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Study on improving anaerobic co-digestion of cow manure and corn straw by fruit and vegetable waste: Methane production and microbial community in CSTR process

Xuemei Wang, Zifu Li*, Xue Bai, Xiaoqin Zhou, Sikun Cheng, Ruiling Gao, Jiachen Sun

School of Energy and Environmental Engineering, Beijing Key Laboratory of Resource-Oriented Treatment of Industrial Pollutants, International Science and Technology Cooperation Base for Environmental and Energy Technology of MOST, University of Science and Technology Beijing, Beijing 100083, PR China

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ABSTRACT

Based on continuous anaerobic co-digestion of cow manure with available carbon slowly released corn straw, the effect of adding available carbon quickly released fruit and vegetable waste (FVW) was explored, meanwhile microbial community variation was studied in this study. When the FVW added was 5% and 1%, the methane production of the cow manure and corn straw was improved, and the start-up process was shortened. With higher proportion of FVW to 5%, the performance was superior with a mean methane yield increase of 22.4%, and a greater variation of bacterial communities was observed. FVW enhanced the variation of the bacterial communities. The microbial community structure changed during fermentation and showed a trend toward a diverse and balance system. Therefore, the available carbon quickly released FVW was helpful to improve the anaerobic co-digestion of the cow manure and available carbon slowly released corn straw.

1. Introduction

According to the report of the State Council General Office, the per capita animal-derived food consumption in China increased from 92 kg in 2000 to 147 kg in 2013 and is expected to rise to 180 kg in 2020, which could lead to the extension of the animal husbandry industry. According to the 13th Five-Year Plan of China, animal husbandry is encouraged to transform from household pattern into large-scale intensive farms. However, the pollution problem of the livestock manure is becoming more serious. The COD emission of animal husbandry industry in 2013 reached 1.12×10^7 t, exceeding the total COD amount of all the industry according to the report of the State Environmental Protection Administration.

Anaerobic digestion is a suitable practice to make waste profitable, but it is trapped by ammonia inhibition as manure fermentation alone (Shah et al., 2015). The anaerobic co-digestion of the manure with other organic wastes can effectively regulate nutrient supply and improve buffering capacity, eventually enhancing the methane production.

The intensive farms are mostly located in the rural or suburban areas in China, which are surrounded by farmlands, orchard, or large fruit and vegetable markets. In the view of material accessibility, anaerobic co-digestion of livestock manure with crop straw is an available method to utilize wastes and solve pollution problem. Some studies have shown that methane production and process performance are significantly improved in the anaerobic co-digestion of straw and manure (Shah et al., 2015). In the batch experiment on anaerobic co-digestion of cow manure and corn stover (1:3, in TS), the methane yield was 173.43 mL·CH_4/g·VS during 50 days of fermentation (Wei et al., 2015). In the continuously stirred tank reactor (CSTR) experiment on anaerobic co-digestion of cow manure and corn stover (4:1, ww), the highest biogas yield was 497 mL·biogas/g·TS under the hydraulic retention time (HRT) of 40 days (Yue et al., 2013). However, crop straw, as the main carbon source in the anaerobic co-digestion of cow manure and corn straw, is difficult to degrade because of its complex lignocellulosic structure (Ghaffar and Fan, 2013; Hu et al., 2017). The new concept of available carbon is introduced for a better understanding of the different feedstocks roles that are played in anaerobic co-digestion, which means that the carbon derived from the feedstocks shall be available during the anaerobic digestion process to provide carbon sources for the metabolism of microbes. In the hydrolysis phase of anaerobic digestion, crop straw is the available carbon slowly released substrate (ACSRS). The improvement of hydrolysis process is crucial to enhance the anaerobic co-digestion of livestock manure and crop straw.

Fruit and vegetable waste (FVW) is known as large amounts of accessible organic wastes; its landfill disposal is difficult because of its

* Corresponding author.
E-mail address: zifulee@aliyun.com (Z. Li).

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very high perishability (Scano et al., 2014). As the available carbon quickly released substrate (ACQRS), FVW is rapidly acidized and provides available carbon in the hydrolysis phase of anaerobic digestion. Taking FVW as the main substrate, acidification problem may destroy the anaerobic digestion system. In the batch experiment on the anaerobic co-digestion of the pig manure and vegetable processing wastes, the buffer capacity of manure was unable to avoid inhibitory effects associated with total volatile fatty acids accumulation if the co-substrates ratio was not adequate (Molinuevo-Salces et al., 2010). If taking FVW as an additive for improving the anaerobic co-digestion of manure and straw, crop straw could provide slowly released available carbon and FVW could provide quickly released available carbon, and the hydrolysis process might be improved. And the mixture of the three substrates may be a good solution to balance the feedstocks, among which, the crop straw could supply plenty of carbon to balance the excess nitrogen of manure, and its difficulty of hydrolysis could be made up by the rapid acidification of FVW (Sawatdeenarunat et al., 2015).

In the anaerobic co-digestion process, the process stability is as important as the biogas production, both can be indicated through the analysis of bacterial community and archaeal community structures. Microorganisms are the core of the anaerobic digestion as it is a biochemical process in which a great variety of microbial groups are involved, and the success of any anaerobic co-digestion process depends crucially on their growth and metabolism (Wang et al., 2017; Heeg et al., 2014). Through targeting the 16S rRNA gene, detailed pictures of the microbial community compositions were being documented and analyzed. The phyla *Proteobacteria, Chloroflexi*, *Firmicutes*, *Spirochaetes*, and *Bacteroidetes* in the dominant bacteria and the classes *Methanomicrobia*, *Methanobacteria*, and *Thermoplasmata* of phylum *Euryarchaeota* in the dominant archaea are the typical phylotypes found in anaerobic digesters (Hao et al., 2016; Narihiro and Sekiguchi, 2007). However, the hydrolytic bacteria and methanogenic archaea differ widely in ambience with different feedstock, pH, temperature, etc., and the microbial community analysis of anaerobic co-digestion of manure and straw that were improved by FVW is seldom reported. The analysis of microbial community in the anaerobic co-digestion of manure and straw that are improved by FVW is needed to explore its process stability and biogas production.

For husbandry farm anaerobic co-digestion project, the acquisition of straw or FVW is difficult and rather expensive sometimes. And the biogas production and operation stability are both important in a real project. In this study focused on the anaerobic co-digestion process with manure as the main substrate and a small quantity of straw ACSRS; its improvement with the addition of the FVW ACQRS was also explored. Based on the experimental results in the laboratory and considering the demand of real farm-scale project, the effect of the addition of FVW on biogas production was analyzed, and the stability and microbial community in the start-up stage as well as stable operation stage were investigated.

2. Material and methods

2.1. Substrates

Cow manure and corn straw were used as feedstocks for co-digestion in this study. The cow manure was obtained from a farm in the suburb of Beijing. The corn straw was collected from the stubble field near the farm. The FVW was collected from the free market on the campus. The corn straw and FVW were chopped into smaller pieces, with the length of less than 5 mm. The initial characterizations of the cow manure, corn straw, and FVW are shown in Table 1. They were stored in a bag in the refrigerator at −20 °C before experiments.

2.2. CSTR test

The substrate mixture ratios were conditioned by the material supply. For studied farm, the manure was main substrate, co-digested with a small quantity of straw. The experiments consisted of group A, group B and group C, details of which were shown in Table 2. The dry matter ratio of the cow manure and corn straw was 10:1. Group C was used as the control. In group A, the FVW dosage was 5% of cow manure (TS), while the FVW dosage was 1% in group B. As shown in Fig. 1, the anaerobic co-digestions were conducted in a continuous stirred tank reactor with the volume of 1 L at the temperature of 38 ± 0.5 °C. The HRT was 15 days. The microbial analysis was conducted to explore the microbial community structure and the stability of the anaerobic co-digestion of cow manure and corn straw with the addition of FVW. The DNA samples were obtained about every half of the HRT from the start-up stage to the stable operation stage until there wasn't much variation in the microbial community and biogas production. Therefore, it was sampled at the 8th (S1), 15th (S2) and 24th (S3) day during the semi-continuous fermentation process for microbial analysis in group A and group B.

2.3. Analytical methods

TS and VS were measured according to Standard Methods (APHA, 2012). pH was analyzed with a Hach pH meter (HQ30d). C and N were analyzed with a Vario EI Elemental Analyzer (Germany). The biogas production amount was measured by Ritter biogas flowmeter and the methane content was analyzed by a Geotech methane analyzer.

Denaturing gradient gel electrophoresis (DGGE) fingerprinting and sequence analysis were conducted to examine the bacterial and archaeal community structures. The analysis procedure was described in the previous study (H. Wang et al., 2016; X. Wang et al., 2016). DNA was isolated from the samples by using an automated nucleic acid extractor (DN2701, Aidlab, China) according to the manufacturer's instructions. The primers were as follows: the bacteria-specific (BAC338F: ACTCC TACGG GAGG CAG and BAC805F: GACTA CCAGG GTATC TAATC C) and archaea-specific (ARC787F: ATTAG ATACC CSBGT AGTCC and ARC1059R: GCCAT GCACC WCCTC T). PCR was conducted using T-gradient (Biometra, Germany). The PCR protocol was: (1) initial denaturation at 94 °C for 3 min; (2) 35 cycles of 94 °C for 35 s, 50 °C for 35 s, and 72 °C for 70 s; and (3) final extension at 72 °C for 10 min (Lee and Kim, 2008; Shin et al., 2010). Resulting sequences were compared with the reference database in GenBank. The similarity coefficients of the bacterial and archaeal communities were computed based on the Dice's index of similarity (Dice, 1945). The Shannon–-Weaver index was calculated following the Shannon index (Shannon et al., 1981; Valipour, 2016).

2.4. Statistical analysis

All the statistical analyses of the experimental data were performed using Microsoft Excel 2010 and Origin 9. The collected data values represent the average of the triplicate experimental measurements.

3. Results and discussion

3.1. Biogas production

The continuous fermentation process was divided into start-up stage and stable operation stage. Every 15 days was one fermentation cycle, as HRT was 15 days. The daily biogas production and methane production are shown in Fig. 2. During the stable operation stage, the mean daily methane productions of the second fermentation cycle were 509.00 ml/day in group A and 456.16 ml/day in group B, while it was 434.17 ml/day in group C without FVW addition. And the specific methane yields were 202.06 mL/g·VS, 174.98 mL/g·VS, and