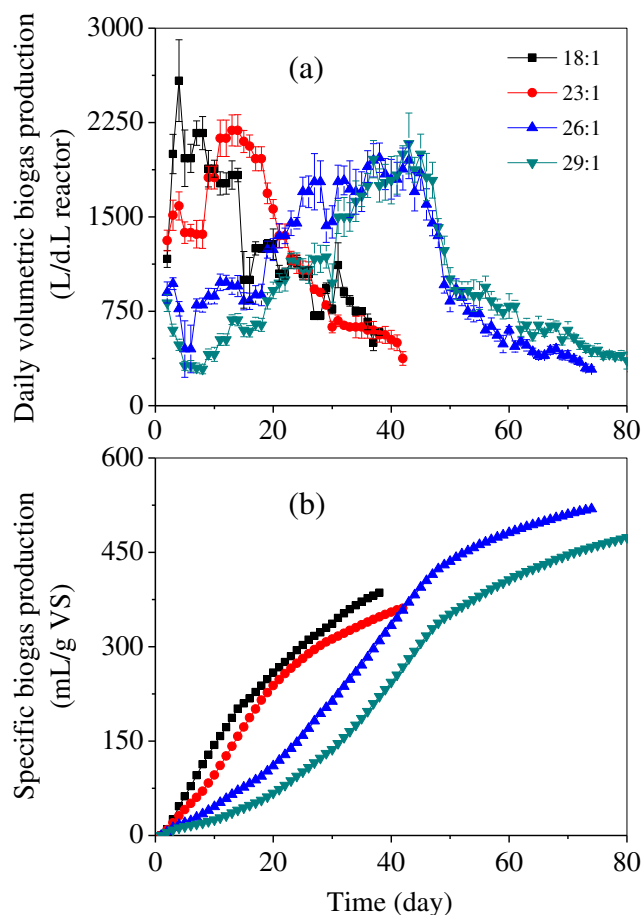
### Dry co-digestion under mesophilic conditions

#### Effect of C/N ratio

C/N ratio is an important factor affecting the performance of anaerobic treatment system. Figure 1 shows the biogas production at various C/N ratios. As shown in Fig. 1a, the startup of dry co-digestion was obviously affected by the C/N ratio. Low C/N ratios had a shorter startup time, and the reactors quickly reached the highest daily biogas production. For example, at the C/N of 18:1, the maximum daily biogas production appeared on the 4th day after digestion, while at C/N ratios of 23:1, 26:1, and 29:1, the maximum daily biogas production appeared approximately on the 13th, 38th, and 43rd days respectively. The phenomenon can be attributed to the difference in the substrate composition. The assay with lower C/N ratio contained higher percentage of sewage sludge, which contributed to more easily biodegradable organic matter, such as digestible microbial protein. The assay with higher C/N ratio contained higher percentage of rice straw. Because the cellulose and hemicellulose are covered

by lignin, rice straw is recalcitrant to be decomposed by microorganisms (Hu et al. 2008). Based on the performance of biogas production, it can be deduced that the carbon source contained in the rice straw was not effectively utilized by microorganisms at the beginning of the co-digestion under mesophilic conditions.

Figure 1b shows specific biogas production (biogas production per gram of VS). It can be found that at low C/N ratios, there were high biogas production rates, which might be due to the easy decomposition of sewage sludge. However, for the specific biogas production, the biogas yield at C/N ratios of 26:1 and 29:1 was higher than that at 18:1 and 23:1, as listed in Table 6. The low biogas yield at low C/N ratio may be due to the inhibition of ammonia to the anaerobic microbial activities. The highest yield of 520 mL per gram of VS was obtained at C/N ratio of 18:1, while the corresponding yield at C/N ratio of 26:1 was 375 mL per gram of VS, but they all were higher than the digestion with single substrate, as listed in Table 5. Therefore, it can be concluded that the co-digestion enhanced the degradation efficiency of VS and biogas production. The C/N ratio of 26:1 was selected for the experiment in the third part.



**Fig. 1** Biogas production at various C/N ratios under mesophilic condition ( $35\pm 3\text{ }^{\circ}\text{C}$ ), (a) daily volumetric biogas production, (b) specific biogas production

#### Effect of moisture content

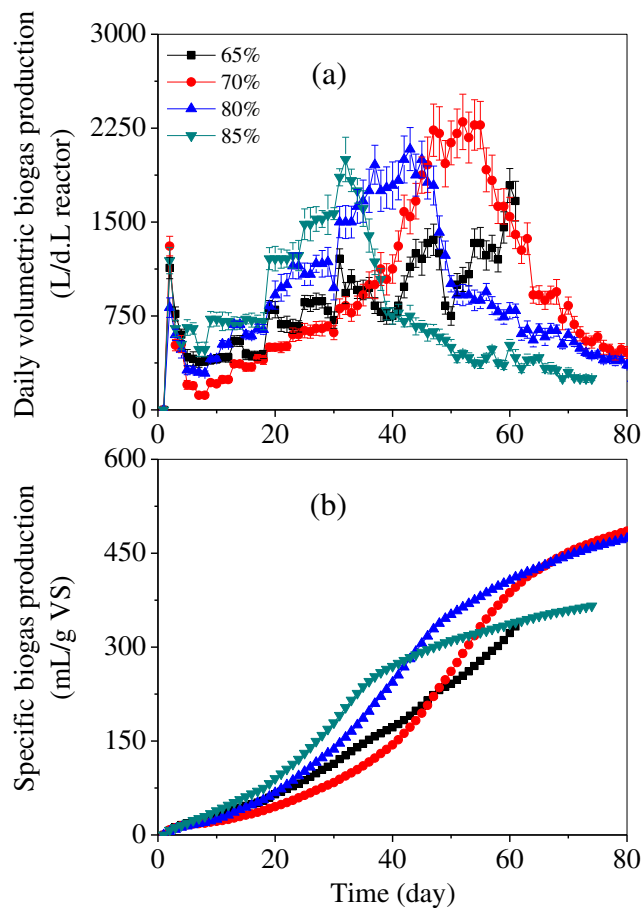
The moisture content influences the transfer and diffusion of mass and energy in the anaerobic digesters (Bollon et al. 2011; Le Hyaric et al. 2012; Pommier et al. 2007). Figure 2 depicts the biogas production of mesophilic anaerobic digestion at moisture contents of 65, 70, 80, and 85 % respectively. It

**Table 6** Degradation efficiency and biogas yield of dry co-digestion of sewage sludge and rice straw under mesophilic conditions ( $35\pm 3\text{ }^{\circ}\text{C}$ )

Factors		Degradation efficiency of VS (%)	Biogas yield <sup>a</sup> (mL/g VS)
C/N ratio	18:1	44.5±2.7a	375±21a
	23:1	43.0±3.2a	361±19a
	26:1	60.1±4.3b	520±34b
	29:1	55.7±3.8b	474±38b
Moisture content (%)	65	40.2±2.6a	333±24a
	70	57.8±4.1b	485±32b
	80	55.5±4.8b	475±27b
	85	42.4±3.9a	361±33a
Initial pH	7.0	47.6±4.4a	402±45a
	8.0	43.1±3.4a	360±26a
	9.0	44.2±3.7a	380±32a
	10.0	43.1±3.8a	365±37a

The different lowercase letters are considered statistically significant ( $p<0.05$ )

<sup>a</sup> Biogas yield per initial gram of VS



**Fig. 2** Biogas production at various moisture contents under mesophilic condition ( $35\pm 3$  °C), (a) daily volumetric biogas production, (b) specific biogas production

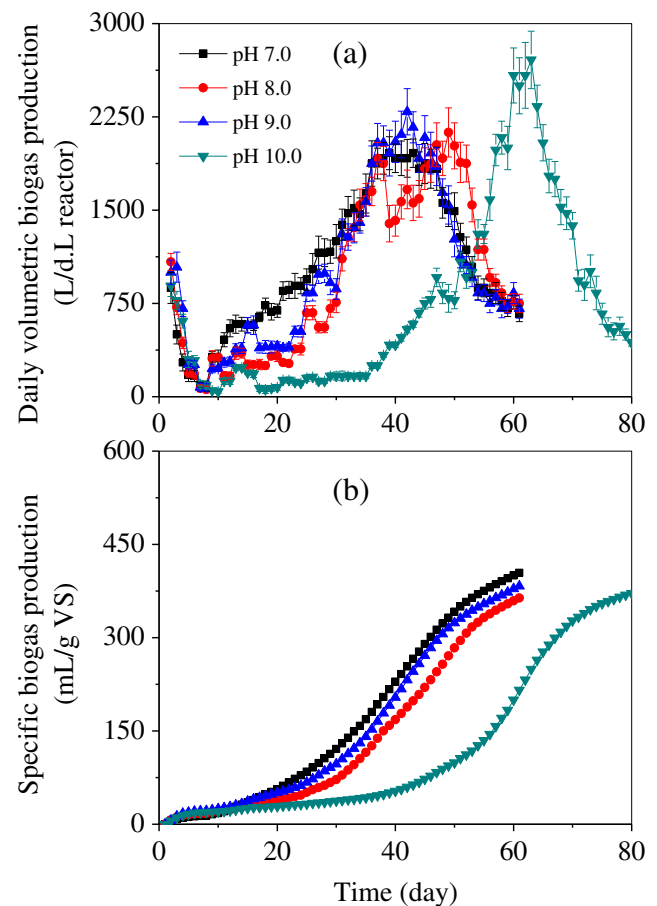
can be found that with the increase of moisture content, the startup time was shortened. At the assay of 65 % moisture content, the highest daily biogas production was still not reached until the 60th day of the operation. Moreover, the biogas production was not stable with 65 % moisture content. Bollon et al. (2013) investigated the diffusion coefficients of the feedstock of dry anaerobic digestion and found that effective diffusion coefficients decreased drastically when TS contents increased from 8 to 25 %. Le Hyaric et al. (2012) reported that in the batch mesophilic dry co-digestion, the low moisture content affected the degradation kinetics and extended the digestion time. Previous investigation indicated that the mass diffusion was seriously limited by the dry feedstock at 65 % moisture content (Bollon et al. 2011; Garcia-Bernet et al. 2011), which was consistent with the result obtained in this study. The highest daily biogas production at the moisture contents of 65, 70, 80, and 85 % occurred on the 61st day, 47th day, 38th day, and 31st day, respectively. Therefore, moisture content is a very important factor for the economic operation of dry anaerobic digestion system.

Figure 2b shows the specific biogas production. At the assay of 85 % moisture content, there was a high biogas

production rate at the early stage, but after approximately 40 days, the specific biogas production was exceeded by the assays of other moisture content. As listed in Table 6, in all four moisture content levels, high specific biogas yield of 475–485 mL/g VS was obtained at moisture contents of 70 and 80 %, and a higher biogas production rate was observed at moisture content of 80 %. Therefore, 80 % moisture content was selected in the third part of the experiment.

#### Effect of initial pH

Figure 3 shows the biogas production at various initial pH values. As shown in Fig. 3a, biogas production was not obviously affected by the initial pH 7.0, 8.0 and 9.0, but at initial pH 10.0, the startup was seriously delayed. pH is one of most important factors influencing microorganisms' activities, and the optimal pH for anaerobic digestion is in the range of 6.5–7.5 (Vavilin et al. 2003). However, the initial pH 8.0 and 9.0 did not inhibit the microbial activity. This might be due to the buffer capacity of sewage sludge, which results in the pH coming back to normal pH values with the proceeding of digestion. However, at pH 10.0, the methanogens' activities



**Fig. 3** Biogas production at various initial pH values under mesophilic condition ( $35\pm 3$  °C), (a) volumetric daily biogas production, (b) specific biogas production

were seriously inhibited, and a long time was needed to recover the methanogens' activities, as shown in Fig. 3b. However, the specific biogas yield was not obviously influenced by the initial pH ( $p > 0.05$ , Table 6).

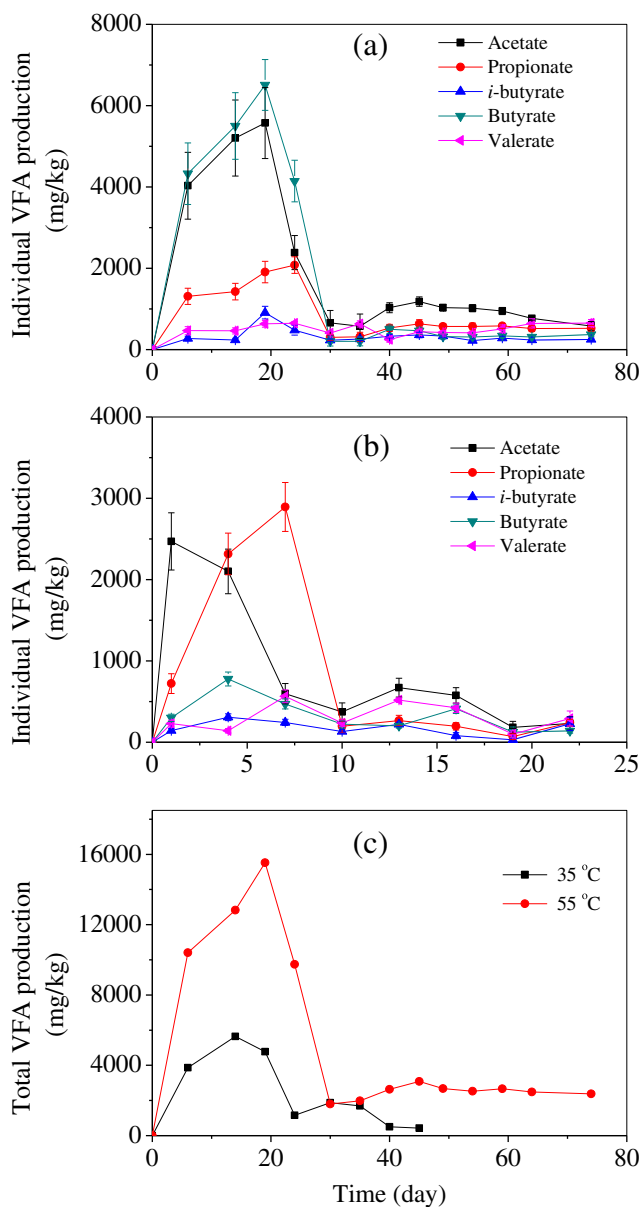
**Dry co-digestion under mesophilic and thermophilic conditions**

Temperature is an important factor influencing the degradation rate and digestibility of the substrate for the anaerobic digestion (Mu et al. 2006, 2007). From the mesophilic dry co-digestion, the C/N ratio of 26:1, moisture content of 80 %, and naturally initial pH was selected to compare the performance of dry co-digestion of sewage sludge and rice straw between mesophilic and thermophilic conditions. The composition of the substrates is listed in Table 4. The dry co-digestion was operated at  $35 \pm 3$  and  $55 \pm 3$  °C respectively.

*VFA production*

Figure 4 shows the profiles of individual and total VFAs production of co-digestion of sewage sludge and rice straw under mesophilic and thermophilic conditions. The accumulation of individual VFA was detected in the first 18 days in the mesophilic digestion, as shown in Fig. 4a. The main individual VFA was acetate and butyrate with highest concentrations of 5500 mg/kg wet weight and 6500 mg/kg wet weight, respectively. The highest total VFAs concentration was over than 15,000 mg/kg wet weight, as shown in Fig. 4c, indicating that serious inhibition might occur during mesophilic dry co-digestion, which can partly explain the low biogas yield at low C/N ratios in the second part. The accumulation of VFAs is due to the two-stage fermentation of organic matter during anaerobic digestion (Li and Yu 2011). In the first stage, the organic matter is hydrolyzed and acidified into VFAs (Yue et al. 2007), which is further converted into methane and carbon dioxide by methanogens in the second stage (Mu et al. 2007). After 18 days, the concentration of individual VFA began to decline, which was because the VFAs generated were less than the consumed, suggesting that the inhibition was mitigated with the extension of the incubation time.

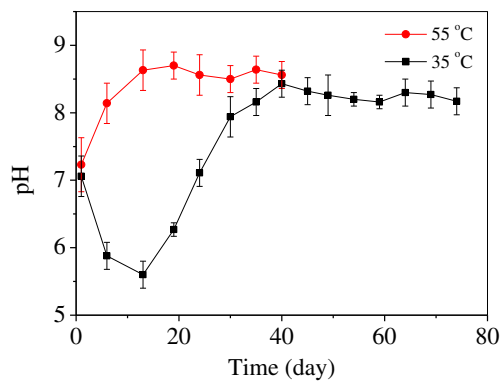
Figure 4b shows the profile of individual VFA production under thermophilic conditions. Compared with mesophilic condition, the concentrations of individual VFA were far low in the thermophilic digestion. The main VFA was acetate and propionate, which was different from the mesophilic dry co-digestion. The highest total VFAs concentration was as low as 5500 mg/kg wet weight, which appeared on the 14th day. The total VFAs concentration then decreased to less than 2000 mg/kg after 20 days. The low total VFAs in the reactor suggest that there was no obvious inhibition occurred under thermophilic dry co-digestion.



**Fig. 4** Profiles of VFAs production, (a) under mesophilic condition ( $35 \pm 3$  °C), (b) under thermophilic condition ( $55 \pm 3$  °C), and (c) total VFAs

*pH variation*

Figure 5 shows the pH variation of the dry co-digestion under mesophilic and thermophilic conditions. As shown in Fig. 5a, the pH in the mesophilic digestion first declined from 7.0 at day 0 to 5.6 at day 17, corresponding to the formation and accumulation of VFAs in the reactor in Fig. 4a. Previous investigation has observed that the  $pH < 6.0$  in anaerobic digestion reactor seriously inhibited the activity of methanogens (Hu and Yu 2005). After day 17, the pH gradually rose from 5.6 to 8.3 on day 40, indicating that the accumulated VFAs were utilized and converted by methanogenic microorganisms. While for thermophilic dry co-digestion, there was no



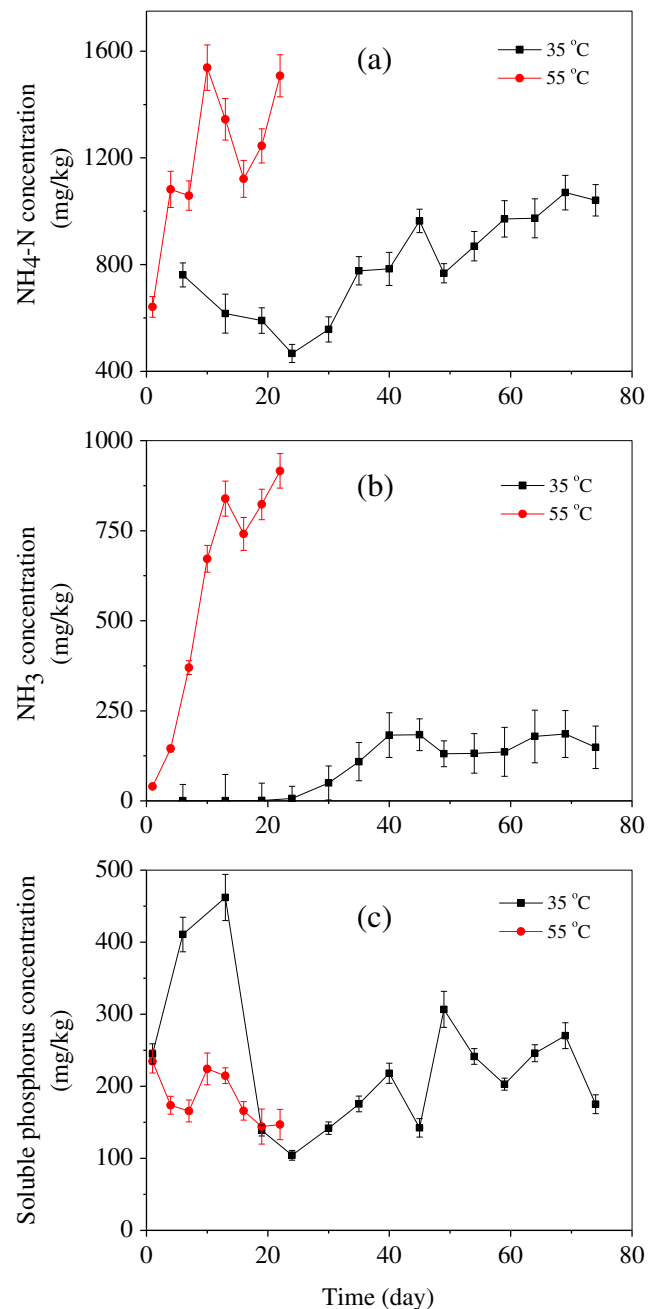
**Fig. 5** pH variation of dry co-digestion under mesophilic ( $35\pm 3$  °C) and thermophilic ( $55\pm 3$  °C) conditions

pH decrease during the incubation. On the contrary, the pH in the reactor began to increase from 7.1 at the beginning of the digestion to 8.7 on day 11, and then leveled at around 8.6. The increase of pH in the reactor was attributed to the formation of  $\text{NH}_4\text{-N}$  released from the degradation of protein and nucleic acids.

#### Release of $\text{NH}_4\text{-N}$ and soluble phosphorus

Figure 6 shows the evolution of  $\text{NH}_4\text{-N}$  and soluble phosphorus in the reactors. As shown in Fig. 6a, for the mesophilic dry co-digestion, the concentration of  $\text{NH}_4\text{-N}$  on the 6th day reached 750 mg/kg, and then gradually declined to 450 mg/kg on the 23rd day of the incubation. While for the thermophilic co-digestion, the concentration of  $\text{NH}_4\text{-N}$  increased from 460 mg/kg on the 1st day to 1530 mg/kg on the 10th day and then fluctuated around 1500 mg/kg. Ammonia-nitrogen in the digester comes from the decomposition of organic matter such as protein and nucleic acids (Gonzalez-Fernandez and Garcia-Encina 2009). Thermophilic condition enhanced the degradation of organic matter, which resulted in the rapid accumulation of  $\text{NH}_4\text{-N}$  in the reactor. It has been reported that high concentration of  $\text{NH}_4\text{-N}$  inhibits the activities of anaerobic microorganisms, especially for methanogens in thermophilic anaerobic digestion, because high temperature results in more release of  $\text{NH}_4\text{-N}$  in the form of free ammonia (Fig. 6b) (Rajagopal et al. 2013). However, no obvious inhibition occurred in this study, which might be due to the absorption of fiber in the rice straw to ammonia, like the medication of carbon fiber textiles to ammonia toxicity for microorganisms in the wet anaerobic digestion systems (Sasaki et al. 2010, 2011).

Figure 6c shows the soluble phosphorus concentration during the mesophilic and thermophilic dry co-digestion. The rapid release of soluble phosphorus was found at the initial stage of mesophilic digestion. In the activated sludge process, the anaerobic process enhanced the release of phosphorus into the aqueous solution, which can explain the increase of



**Fig. 6** Profile of concentrations of  $\text{NH}_4\text{-N}$  (a),  $\text{NH}_3$  (b), and soluble phosphorus (c) under mesophilic ( $35\pm 3$  °C) and thermophilic ( $55\pm 3$  °C) conditions

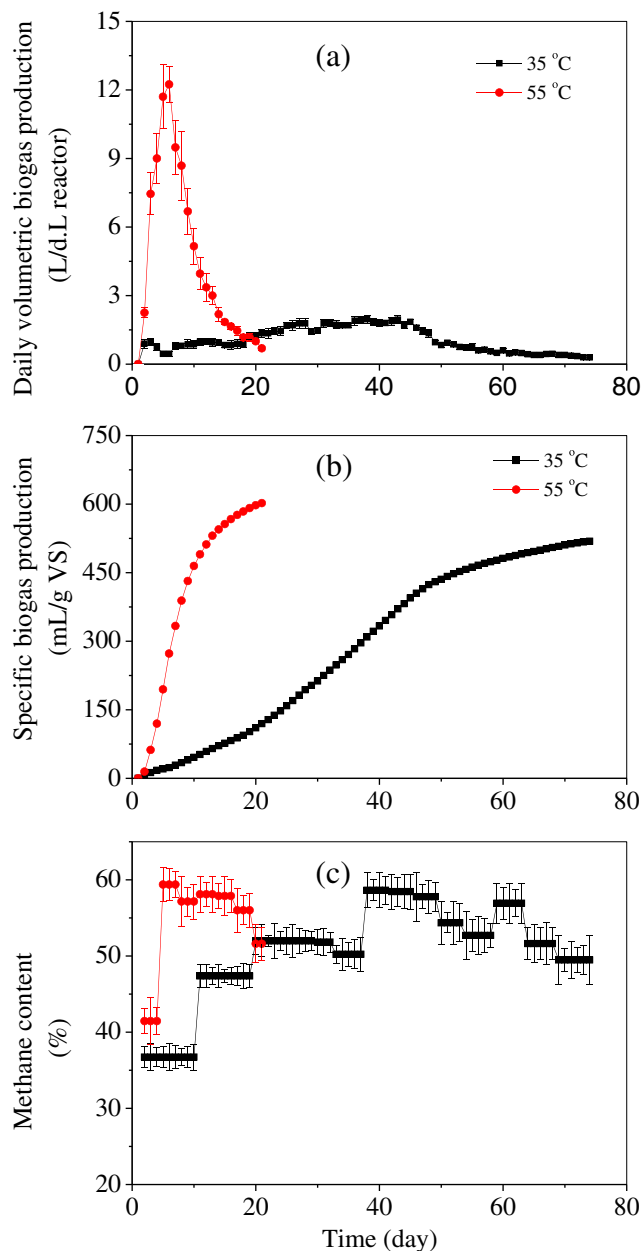
soluble phosphorus concentration in the reactors. While for the thermophilic anaerobic digestion, the concentration of soluble phosphorus showed a slight fluctuation at a lower concentration as compared with mesophilic digestion. Combined the VFAs production and  $\text{NH}_4\text{-N}$  formation, it can be deduced that sewage sludge and rice straw were rapidly broke down at the initial stage of thermophilic digestion, but the rapid increase of soluble phosphorus was not observed in this study. This may be because the released phosphorus reacts with other chemicals to form insoluble phosphoric compounds under



alkaline conditions for thermophilic digestion (as shown in Fig. 5).

*Biogas production*

Figure 7 shows the biogas production and methane composition of co-digestion under mesophilic and thermophilic conditions. Daily biogas production was significantly affected by the operation temperature ( $p < 0.05$ ). As shown in Fig. 7a, the maximum daily biogas production under thermophilic



**Fig. 7** Biogas production of dry co-digestion under mesophilic (35±3 °C) and thermophilic (55±3 °C) conditions, (a) daily volumetric biogas production, (b) specific biogas production, and (c) methane content

conditions was 12.2 L/day L, as observed on the 11th day of the digestion, while under mesophilic conditions, the maximum value was only 2.0 L/day L. Compared with mesophilic digestion, the treatment duration was reduced by 3 times from average 75 days under mesophilic conditions to average 25 days under thermophilic conditions, indicating that thermophilic dry co-digestion can significantly improve the utilization efficiency of reactors.

Figure 7b shows specific biogas production under both digestion conditions. Under mesophilic conditions, the specific biogas yield reached 518 ml/g VS for mesophilic digestion. While under thermophilic condition, the yield increased to 602 ml/g VS, indicating that biogas production was significantly enhanced by the increasing treatment temperature ( $p < 0.05$ ). More importantly, the degradation efficiency correspondingly increased from 60.1 % under mesophilic conditions to 70.2 % under thermophilic conditions ( $p < 0.05$ ), as listed in Table 7.

The methane content in the biogas is shown in Fig. 7c. For the mesophilic dry co-digestion, the methane content gradually increased from 36 % at the beginning to 59 % on the 38th day. While for the thermophilic dry co-digestion, the methane content rapidly increased from 41 % at the beginning to 60 % at the 5th day, and then fluctuated and declined gradually. The methane content in the biogas was affected by the composition of the decomposed feedback. Protein has a higher methane production potential than carbohydrate (Yu and Fang 2003). As compared with mesophilic digestion, thermophilic digestion had high methane content at the beginning of digestion, meaning that all the composition of the substrates, especially microbial protein was quickly broken down. Therefore, thermophilic co-digestion significantly enhanced the degradation efficiency of VS and biogas production from the dry co-digestion of sewage sludge and rice straw.

**Conclusions**

The co-digestion of sewage sludge and rice straw under mesophilic conditions was significantly affected by C/N ratio and moisture content. Substrates at low C/N ratio resulted in a

**Table 7** Comparison of co-digestion of sewage sludge and rice straw between mesophilic and thermophilic conditions

Treatment temperature (°C)	Degradation efficiency of VS (%)	Yield <sup>a</sup> (mL/g VS)
35±3	61.0±3.8a	518±26a
55±3	70.2±2.9b	602±38b

The different lowercase letters are considered statistically significant ( $p < 0.05$ )

<sup>a</sup> Biogas production per initial gram of VS

high biogas production rate and a low specific biogas yield. At the moisture content of 65 %, the biogas production was inhibited, and the digestion system was in an unstable state. At the moisture content of 70 and 80 %, the dry co-digestion of sewage sludge and rice straw obtained high degradation efficiency and specific biogas yield. In comparison with mesophilic digestion, thermophilic digestion resulted in the higher biogas production rate and specific biogas yield accompanied with high  $\text{NH}_4\text{-N}$  accumulation and concentration. It is very interesting that the activity of methanogens was not inhibited by the high  $\text{NH}_4\text{-N}$  concentration in the substrate, but the mechanism of the medication effect by rice straw needs further investigation. The disposal of sewage sludge and rice straw is a big challenge in China. The dry co-digestion of sewage sludge and rice straw under thermophilic conditions not only reduces the volume of the wastes, but also recovers biogas as energy. The recovered energy can be used for electricity generation, which will reduce the greenhouse gas emission from wastewater treatment plants.

**Acknowledgments** This research was partially supported by the Key Special Program on the S&T for the Pollution Control (2012ZX07103-001), the NSFC (51078122, 41372246), and the Program for New Century Excellent Talents in University (NCET-11-0624).

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