Enhanced Methane Production from Anaerobic Co-Digestion of Wheat Straw and Herbal-Extraction Process Residues

Yonglan Xi, Zhizhou Chang,* Xiaomei Ye, Jing Du, Guangyin Chen, and Yueding Xu

The efficient biosynthesis of methane from renewable biomass resources is discussed in this paper. Herbal-extraction process residues (HPR) are an excellent raw material for anaerobic digestion because of their abundant trace elements and fermentation stability. Anaerobic co-digestion of wheat straw with HPR was evaluated at HPR/wheat straw ratios (based on total solids (TS), of wheat straw) of 3%, 5%, and 10% with anaerobic sludge at 35±1 °C during 30-d anaerobic digestion. The best performance was achieved with 5% HPR added to the reactor, with cumulative methane production of 13,130 mL and cumulative methane yield of 260.5 mL/g TS_{added}, respectively. Cumulative methane production increased by 31.4% compared to the 9995 mL achieved in mono-digestion with wheat straw. Furthermore, higher activities of protease and total dehydrogenase and higher ATP levels were displayed during the co-digestion process. The high methane yield in this study demonstrates the great potential of co-digestion of renewable biomass as a feedstock for the economical production of methane.

Keywords: Wheat straw; Herbal-extraction process residues; Anaerobic co-digestion; Methane

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INTRODUCTION

With the continued increase in energy demand over the past few decades, researchers have been forced to explore alternate renewable sources of energy and develop novel technologies to harness green energy. Biogas is a versatile and environmentally friendly fuel used for heat and power production or, in the form of purified methane, as a vehicle fuel (Saha *et al*. 2015). As fossil-fuel reserves are declining, anaerobic digestion (AD) is becoming a great alternative renewable energy source and the AD of organic waste to produce biogas is a promising climate change mitigation option and is considered a sustainable treatment technology (Pantaleo *et al*. 2013; Sawatdeenarunat *et al*. 2015). Because of the wide availability of various agricultural residues and processing byproducts, the cost of lignocellulosic feedstocks for conversion to biogas is especially low.

Wheat straw is one of the most abundant agricultural residues in the world and is one of the main crop residues abundant in China. It is the third-most abundant crop residue after rice straw and corn stover. The annual total output of wheat straw in China is 1.09×10^8 t, and an average 1.3 to 1.4 kg of wheat straw is left over following the production of 1 kg of wheat grain (Montane *et al*. 1998). This makes wheat straw an

attractive feedstock for conversion to methane and other value-added products. Wheat straw contains 35% to 45% cellulose, 20% to 30% hemicellulose, and 8% to 15% lignin. It is difficult, if not impossible, to treat wheat straw individually *via* AD because of the physical shielding of cellulose by non-digestible lignin. Lignin is a barrier to efficient hydrolysis, which is a significant problem when considering using lignocellulose for biofuel production. To enhance biogas production from the AD of wheat straw, the cellulosic biomass must first be subjected to pretreatment to increase its accessible surface area. Chemical, thermal, ultrasound, and enzymatic pretreatments can be applied to achieve synergetic effects that make the AD process profitable (Rollini *et al*. 2014; Toquero and Bolado 2014; Kratly and Jirout 2015). However, the high cost of these pre-treatments limits their implementation on a large scale. Because of the high lignocellulose content of wheat straw, it is not used in mono-fermentation for biogas production. The formation of methane from organic materials can be carried out by a mixed microbial community under anaerobic conditions and the poor nutrient and buffer content of wheat straw can influence the microorganic and enzymatic transformations of the AD process (Bahar *et al*. 2013). Addition of trace metals to the biogas process performance often improves biogas production during the mono-digestion of organic matter. The roles of trace elements in anaerobic processes are significant. Some studies have shown that trace metals are essential constituents of cofactors and enzymes and that their addition to anaerobic digesters increased methane production (Demirel and Scherer, 2011; Mao *et al*. 2015). Thus, the AD of wheat straw requires an extra source of trace elements. Although supplementation of micro-nutrients and trace elements could be a simple way to achieve AD process stabilization and efficient biogas generation, the economic feasibility of trace element addition is dependent on their cost.

Co-digestion is believed to help remedy nutrient deficiency and poor buffer capacity in biogas reactors, thereby overcoming the deficiencies of mono-digestion, improving the microbial activity in the AD process, and increasing the efficiency of AD and biogas production (Suraju *et al*. 2014; Zhang *et al*. 2014). However, very limited information exists about solid wastes rich enough in trace elements to meet the requirements of anaerobic biogas digesters fed with various energy crops. One strategy to address this issue might be the co-digestion of wheat straw and materials with a high amount of trace elements to obtain a mixture with balanced overall nutrition.

Many trace elements, including heavy metals, are contained in raw Chinese herbal medicine (Harris *et al*. 2011). With the rapid development of the Chinese herbal medicine industry, herbal-extraction process residues (HPR) are one of the major solid organic wastes generated in China; over 10 million tons of HPR are produced per year (Wu *et al*. 1998). The main method to dispose of HPR is in sanitary landfills, which could potentially cause secondary pollution. Because of the abundance of trace elements, cellulose, hemicellulose, lignin, and protein in HPR, several research groups have tried to use microwave-assisted alkaline pretreatment to produce methane from AD using HPR (Cheng and Liu 2010). Wang *et al*. (2013) reported that HPR can be used as a raw material for AD production of methane, but with low fermentation efficiency. The specific methane yield was only 211 mL methane/gram volatile solids. Anaerobic co-digestion of different organic materials may enhance the stability of the anaerobic process, because the nutritional medium is better, and exhibit more stable biogas production. It is a promising technology for improving digester performance. However, details about its function are not well known. HPR can therefore be considered an excellent raw material for anaerobic co-digestion. Both wheat and HPR are abundant and

have potential for biogas production through co-digestion. This suggests that combining wheat straw and HPR at an appropriate ratio could have potential advantages to optimizing biogas production.

The objective of this study was to develop the direct anaerobic co-digestion of wheat straw and HPR with anaerobic sludge in the biogas production. Some key AD process parameters were investigated. More specifically, the methane production, the concentrations of volatile fatty acids (VFAs), the lignocellulose contents of the wheat straw before and after AD, the impact of the HPR nutrient content on key enzyme activities, and the ATP levels in the AD process were used as the indicators for process stability. They were investigated during a 30-d anaerobic digestion period in batch anaerobic reactors operated under mesophilic conditions. To our knowledge, this is the first use of wheat straw co-digestion with HPR for the economical production of methane.

EXPERIMENTAL

Chemicals and Materials

All chemicals were of reagent grade and were purchased from either Sinochem (Shanghai, P.R. China) or Fluka Chemical (Buchs, Switzerland). CO_2, H_2, and N_2 were obtained from Nanjing Special Gases Factory (Nanjing, P.R. China).

Feedstock and Inoculums

Wheat straw was freshly collected from a farm yard in Luhe District, Nanjing, Jiangsu Province, China at the end of May 2013. It was cut into particles approximately 2 to 3 mm in size using a grinder (Hummer 900, USA). Raw herbal-extraction process residues (HPR) were obtained from Haichang Chinese Medicine Corporation (Nanjing, China) and were milled into a powder using an herbal medicine grinding machine (FW177, Taisite, China). After being air-dried, the straw particles and the HPR powder were stored at 4±0.5 °C until use. Table 1 shows the chemical parameters of the wheat straw, HPR, and anaerobic sludge.

The inoculum of anaerobically digested sewage sludge was taken from a wastewater treatment plant (Yangzi Petrochemical Co., Ltd, Nanjing, China). Glucose was fed to the sludge at 1.5 g/L·d at 35±1 °C for one month, after which the feeding of glucose was stopped. When no biogas production was observed for one week, the seed culture was thoroughly mixed and filtered through a screen with a pore size of 833 μm (20-mesh). This was done to ensure the removal of easily degradable organic matter still present in the inoculum and to remove dissolved methane.

Batch Assay Methane Fermentation Setup

All experiments were conducted in a sequencing batch model with a total volume of 1000 mL. The active volume of the reactor was 800 mL (Xi *et al*. 2014). The reactors were fed with wheat straw with anaerobic sludge main containing a total solids contents of 6%. The experiments were carried out at a mesophilic temperature of 35±1 °C. After the feedstock was added to the reactors, they were sealed immediately with butyl rubber stoppers, and the batch assay methane fermentation reactors were carefully checked for leakage and flushed with pure nitrogen (99.9%) for 3 min to ensure anaerobic conditions. An outlet in the stopper was used to collect the biogas in gas-tight glass jars. Each

digester of static culture was manually mixed once a day to avoid stratification. Wheat straw used in mono-digestion was denoted CK (control). In co-digestion, the amount of wheat straw added to each digester was kept constant (6% TS), while the amount of HPR added varied. The HPR/wheat straw ratios (based on the TS of wheat straw) of digestions H3, H5, and H10 were designed as 3%, 5%, and 10%, respectively, corresponding to HPR amounts of 1.8, 3, and 6 g TS/L in the reactors. The initial carbon-to-nitrogen ratio (C/N) of 30:1 was maintained by the addition of carbamide to each reactor.

Batch experiments were conducted in triplicate to determine the biogas production rates of wheat straw for 30 d. During anaerobic digestion (AD), biogas samples were collected daily, and liquid samples were measured from the control digester in 3-day intervals for process stability investigation.

Table 1. Characteristics of Substrate Used in the Reactors

Parameter	Herbal-Extraction	Wheat straw	Anaerobic sludge
Total solids (TS, %)	92.31±0.70 [a]	90.01±2.03	5.11±0.03
Volatile solids (VS, %)	81.02±1.03	89.26±0.63	68.47±1.44
Total carbon (mg/g TS)	525.10±0.24	479.83±0.02	497.63±0.02
Total nitrogen (mg/g TS)	20.03±0.46	5.34±0.18	14.25±0.13
Carbohydrate (/TS)	67.41±1.08	54.62±0.37	NA[b]
Protein (/TS)	4.56±0.64	3.41±0.32	NA
Cellulose (%)	26.21±1.30	39.21±0.11	NA
Hemicelluloses (%)	21.64±0.82	28.32±0.30	NA
Lignin (%)	12.26±0.57	13.29±0.17	NA
Mg (%, d.b.)	2.74±0.26	0.83±0.31	0.03±0.01
Ca (%, d.b.)	1.96±0.18	0.11±0.03	0.15±0.02
Fe (%, d.b.)	1.63±0.20	0.05±0.01	0.18±0.06
Mn (ppm)	286.19±40.62	49.02±4.05	26.37±0.12
Zn (ppm)	932.56±87.22	65.51±11.03	567.68±20.16
Cu (ppm)	632.33±35.41	—[c]	216.13±22.68
Ni (ppm)	—	4.05±1.03	19.63±0.21
As (ppm)	18.15±3.32	—	18.51±2.33
Mo (ppm)	8.01±1.63	—	1.29±0.07
V (ppm)	—	7.51±1.53	—
Cd (ppm)	26.44±5.98	—	—
Sb (ppm)	6.43±0.99	—	—
Pb (ppm)	78.27±13.78	—	26.49±3.78

[a] Each value is an average of three replicate measurements and is represented as the mean ± standard deviation
[b] No analysis
[c] Concentration lower than the detection limit

Analytical Methods

The daily biogas production was obtained directly from the volume of displaced, saturated $NaHCO_3$ solution in the graduated cylinder after the mixture was manually stirred. The methane concentration in the biogas was analyzed using a gas chromatograph (GC 9890A, Renhua, China) equipped with a TCD (thermal conductivity detector), a TDC-01 column (Φ 4 mm × 1 m, Shimadzu, Japan), and using hydrogen as the carrier gas. The injector, oven, and detector temperatures were 100, 150, and 120 °C,

respectively. The flow rate of the carrier gas was 50 mL/min, and the sample injection volume was 0.5 mL.

The total solids (TS) and volatile solids (VS) were measured in accordance with the standard methods of the APHA (APHA 1998). The total carbon (TC) and total nitrogen (TN) contents were analyzed by a CHN (carbon, hydrogen, nitrogen) analyzer vario EL (Perkin Elmer, USA). The protein content was calculated with a conversion factor of 6.25. The carbohydrate content was calculated as the fraction of VS remaining after the subtraction of protein and lipids (Li *et al*. 2009). The pH was directly measured from the liquid samples with a digital pH meter (FE20K, Mettler-Toledo, Switzerland). The lipids content was determined by a Soxhlet system at 65 °C with more than 60 circulations using methylene dichloride as the extractive reagent. The sample weights before and after extraction were used to calculate the lipids content. For the determination of the major and trace metal element contents, dried samples were pretreated with a mixture of HNO_3/H_2O_2/HF, followed by neutralization with H_3BO_3, and the resulting clear solution was analyzed by inductively-coupled plasma atomic spectrometry (ICP-OES, Thermo Fisher CAP 6200), according to standard procedures. The contents of cellulose, hemicellulose, and lignin were determined by sequential fiber analysis using Goehring and Van Soest's method with an FIWE Cellulose Analyzer (Velp Scientifica Company, Italy) (Van *et al*. 1991). The content of volatile fatty acids (VFAs) was determined by a gas-liquid chromatograph (Model GC-2014, Shimadzu, Japan, fitted with an FID (flame ionization detector), a TCD (thermal conductivity detector), and a 30 m × 0.53 mm × 1 μm Stabilwax DA column. The injector and detector temperatures were 150 and 240 °C, respectively.

The protease activity was analyzed using the Folin-phenol reagent method (Ledoux and Lamy 1986). The total dehydrogenase activity assay was based on the reduction rate of triphenyl-tetrazolium chloride (TTC) to triphenyl-formazan (Feng *et al*. 2009). For the coenzyme F_{420} assay, a 2-mL sample from the digester was first disintegrated and suspended in a Bead Bug (D 1030, Benchmark, USA) at 500 oscillations/min for 100 s and was then centrifuged at 10,000 rpm and 4 °C for 15 min to remove the waste debris. The suspensions were measured using a fluorescence/ luminescence spectrometer (LS 55, Perkin Elmer, USA) under the synchronous scan regime. The difference between the excitation and emission wavelengths was 20 nm. The excitation wavelength was changed from 360 to 480 nm and the emission wavelength was synchronously changed from 380 to 500 nm. Fluorescence at 420 nm was used in these experiments.

Analysis of ATP was based on previous studies (Shanmugan and Horan 2009a; Liang *et al*. 2013) involving the quantification of luminescence released from the reaction of luciferase with ATP. Samples were diluted with 20 mM Tris-EDTA at pH 7.75, boiled for 30 min, and equilibrated to room temperature. Supernatant ATP was recovered by centrifugation at 6000 rpm for 5 min. The ATP concentrations were then measured using a BacTiter-Glo™ Microbial Cell Viability assay kit on a GloMax®-Multi+Detection System (Promega, Madison, WI, USA).

Statistical Analysis

All analytical results were conducted at least in triplicate. The values of the different parameters were expressed as the mean and standard deviation. The standard deviations were analyzed using Microsoft Excel 2003 for Windows.

RESULTS AND DISCUSSION

Biomethane Production Potential at Different HPR/Wheat Straw Ratios

The co-digestion of wheat straw and HPR was investigated at various mixing ratios. The methane volume produced was recorded in batch mode. Figure 1a shows the daily methane production of CK, H3, H5, and H10 in the four separate groups of experiments. Two obvious peaks were observed for daily methane production in all of the reactors during their 30-d operation. The first appeared on the second day in most of the experimental groups. For wheat straw co-digestion with HPR in the anaerobic bottles, the methane productions were 1144.6, 1343.6, and 1303.9 mL/d (groups H3, H5, and H10), respectively. The first peak for CK appeared later, on the third day, and methane production was lesser (867.6 mL/d). The second peaks in the CK group appeared on the twelfth day, but for the other groups co-digested with HPR, they appeared on the eighth day. In comparison with CK, the peaks of H3, H5, and H10 appeared much earlier. The cumulative methane productions are shown in Fig. 1b. After the 30-d anaerobic digestion, the cumulative methane productions of the CK, H3, H5, and H10 groups were 9994.8, 11,704.6, 13,130, and 12,521.1 mL, respectively. There was a significant increase in the methane production from the co-digestion of wheat straw and HPR. Compared with CK, the methane productions for H3, H5, and H10 were 17.1%, 31.4%, and 25.3% higher, respectively. Therefore, H5 was the best addition ratio for methane production, and for this case, the cumulative methane yield was 260.5 ml/g-TS_{added} during the 30-d operation. The results showed that mono-digestion of wheat straw alone may result in significantly low methane yield and that adding to the anaerobic digestion process was effective in improving methane production.

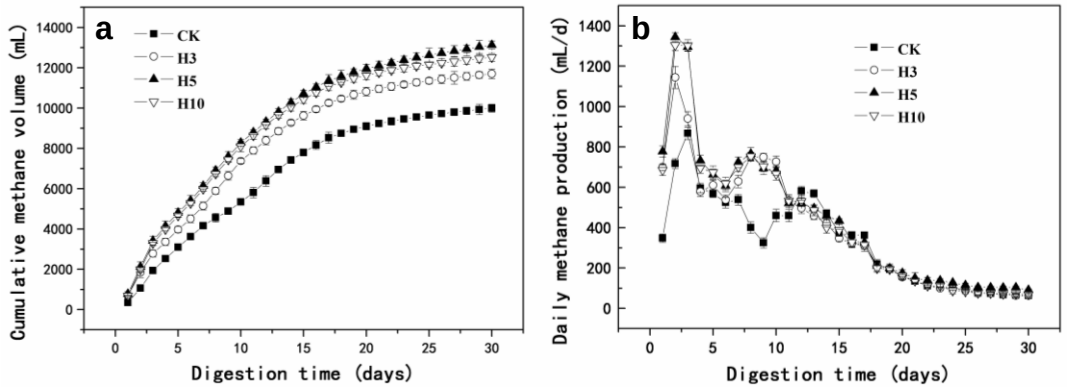

Fig. 1. Daily methane production and cumulative methane volume in batch fermentation from wheat straw or anaerobic co-digestion with HPR in 1-L anaerobic reactors. The plotted data were the averages from parallel experiments (CK: 0 g TS_{HPR}/L; H3: 1.8 g TS_{HPR}/L; H5: 3 g TS_{HPR}/L, H10: 6 g TS_{HPR}/L).

Effect of Wheat Straw Co-Digestion with HPR on VFAs and Lignocellulose Content

VFAs is a key index describing the process of anaerobic digestion. During the anaerobic co-digestion of wheat straw and HPR, VFAs were produced rapidly from the bio-degradable feedstock. High VFAs concentrations in the AD system caused the pH to drop if the buffer capacity of the system was not sufficient, leading to a reduced VFAs consumption rate and inhibiting the methanogens. Figure 3 displays the VFAs production and variation during the anaerobic co-digestion of wheat straw and HPR. The VFAs

concentrations increased significantly in the first 6 d due to hydrolysis of organic matter in all reactors, and reached maximum values of 1716, 1918, 2028, and 1989 mL (CK, H3, H5, and H10), respectively. The concentration of VFAs in groups of H3, H5, and H10 were significantly higher than those in CK.

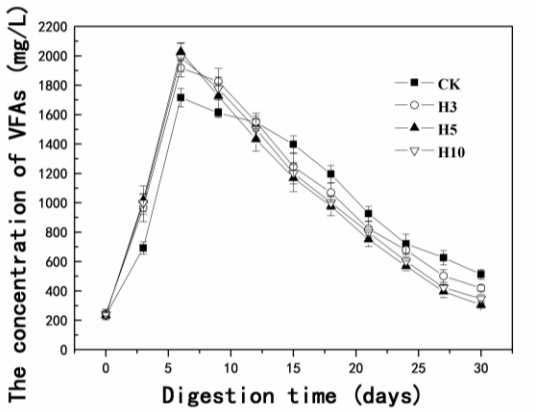

Fig. 2. Concentration of VFAs in batch fermentation from wheat straw anaerobic co-digestion with HPR in 1-L anaerobic reactors. The plotted data were the averages from parallel experiments. (CK: 0 g TS_{HPR}/L; H3: 1.8 g TS_{HPR}/L; H5: 3 g TS_{HPR}/L, H10: 6 g TS_{HPR}/L). VFAs, volatile fatty acids.

After six days, the VFAs concentration gradually declined. Magnesium and iron were the main trace elements in the HPR (Table 1). There have been many studies indicating that the supplementation of magnesium and iron could be an alternative for releasing accumulated VFAs (Kim *et al*. 2002). In contrast, the rate of consumption of VFAs during the co-digestion of wheat straw and HPR was faster than that during mono-digestion of wheat straw. The final VFAs concentration in the reactors with the mono-digestion of wheat straw was 513 mg/L, which was higher than in other cases (between 305 and 419 mg/L). No methanogenesis inhibition was caused by the accumulation of VFAs in the case of wheat straw co-digestion with HPR, and the bacteria consumed VFAs quickly in the anaerobic co-digestion system. Thus, co-digestion could improve the buffer capacity and result in increased acceptable organic loadings in comparison with singular digestion. All these results are consistent with those of a previous study (Kim *et al*. 2002).

The degradation of organic components (cellulose, hemicellulose, and lignin) are important for methane production. Table 2 shows the characteristics of the AD performance in the reactors.

Table 2. Anaerobic Digester Performance Evaluation in the Reactors[a]

Item Unit	Methane Content (%)	Methane Yield (mL/g TS_{added})	Residual Content of Cellulose (TS, %)	Residual Content of Hemicellulose (TS, %)	Residual Content of Lignin (TS, %)
CK	55.3±0.6	208.2±3.2	22.9±0.8	25.4±0.1	22.8±0.3
H3	54.3±1.2	236.7±2.1	21.4±0.9	25.9±0.4	24.7±0.3
H5	54.6±1.6	260.5±1.6	19.2±1.1	24.3±1.2	31.6±0.1
H10	55.0±0.9	237.1±0.8	19.9±1.6	25.1±0.7	29.9±0.6

[a] Each value is an average of three parallel replicates and is represented as mean ± standard deviation (CK: 0 g TS_{HPR}/L; H3: 1.8 g TS_{HPR}/L; H5: 3 g TS_{HPR}/L, H10: 6 g TS_{HPR}/L)

After a 30-d AD, among the three organic components in wheat straw, cellulose exhibited degradation rates ranging from 19.2% to 21.4% for co-digestion and 22.9% for mono-digestion (the initial content was 39.21%, as shown in Table 2). The hemicelluloses content was slightly decreased, ranging from 24.3% to 25.9% (the initial content was 28.32%). Lignin is generally not degradable during digestion; thus, after AD, the lignin content increased significantly in all reactors. Cellulose contributed the most to methane generation. The methane yield was the highest in the H5 group, as was the degradation rate of cellulose (19.2%).

Effect of Wheat Straw Co-Digestion with HPR on Enzymatic Characterizations

HPR contains many trace elements (shown in Table 1). Microorganisms need these trace elements as building blocks for growth and to support enzymatic activities during the AD process (Mao *et al*. 2015). The analysis of the key enzyme activities during AD (Fig. 3) showed that both protease activity and dehydrogenase activity were significantly increased in the groups of wheat straw co-digested with HPR, while the activity of coenzyme F_{420} was not. Results showed that an adequate level of HPR addition could accelerate the hydrolysis of wheat straw during co-digestion with HPR and that the level of microbial activity was high, resulting in faster completion of the AD process. Because of the accelerated hydrolysis rate, VFAs were produced and helped to synthesize methane rapidly, and the peaks in the gas production were reached quickly. These results are consistent with the above results regarding the daily methane production.

Coenzyme F_{420} activity is a measure of methanogens activity. There were no obvious differences for coenzyme F_{420} activity within the four experimental groups, as shown in Fig. 3c. This phenomenon was consistent with the methane content results shown in Table 2. The methane content was not clearly different in all reactors. The higher methane production rate could be due to the excellent hydrolysis efficiency derived from the cell activity. The total dehydrogenase activity reflects the level of microbial activity in the AD process, which can be as used an indirect indicator reflecting the microbial quantity. The adenosine triphosphate (ATP) needed for cell growth was derived from substrate-level phosphorylation, which is discussed in the following section.

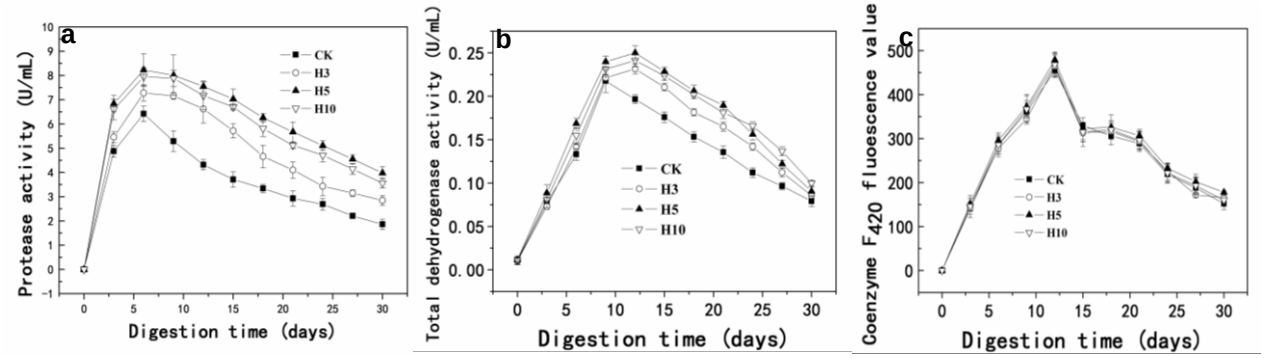

Fig. 3. Change in enzyme activities of protease, dehydrogenase and coenzyme F_{420} during anaerobic digestion in batch fermentation from wheat straw anaerobic co-digestion with HPR in 1-L anaerobic reactors. The plotted data were the averages from parallel experiments. (CK: 0 g TS_{HPR}/L; H3: 1.8 g TS_{HPR}/L; H5: 3 g TS_{HPR}/L, H10: 6 g TS_{HPR}/L).

Effect of Wheat Straw Co-Digestion with HPR on ATP

Mono-digestion of energy crops lacks essential trace elements. Many enzymes and co-enzymes need a minimal amount of certain trace elements for their activation and activity (Appels *et al*. 2008). The level of ATP in a digester has been used to assess both cell viability and biomass yield, thus providing important information on the behaviour of the organisms during the AD process. The ATP level can be used as a rapid technique to evaluate the methane generation potential. Meanwhile, the extent of ATP formation can be controlled by the level of trace elements present in the medium. During anaerobic bacterial growth, organic intermediates can serve as electron acceptors and maintain the overall redox balance. Under these conditions, the ATP needed for cell growth is derived from substrate-level phosphorylation.

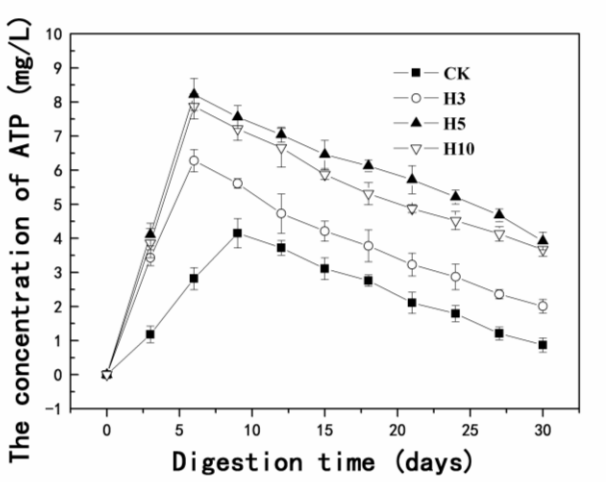

Fig. 4. Concentration of ATP in batch fermentation of wheat straw in anaerobic co-digestion with HPR in 1-L anaerobic reactors. The plotted data were the averages from parallel experiments. (CK: 0 g TS_{HPR}/L; H3: 1.8 g TS_{HPR}/L; H5: 3 g TS_{HPR}/L, H10: 6 g TS_{HPR}/L).

Table 1 shows the metals found in the HPR. Magnesium, calcium, and iron were the main trace elements in the HPR, and their contents on a dry basis were 2.74%, 1.96%, and 1.63%, respectively. The addition of magnesium and calcium ions as energy supplements has been shown to enhance methane production and prevent foaming during the AD process (Reda *et al*. 2008). Magnesium ions generally influence ATP formation: the extent of ATP formation is controlled by the number of magnesium ions present in the medium. At high magnesium concentrations, ADP import, ATP-synthesis by ATP synthase, and ATP export increased (Busch and Ninnemann 1997; Paczosa-Bator *et al*. 2006). Magnesium likely plays a central role in adenine nucleotide-based energy metabolism. Generation of VFAs (*e.g*., acetic acid) has been noted to be accompanied by ATP formation. During anaerobic bacterial growth, organic intermediates such as acetic acid serve as electron acceptors and maintain the overall redox balance. Under these conditions, the ATP needed for cell growth is derived from substrate-level phosphorylation. In addition to the decreased cell viability, low ATP supply for methane production may have also contributed to the decrease in methane yield and productivity. Figure 3 shows the profile of ATP generation during the reaction process. In all groups, the ATP concentration first increased, and then decreased. It rapidly increased from about 0.3 mg/L to maximum values of 4.15, 6.28, 8.22, and 7.87 mg/L, respectively. Similar values have been reported by others (Shanmugan and Horan 2009a,b).

The peak time of CK occurred on the ninth day, and all others occurred on the sixth day, after which time the activity decreased. The levels of ATP in the wheat straw co-digestion with HPR were higher than those in CK. An insufficient supply of energy in the form of ATP has been found to decrease cell viability. As a result, the specific productivity and yield of methane decreased with continuous fermentation (CK). Different levels of ATP could probably be attributed to the bacterial population change *via* adaptive evolution. The highest level of ATP appeared in H5, indicating that after the appropriate proportion of HPR was added, more ATP was produced than in CK. In the case of a high concentration of total VFAs outside the cells, the removal of electrons and VFAs from cells by bacteria was enhanced. This was done to avoid metabolism cessation when VFAs accumulated. As a result, the H5 group, with the highest level of ATP, exhibited the strongest ability VFAs metabolism. This indicates that the bacteria in wheat straw co-digestion with HPR were better tolerated than in CK, improving digester stability, as concluded in a previous study (Zhang *et al*. 2013). It also indicates that HPR supplementation in the AD process could improve the level of ATP in the fermentation system, which could be increased by some trace elements in the HPR (in particular, magnesium) and efficiently induced by bacteria *via* VFA metabolism. Thus, methane yield was improved.

In this study, the co-digestion of wheat straw and HPR was proven to be a feasible strategy for using HPR to enhance the performance of wheat straw AD.

CONCLUSIONS

1. After a 30-d batch anaerobic co-digestion process, the cumulative methane production was increased by 31.4% as compared to that of mono-digestion with wheat straw.
2. Further analysis of the key enzyme activities revealed that the co-digestion led to high efficiencies of biodegradation during AD.
3. The measured ATP content for biomass activity was well-correlated with the methane yield.
4. The balance of nutrients in the fermentation process and the trace elements present played important roles in the performance enhancement achieved *via* the anaerobic co-digestion of wheat with HPR.

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