Synergistic and Pretreatment Effect on Anaerobic Co-Digestion from Rice Straw and Municipal Sewage Sludge

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Anaerobic digestion is considered to be a priority disposal technology for rice straw and sewage sludge. In this study, the synergistic and alkali-treat effect on co-digestion of rice straw and sewage sludge was investigated. The results indicated that the co-digestion of alkali-treated rice straw and sewage sludge had the best biogas yield of 338.9 mL/gVS, which was 1.06 and 1.75 times that of either alkali-treated rice straw or sewage sludge alone, respectively. The actual biogas and methane yields of a co-digestion group with raw rice straw and sewage sludge (G4) increased 26.39% and 24.79% relative to the theoretical calculation based on raw rice straw digestion (group G2) and sewage sludge digestion (group G5), suggesting that a synergistic effect occurred during the co-digestion process. The maximum concentration of volatile fatty acids (VFA) was 4860 mg/L on the 4th day in the sewage sludge group. Xylanase activity reached a maximum of 10.55 U/mL on the 6th day in the alkali-treated rice straw group, while the concentration of protease enzyme was relatively higher in the sewage sludge group than in others. The removal rates of cellulose and hemicellulose in groups with alkali treatment were 32.25% and 36.96% (G1) and 40.86% and 41.61% (G3), higher than that of groups without treatment.

Keywords: Rice straw; Sewage sludge; Co-digestion; Synergistic effect; Biogas

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INTRODUCTION

The annual production of rice straw ranges between 180 and 270 million tons in China (Ye *et al*. 2013). However, few effective measures have been applied to treat such large-scale waste, and most of it is openly burned (Gu *et al*. 2014), which causes serious air pollution, such as fog and haze. Biomethane technology has been attracting increased attention because not only can it dispose of rice straw, but it also constitutes a biofuel source (Chandra *et al*. 2012). However, the direct utilization of rice straw by microorganisms is difficult because of its unbalanced carbon to nitrogen properties and recalcitrant lignocellulosic structure (Himmel *et al*. 2007). Therefore, attention has been increasingly focused on improving the hydrolysis stage by pretreatment (Cheng *et al*. 2011).

Municipal sewage sludge is a by-product from wastewater treatment plants, representing up to about 50% of the entire operating costs, and its appropriate disposal is an issue of particular concern (Silvestre *et al*. 2014). Anaerobic digestion technology has been shown to be a good candidate for sewage sludge treatment, but the low carbon and nitrogen ratio presents a serious problem that leads to ammonia inhibition during the digestion process (Noutsopoulos *et al*. 2013). To obtain a highly economical and efficient biogas production from sewage sludge, a supplemental nitrogen source is needed.

Co-digestion has been successfully employed for rice straw or sewage sludge, such as rice straw with kitchen waste, rice straw with piggery wastewater, sewage sludge with the organic fraction of municipal solid waste, and sewage sludge with pig manure (Mussoline *et al*. 2012; Cabbai *et al*. 2013; Ye *et al*. 2013). Moreover, the co-digestion of rice straw and sewage sludge was investigated under mesophilic and thermophilic conditions; the results indicated that a decrease in ammonia nitrogen concentration and an improvement in the methane yield was achieved during the co-digestion process (Komatsu *et al*. 2007). However, the synergistic effect of co-digestion rarely has been elaborated upon. The aim of this study was to (1) investigate the potential of biogas production from a mixed substrate of rice straw and sewage sludge, (2) evaluate the effect of alkali treatment of rice straw on the co-digestion, and (3) estimate the synergistic effect of the co-digestion process from process parameters.

EXPERIMENTAL

Materials

Rice straw and municipal sewage sludge

Rice straw was collected from a rice field in Wuxi (Jiangsu province, China) and was cut into 1- to 2-cm pieces. Dewatered municipal sewage sludge was taken from a sewage treatment plant in Wuxi. Dilute alkali pretreatment was used for the rice straw (Cui *et al*. 2013). Rice straw and a NaOH solution (5% mass fraction) with a solid/liquid ratio of 1:10 (g:mL) were mixed in a reaction bottle at room temperature for 24 h. After that, rice straw was washed to neutrality and finally dried to a constant weight at 70 °C. Substrate characteristics are shown in Table 1.

Table 1. Characteristics of Rice Straw and Municipal Sewage Sludge

Substrate	Total Solid (TS) (%wet weight)	Volatile Solid (VS) (%wet weight)	VS/TS (%)	Cellulose (% TS)	Hemi-cellulose (% TS)	Lignin (% TS)	C/N
Raw rice straw	91.98±3.23	77.26±2.14	83.99±2.68	33.52±1.46	25.21±1.02	10.52±0.48	47.64±2.12
Alkali treated rice straw	90.31±3.02	78.71±2.01	87.16±2.52	45.11±1.68	11.2±0.46	1.24±0.08	47.64±2.13
Municipal sewage sludge	16.48±0.78	8.77±0.36	53.22±0.57	-	-	-	6.68±0.32

Inoculum

Anaerobic methane sludge was obtained from an anaerobic digestion reactor at Jiangsu Clean Environmental Technology Co., Ltd. (Suzhou, China); the working volume of the reactor was 1500 m^3. The total solids (TS) and volatile solids (VS) of the sludge were 6.81% and 4.52%, respectively.

Experimental Setup

The mixed substrate was made up of rice straw and municipal sewage sludge at a ratio of 1:1 based on VS. Anaerobic methane sludge was used at an inoculum to substrate (I/S) ratio of 1:1 based on VS. Six different substrates were used in the experiments. Group 1 (G1) was alkali-treated rice straw, group 2 (G2) was raw rice straw, group 3 (G3) was mixed substrate with alkali-treated rice straw, group 4 (G4) was mixed substrate with raw rice straw, group 5 (G5) was municipal sewage sludge, and group 6 (G6) was the mixed substrate control, having the same composition as G4 except that it was not inoculated with anaerobic methane sludge when the experiment started (Table 2). The loading rate of each group was 20 gVS/L.

Anaerobic sludge and different substrates were introduced to a reaction bottle with a volume of 500 mL, the headspace of the reaction bottles was purged with nitrogen to maintain the anaerobic environment, and the reaction temperature was kept at 35 °C.

Table 2. Experimental Design

Group	Rice straw with alkali treatment	Raw rice straw	Mixed substrate with alkali-treated rice straw	Mixed substrate with raw rice straw	Municipal sewage sludge	Mixed substrate control
Code	G1	G2	G3	G4	G5	G6
Rice straw content (gVS)	10	10	5	5	0	5
Municipal sewage sludge content (gVS)	0	0	5	5	10	5

Methods

Analytical methods

The TS, VS, and soluble chemical oxygen demand (SCOD) were determined according to standard methods (APHA 1999). Carbohydrate content was determined by the phenol sulfuric acid method (Dubois *et al*. 1956). Total nitrogen was determined by the Kjeldahl method (Zhao *et al*. 2012). Protein was analyzed by the Folin-phenol method (Lowry *et al*. 1951). Cellulose, hemicellulose, and lignin were estimated according to the acid detergent method, as described by Goering and Vansoest (1970). Protease enzyme measurement was based on the protein transition to amino acid (Kardos *et al*. 2011). Filter paper activity was determined as described by Ghose (1987). Xylanase activity was measured using birchwood 4-O-methyl glucuronoxylan as a substrate according to the method described by Bailey *et al*. (1992). The content of methane was detected by gas chromatography, and volatile fatty acids (VFA) were measured by high performance liquid chromatography as in the authors' previous study (Zhao *et al*. 2012).

RESULTS AND DISCUSSION

Biogas and Methane Performance in Each Group

The cumulative biogas yield for different groups is plotted in Fig. 1a. The whole duration of biogas production lasted about 30 days. The biogas increased rapidly during the initial time, while it remained stable at the end of reaction for each group. The data indicated that G6 had the lowest cumulative biogas yield of 54.9 mL/gVS. The alkali-treated groups G1 and G3 had biogas yields of 319.6 and 338.9 mL/gVS, representing increases of 25.88% and 19.71%, respectively, compared to that of the raw rice straw groups G2 and G4. Figure 1a shows that the mixed substrate group had a better performance than the single substrate group. Zhang *et al*. (2013) suggested that co-digestion is a promising method for improving biogas production. Dai *et al*. (2013) found that the system stability was improved with the use of co-substrates, and biogas production was enhanced. These reports are in agreement with the present results.

The daily biogas generation yield of each group is shown in Fig. 1b. G1 and G3 exhibited a rapid increase in the first 2 days, reaching maxima of 63.53 and 56.88 mL/gVS.d, respectively. The daily biogas peak time occurred relatively early in the mixed group G4 compared to the raw rice straw group G2, at the 8th and 14th day, respectively. The co-digestion group exhibited a shorter reaction time and improved biogas yield compared to any single substrate group, whether alkali-treated or not. Zhang *et al*. (2013) suggested that the C/N ratio in the reaction process could be better balanced in the co-digestion system than with a single group, allowing the anaerobic reaction to start more quickly. The data also revealed that alkali treatment can increase the digestion potential of rice straw, which is consistent with the finding of Song *et al*. (2013).

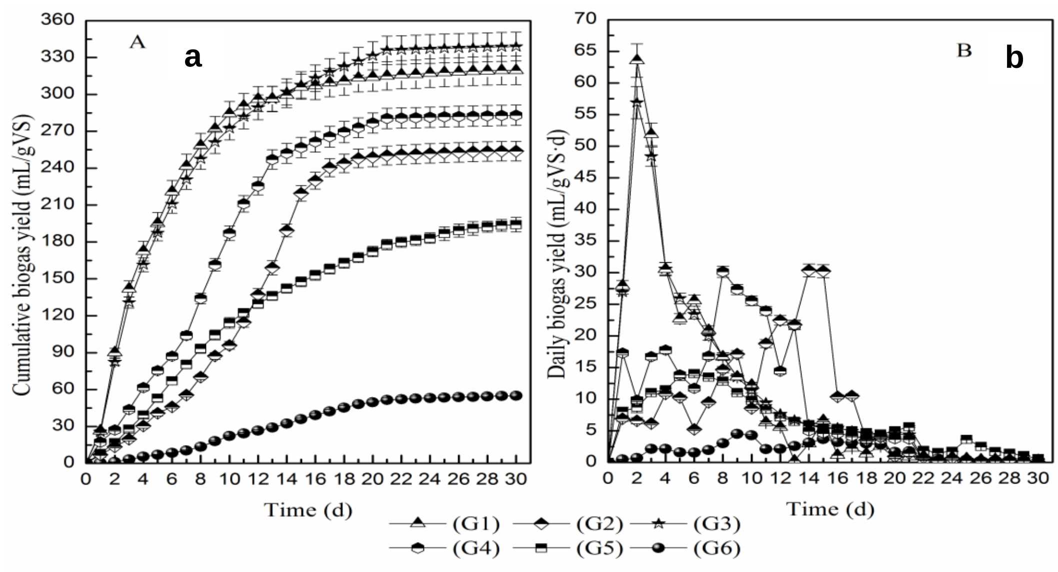

Fig. 1. The change of (a) cumulative biogas and (b) daily biogas in each group

The methane content and cumulative methane yield in each group are indicated in Fig. 2. The methane content was measured every day, and the average methane content in each group was about 50%. The other components of biogas were carbon dioxide, nitrogen, and hydrogen, which were present at about 30%, 8% and 4%, respectively. The primary composition of rice straw is complex carbohydrate matter such as cellulose, hemicellulose, and lignin, while sewage sludge is mostly composed of proteins. Angelidaki and Sanders (2004) indicated that the methane produced by protein degradation was relatively higher than that of carbohydrates.

The cumulative biogas and methane amounts were different when the loading rate was 20 gVS/L in each group. This suggests that the synergistic effect appeared in the mixed digestion groups and that co-digestion can produce more bioenergy than digestion of either single biomass type. Taking the biogas and methane yield from raw rice straw, alkali-treated straw, and sewage sludge digestion as standards, the biogas and methane generation in mixed groups was calculated in accordance with the VS content (Table 3). Interestingly, the actual biogas and methane yields in the mixed substrate group were greater than the theoretically calculated value, whether alkali-treated or not. The actual biogas and methane yields increased by 31.95% and 36.72%, respectively, for G3 and 26.39% and 24.79%, respectively, for G4, compared to the theoretical calculate values. The results showed that the co-digestion was not a simple superposition of two substrates, but had a synergistic effect. Co-digestion can regulate C/N, provide appropriate microbial nutrients, adjust the alkalinity, and provide buffering capacity (Mata-Alvarez *et al*. 2011).

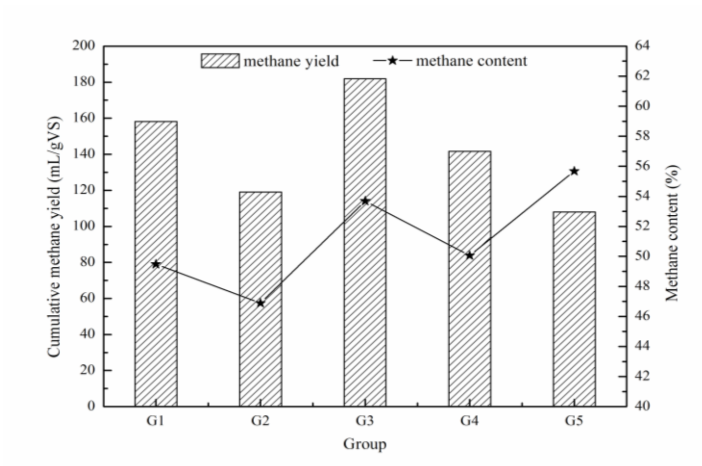

Fig. 2. Methane content and cumulative methane yield

Table 3. Comparison of Actual and Theoretical Cumulative Biogas and Methane Yield

Groups	G1	G2	G3	G4	G5
Cumulative biogas yield/mL (actual value)	3195.9	2538.6	3389.0	2830.7	1940.7
Cumulative biogas yield/mL (theoretical value)	3195.9	2538.6	2568.3	2239.7	1940.7
Biogas increase rate (%)	0	0	31.95	26.39	0
Cumulative methane yield/mL (actual value)	1581.3	1190.3	1819.6	1417.0	1080.6
Cumulative methane yield/mL (theoretical value)	1581.3	1190.3	1330.9	1135.5	1080.6
Methane increase rate (%)	0	0	36.72	24.79	0

Soluble COD, Protein, and Carbohydrate Variation in the Digestion Process

Figure 3 presents the profile of SCOD in the reaction process. The SCOD of each group first increased and then decreased. This was probably because the organic components in the substrates disintegrated into soluble matter in the initial time period, while they were later utilized to generate biogas. G1 and G3 had better hydrolysis performance than the other groups, reaching maxima of 3524 and 3816 mg/L, respectively, at the 4th day; this was attributed mostly to the destruction of the internal structure of rice straw after the alkali treatment. Furthermore, the maximum concentration of COD in alkali-treated rice straw group (G1) was 370 mg/L more than raw rice straw group (G2), while the mixed digestion group (G4) was only 100 mg/L higher than the single rice straw group (G2), which suggested that the alkali pretreatment had a bigger influence on the SCOD release than the co-digestion.

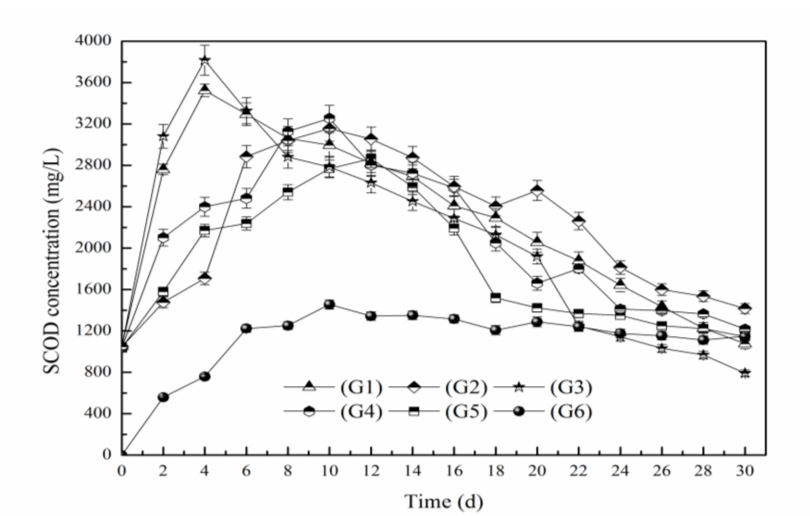

Fig. 3. SCOD concentration during the fermentation process

Soluble proteins and carbohydrates are the primary fermentation substrates that allow acid-producing microorganisms to produce various types of volatile fatty acids (Okamoto *et al*. 2000). Therefore, the variation in protein and carbohydrate concentrations is an important index during the anaerobic digestion process. The concentration of protein in G5 was much higher than it was in the other groups, probably because of the high proportion of protein in sewage sludge itself (Fig. 4a). Its concentration increased sharply during the first 4 days to 1262 mg/L, but decreased afterward. The concentrations of proteins decreased from the beginning of the experiment in G1 and G2; this indicated that the alkali pretreatment had no significant effect on the protein degradation.

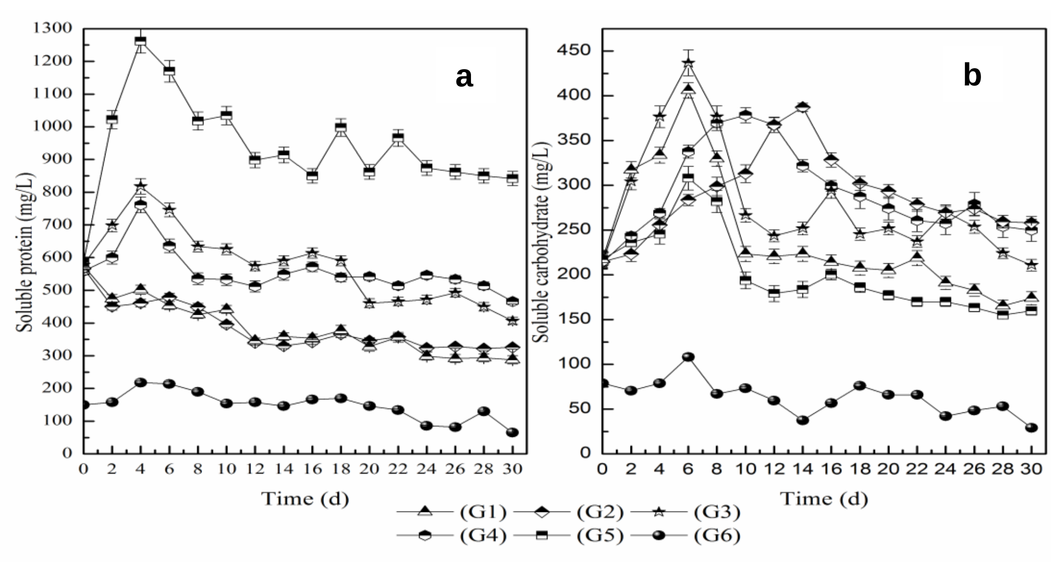

Fig. 4. Change in (a) soluble protein and (b) carbohydrate concentrations in the digestion system

The maximum concentration of soluble protein in co-digestion group G4 reached 761 mg/L, which was higher than the rice straw group (G1). Thus, the co-digestion had a bigger effect on the soluble protein change than alkali pretreatment. In addition, the variations in protein concentration were similar in the co-digestion groups G3 and G4.

The soluble carbohydrate concentration first increased and then decreased (Fig. 4b); the difference was that the concentration of carbohydrates in the rice straw groups was higher than it was in the other groups. This was probably because the major components of rice straw are cellulose, hemicellulose, and lignin. The data showed that the carbohydrates were easier to hydrolyze when adding sewage sludge, as G2 and G4 reached their peak values at the14th day and 10th day, respectively. The concentration of soluble proteins and carbohydrates in G6 was the lowest of the reaction groups, since hydrolysis was inhibited due to no methane microorganisms in the reaction system.

Development of Volatile Fatty Acids during the Reaction Process

Biogas was mainly converted from VFA, and the variation of VFA can be an indicator affecting the methanogenic process (González-Fernández and García-Encina 2009). Figure 5 shows the change in volatile fatty acid concentration during the digestion process, also presenting a trend of first increasing and then decreasing. The generated VFA could not be utilized for biogas production in G6 due to the lack of inocula, making its VFA content relatively higher than others. The VFA content in this study was lower than that of the activated sludge and corn straw co-digestion system described by Zhou *et al*. (2013).

The data indicate that the concentration of VFA increased after alkali pretreatment of rice straw, whether in a single or mixed group. He *et al*. (2008) indicated that the biodegradability of rice straw was enhanced through NaOH pretreatment, which is in agreement with the present research.

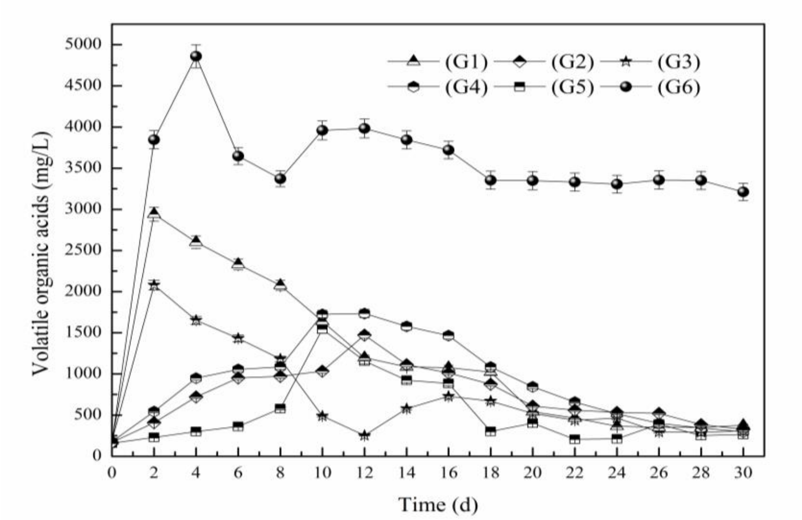

Fig. 5. Change in VFA concentration during the biogas production process

Enzymatic Characterization of the Anaerobic System

Cellulose, hemicellulose, and lignin, which are the major components of rice straw, were degraded primarily by microbial enzymes. Protein is the major component of sewage sludge, and its hydrolysis was an important step. The filter paper activity can indicate the reducing sugars produced by enzymatic hydrolysis of cellulose as well as the synergistic effect of cellulose-degrading enzymes (Zhang *et al*. 2010).

Because of the low carbohydrate content in sewage sludge, xylanase and filter paper activity decreased gradually from the onset in G5 (Figs. 6a and 6b). Xylanase activity in each group became zero by the 14^{th} day of anaerobic digestion. This showed that the degradation of hemicellulose in rice straw was almost completed. The hemicellulose in rice straw was more easily utilized by anaerobic microorganisms after alkali treatment.

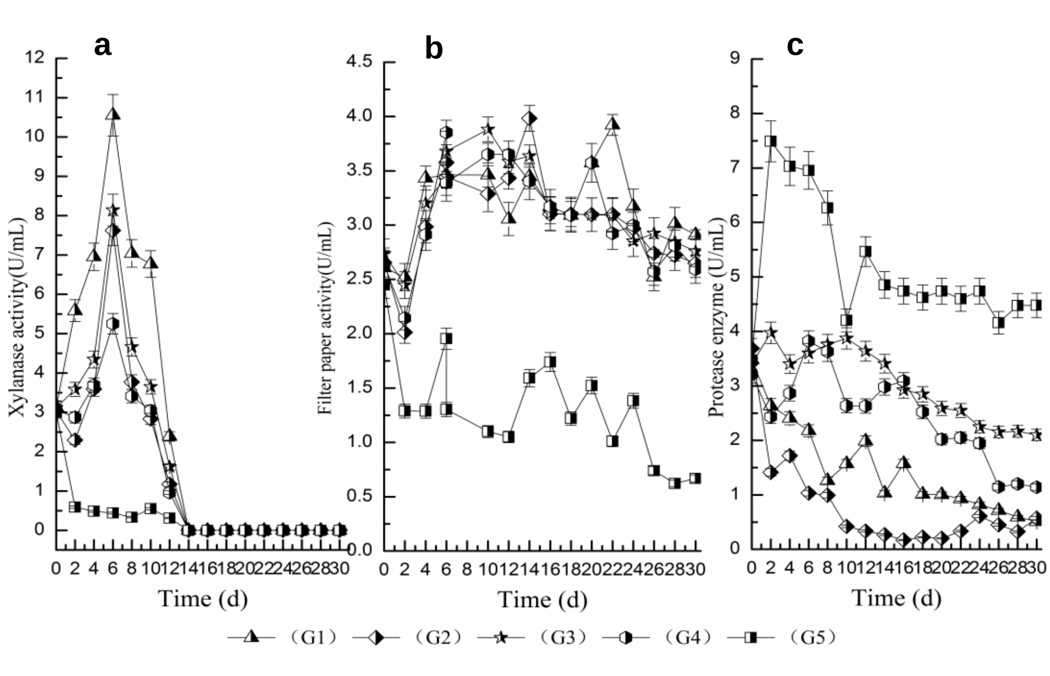

Fig. 6. The variation of (a) xylanase activity, (b) filter paper activity, and (c) protease enzyme concentration

The concentration of filter paper activity declined in the initial 2 days, but it rapidly increased until the 14^{th} day in each group except G5 (Fig. 6b). Moreover, the filter paper activity rapidly declined to the minimum of 0.622 U/mL at the end of reaction in the sewage sludge group due to the low cellulose content. The filter paper activity trend was very similar in groups of G1, G2, G3, and G4, which all contained rice straw.

The protease enzyme concentration was relatively higher in the sewage sludge group than it was in other groups, probably because of the high concentration of nitrogen in sludge. The concentration of protease enzyme in the mixed digestion groups was higher than it was in the other groups, which is the same result as that reported in a previous study (Zhao and Ruan 2013). Furthermore, the mixed group with alkali treatment obtained a higher concentration than did the group without pretreatment.

Substrate Composition Change after the Digestion Reaction

The evaluation of anaerobic digestion efficiency for rice straw mainly depended on the degradation rate of its components and the composition changes before and after the reaction (Table 4). The content of lignin relatively increased because its complex structure prevented its utilization by microorganisms. The removal rates of cellulose and hemicellulose in groups with alkali treatment were 32.25% and 36.96%, respectively (G1), and 40.86% and 41.61%, respectively (G3), which was higher than that of groups without treatment. Furthermore, the removal rate in mixed groups was also greater than that of the single-substrate groups. This showed that the co-digestion of alkali-treated rice straw with sewage sludge could regulate the carbon to nitrogen ratio of the reaction system, benefitting the anaerobic digestion reaction. Cellulose is primarily divided into crystalline and amorphous regions, which constitute the basic skeleton of the cell wall in rice straw. When microorganisms start to degrade the hemicellulose, the degradation of cellulose can be gradually accelerated. At the same time, the skeletal structure was further damaged, and the degradation of hemicellulose was stimulated. Crystalline cellulose can be destroyed by NaOH pretreatment, making it easier to be degraded by microorganisms (Song *et al*. 2013).

Table 4. Composition of Rice Straw Before and After Anaerobic Digestion

Rice straw	Group	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Before digestion	Raw	33.52	25.21	10.52
	Alkali-treated	45.11	11.2	1.24
After digestion	G1	30.56 (32.25)	7.06 (36.96)	1.81 (+)
	G2	25.62 (23.56)	18.69 (25.86)	11.54 (+)
	G3	26.68 (40.86)	6.54 (41.61)	1.93 (+)
	G4	22.84 (31.86)	16.57 (34.27)	12.23 (+)

Note: data in brackets are the removal rate after fermentation (%)

CONCLUSIONS

1. The biogas and methane production yields in mixed substrate with raw rice straw and sewage sludge (G4) increased by 26.39% and 24.79%, respectively, compared to the theoretical calculation values based on raw rice straw (G2) and sewage sludge digestion group (G5), indicating that the co-digestion exhibited a synergistic effect.
2. The maximum biogas yield reached 338.9 mL/gVS in the alkali-treated rice straw and sewage sludge co-digestion group, which was an increase of 19.71% compared to that of co-digestion without pretreatment.
3. The maximum concentration of SCOD was 3816 mg/L at the 4^{th} day in G3. The concentrations of soluble proteins and carbohydrates in mixed substrate control group (G6) were the lowest of all of the reaction groups.
4. Xylanase activity, filter paper activity, and protease enzyme concentration could reflect the biodegradable efficiency of the reaction process. The cellulose and hemicellulose removal rates in the co-digestion group were higher than they were in the single-substrate groups.

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