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Anaerobic codigestion of pretreated wheat straw with cattle manure and analysis of the microbial community

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HIGHLIGHTS

- Wheat straw (WS) pretreated with H_2O_2 was codigested with cattle manure (CM).
- Methanogenic community was measured by the high-throughput sequencing technique.
- The optimal concentration of H_2O_2 for treating WS was 3%.
- A 40:60 ratio of H_2O_2-treated WS mixed with CM produced the highest methane yield.
- Methanogen shifted from acetoclastic to hydrogenotrophic population in digestion.

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ABSTRACT

Wheat straw (WS) was pretreated with four concentrations of H_2O_2 (1%, 2%, 3%, and 4%) and was anaerobically codigested with dairy cattle manure (CM) at various ratios from 100:0 to 0:100. Wet-state H_2O_2 pretreatment effectively enhanced the biodegradability and methane yield of the WS. The optimal concentration of H_2O_2 for treating WS was 3%. The methane yield was higher with the codigestion of CM and H_2O_2-treated WS than with untreated WS and higher than with H_2O_2-treated WS alone or CM alone. A 40:60 ratio of H_2O_2-treated WS mixed with CM produced the highest yield of methane (320.8 mL g volatile solid $(VS)^{-1}$). Results of high-throughput sequencing indicated that the methanogenic community shifted during the codigestion from the acetoclastic methanogens, *Methanosarcina*, to the hydrogenotrophic methanogens, *Methanosphaera* and *Methanoculleus*.

1. Introduction

Environmental problems caused by the burning of fossil fuels and the increasing depletion of resources during the last two decades have led to the development of renewable and sustainable energy sources. The production of biogas by anaerobic digestion (AD) is a cost-efficient and environmentally beneficial bioenergy technology and has thus received much attention (Amon et al., 2007). Lignocellulosic biomass, such as agricultural straw, stalks, waste sludge, and livestock manure, is currently the most common feedstock for AD. Lignocellulosic materials, however, are difficult to enzymatically or bacterially digest because they contain cellulose, hemicellulose, and lignin in complex and cross-linked structures; soluble compounds with low molecular weights available for anaerobic digestion are less abundant (Taherzadeh and Karimi, 2008). Economical and effective pretreatments are thus often needed to enable bacteria to degrade these materials.

Thermal, ultrasonic, chemical, and biological pretreatment can decompose celluloses and hemicelluloses into relatively readily biodegradable components (Laureano-Perez et al., 2005; Dewil et al., 2006; Fernández-Cegrí et al., 2012). Thermal pretreatment can improve biodegradability but requires a substantial amount of energy, and ultrasonic and biological pretreatments are very expensive (Lin et al., 2009). Previous studies have shown that chemical pretreatment is the preferred method for improving the biodegradation of lignocellulosic material (Pang et al., 2008; Guo et al., 2011). Of the chemical pretreatments, hydrogen peroxide (H_2O_2) is commonly used for pretreating agricultural residues because of its strong oxidizing properties. This method effectively digests lignocellulosic biomass, including paper-tube residuals (Teghammar et al., 2010) and various agricultural straws (Song et al., 2012). Most chemical pretreatments, however, presently soak substrates in large volumes of chemical solutions and water, which requires the recycling of chemicals, disposal of waste

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solutions, and sometimes high temperatures and thus requires high investments in facilities, high treatment costs, and potential environmental pollution. Pang et al. (2008) developed a "solid state" pretreatment to improve the biodegradability of corn stover, which used a limited amount of water and produced no waste chemical solutions. Solid-state pretreatment, however, requires long treatment times (three weeks) and has a low pretreatment efficiency. Wet-state pretreatment, in contrast, allows the straw to absorb water thoroughly and can maintain a complete saturating state without losing extra water, which avoids the generation of waste chemical solutions, shortens the pretreatment time, and enhances pretreatment efficiency (Zheng et al., 2009). Pretreatment studies for improving the biodegradability of lignocellulosic biomass have mainly focused on the digestion of single raw materials. The low pHs, suboptimal carbon/nitrogen (C/N) ratios, and poor buffering capacities of single lignocellulosic substrates greatly hinder their digestibility (Zeshan et al., 2012). The codigestion of mixed substrates for biogas production has consequently attracted interest because of its better C and nutrient balance during AD (El-Mashad, 2013).

Codigestion is defined as the digestion of mixtures of at least two waste materials for improving AD efficiency. Many successful codigestions of substrates have increased methane potential substantially compared to the separate digestion of the substrates (Teghammar et al., 2013; González-Fernández et al., 2011). For example, Zhou et al. (2012) reported that the codigestion of corn cover with cow manure increased biogas production by 29.1% relative to the digestion of corn cover alone. Xie et al. (2011) suggested that applying pig manure to grass silage at a ratio of 1:1 would produce a high specific methane yield with a short lag phase. These studies, however, have mostly focused on the technology, such as the effects of operational parameters on the biogas yield and the optimization of the substrate proportions to increase the digestion efficiency. Little information is available for the performance of microbial communities during the codigestion. The characterization of the composition of the microbial community is important for assessing and enhancing digestion efficiency, because the stability and efficiency of AD largely depends on the identity of the active microorganisms (Cho et al., 2013).

The present study evaluated the codigestion performance of H_2O_2-treated wheat straw (WS) with dairy cattle manure (CM) and determined the optimal proportion of the H_2O_2-treated WS and the CM for efficient methane production. The microbial community was also analyzed using the Illumina MiSeq platform, a high-throughput metagenomic sequencing method based on sequencing-by-synthesis technology, to investigate the microbial dynamics of the codigestion.

2. Methods

2.1. Raw material

Wheat straw (WS) was collected from local villagers near Northwest A&F University (Yangling, Shaanxi, China). Prior to use, the straw samples were air dried and cut with a grinder into 20–30 mm segments. CM was obtained from a livestock farm in Yangling. Inoculum was taken from the anaerobic digester treatingcattle manure in a local biogas demonstration village in Yangling, China. The substrates and inoculum were individually homogenized for further use. The chemical characteristics of the substrate and the sludge are shown in Table 1.

2.2. Pretreatment

H_2O_2, purchased from Sinopharm Chemical Reagent Co. Ltd, Beijing, China, was used as the pretreatment reagent. The H_2O_2 was mixed with distilled water to obtain concentrations of 1%, 2%, 3%, and 4% (w/w). Moisture contents of 70%-85% of the ground WS were tested before the pretreatments. The moisture content was calculated as:

$$\text{Mositure content } (\%) = 1 - \frac{\text{dry weight of straw}}{\text{dry weight of straw} + \text{water added}} \times 100\%$$

Preliminary tests indicated that a moisture content of 75% allowed the dried WS to absorb water thoroughly and to maintain a complete saturating state without the loss of water, known as a wet-state pretreatment. This method used a limited amount of water and produced no waste chemicals. Dried corn straw (500 g) was thus soaked in 1.5 L of the prepared H_2O_2 solutions in beakers to produce straw samples with 75% moisture. All beakers were covered with plastic film secured with a plastic ring and were then stored in a chamber at an ambient temperature of 25 ± 2 °C for 7 days. WS soaked in distilled water and stored as above but without chemical pretreatment was used as the control. The straw samples were then removed from the beakers, dried in an electronic oven at 80 °C for 48 h, and refrigerated until compositional determination and the AD experiments to investigate the effect of pretreatment on methane yield. Each pretreatment was conducted in triplicate.

2.3. Digestion experiments

Methane production was determined in two sets of experiments. In the first set, untreated and four H_2O_2-treated (1%, 2%, 3%, and 4%) samples of WS were digested anaerobically in batch flasks to investigate the effect of the pretreatment on the biodegradability and digestibility of the WS. In the second set, the most effective H_2O_2-treated WS from the first set of experiments was codigested with CM at mixed dry-weight ratios of 100:0, 90:10, 80:20, 70:30, 60:40, 30:70, 40:60, 20:80, 10:90, and 0:100. Untreated WS was codigested with CM at the same ratios as controls to investigate the effects of the pretreatments on the performances of the codigestions. The amounts of the substrates in the codigestions are shown in Table 2.

For both sets of experiments, the digestion tests were conducted in batch anaerobic Erlenmeyer flasks. The volume of each flask was 1 L, with a working volume of 0.75 L. The inoculum (200 g) was added to each digester, followed by deionized water to obtain a total solid (TS) content of 8%. The solutions were stirred

Table 1
Chemical characterization of substrate used in the digestion experiments.

	pH value	TS (%)	VS (%)	TC (%)	TN (%)	C/N
Wheat straw	NA	95.2 ± 2.2	86.7 ± 1.8	37.9 ± 1.1	0.43 ± 0.03	88.1 ± 4.5
Cattle manure	6.89 ± 1.0	13.7 ± 1.4	66.2 ± 2.9	17.6 ± 1.4	1.06 ± 0.08	16.6 ± 1.1
Sludge	7.80 ± 0.8	4.86 ± 0.5	67.4 ± 2.4	NA	NA	NA

Value are expressed as the mean ± deviation (n = 3). TS, Total solid; VS, volatile solid; % dry matter, TC, total carbon, % dry matter; TN, total nitrogen, % dry matter; NA = not applicable.