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Study on the bio-methane yield and microbial community structure in enzyme enhanced anaerobic co-digestion of cow manure and corn straw

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HIGHLIGHTS

- Enzymatic treatment could improve anaerobic co-digestion of cow manure and corn straw.
- The direct addition of amylase increased methane yield by 110.79%.
- The cellulase pretreatment increased methane yield by 103.20%.
- The enzymes slightly affected the structure of hydrolysis bacteria community.
- Amylase, cellulase and protease had no harmful effect on microbial community.

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ABSTRACT

The use of enzymes to improve anaerobic co-digestion (AcoD) of cow manure and corn straw was explored in this study, including cellulase pretreatment and direct additions of amylase and protease. The effects of enzymes on microbial community structure were investigated though PCR-DGGE method. Results showed that AcoD with amylase achieved the highest methane yield of 377.63 ml·CH_4/g·VS, which was an increase of 110.79%. The methane increment consumed the amylase of 4.18×10^{-5} g/ml·CH_4. Enzymes mainly affected the bacteria in the hydrolysis stage rather than the bacteria in the hydrogenesis and acetogenesis stage and the archaea in the methanogenesis stage. However, the experimental results demonstrated that enzymes had no negative influence on microbial communities; the predominant microbial communities were similar. Therefore, AcoD with amylase was an effective way to improve the bio-methane yield of cow manure and corn straw.

1. Introduction

With the development of anaerobic digestion technology, the concept of anaerobic co-digestion (AcoD) has aroused wide attention. Initially proposed in the 1980s (Hills, 1980), AcoD mixes different organic wastes to regulate nutrient supply and improve the cushion capacity, aiming to enhance the methane production and avoid inhibition. Agricultural residues, animal manure, municipal solid wastes, and industrial wastes can be utilized maximally in this way (Esposito et al., 2012).

The National Development and Reform Commission has estimated that about 3.8 billion tons of livestock manure and 0.83 billion (83 million) tons of crop straw are produced annually in China (NDRC, 2014). Intensive breeding is inevitable for the development of livestock industries in China, and increasingly serious environmental pollution brought by livestock is worrisome. Crop straw burning is also a serious economical and environmental problem. AcoD of livestock manure and crop straw is an effective method for solving the problems of the livestock industry and agriculture. In AcoD publications, livestock manure (which mainly refers to cow manure and pig manure) co-digested with crop straw is one of the most common combinations (47%) (Mata-Alvarez et al., 2014). Research has shown that AcoD of carbohydrate-rich straw with nitrogen-rich manure has significant implications in maintaining an optimal C/N ratio for methane production (Mata-Alvarez et al., 2014)(Sawatdeenarunat et al., 2015).

However, in AcoD, hydrolysis is considered the rate-limiting step, in which hydrolytic enzymes can attach to the surface of the substrate and convert macromolecular organic compounds

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into small molecules for further degradation (Naran et al., 2016). Therefore, enhancement of hydrolysis via pretreatment or a catalyst is crucial for improving methane production.

As enzymes are essential for improving AcoD, supplying essential enzymes is an effective way to enhance the methane yield. Studies have shown that the use of enzymes via both pretreatment and direct addition is feasible (Krishania et al., 2013; Parawira, 2012). Furthermore, enzymes can act in the presence of various toxic, recalcitrant substrates, microorganisms, predators, and inhibitors of microbial metabolism. Enzymes can remain active under a wide range of conditions (e.g., pH, temperature, and salinity) even if these conditions change rapidly, thereby avoiding the adverse effects that occur on living biomass. With smaller size, higher solubility and mobility, enzymes have much easier access to substrates than microbes (Romero-Güiza et al., 2016).

The application of enzymes in anaerobic digestion was proposed in the early 1990s. Akao et al. (1992) reported the enhanced anaerobic digestion of citrus peels with an enzyme solution containing cellulase and pectinase. Their study resulted in a 50% increase in the amount of limit load for anaerobic digestion (Akao et al., 1992). The studies on the application of enzymes have drawn a great attention since then. The majority of previous studies focused on the cellulase (cellobiohydrolase, endoglucanase and β-glucosidase et al.), hemicellulase (*endo*-xylanase, acetyl xylan esterase and endomannanase et al.) and ligninase (laccase, manganese peroxidase and lignin peroxidase et al.) (Schroyen et al., 2014, 2015; Van Dyk and Pletschke, 2012). In recent studies, cellulase pretreatment increased the methane yield of microalgae by 8%, resulting in a methane yield of 203.0 ± 0.4 ml·CH_4/g·VS (Passos et al., 2016); cellulase and xylanase pretreatment increased the methane yield of the filamentous algae by 17% and 4% respectively, resulting in a methane yield of 133 ± 4 ml·CH_4/g·TS and 118 ± 5 ml·CH_4/g·TS (Ehimen et al., 2013); the mix of laccase and versatile peroxidase pretreatment increased the methane yield of corn straw by 8%, resulting in a methane yield of 223.6 ml·CH_4/g·VS (Schroyen et al., 2015). Protease was mainly used in the treatment of algae for the cell wall hydrolysis. Protease pretreatment raised the methane yield of *Chlorella vulgaris* to 255.6 ± 4.9 ml·CH_4/g·COD, which was an increase of 51% (Mahdy et al., 2014). Amylase pretreatment increased the methane yield of the filamentous algae to 121 ± 9 ml·CH_4/g·TS, which was an increase of 7% (Ehimen et al., 2013).

Considering that cellulose, protein and starch are the main macromolecular organic matters in the livestock manure and crop straw, the cellulase, protease and amylase may be effective in enhancing the AcoD of livestock manure and crop straw. However, the research on the application of protease and amylase in AcoD of livestock manure and crop straw is comparatively rare. This is the gap we need to fill. Furthermore, most researches paid attention to the enzyme pretreatment of substrates instead of adding the enzyme in the digester directly. The protease and amylase are active under the condition of mesophilic anaerobic digestion, but the direct additions of protease and amylase in the digesters are lack of concerns. As the enzyme treatment efficiency was still low in anaerobic digestion, researches still need to be carried out on the development of enzymatic enhancement in anaerobic digestion. This study focused on the AcoD of corn straw and cow manure. Two research methods of enzyme enhancement were analyzed. One was the pretreatment of corn straw by cellulase before mixing with cow manure. The other was the direct addition of amylase and protease in the AcoD system. Furthermore, the effects of the three different enzymes on the AcoD microbial community structure were also investigated.

2. Material and methods

2.1. Substrates

Cow manure and corn straw were used as feedstocks for co-digestion in this study. Raw materials were collected from the stubble field in Daxing District, Beijing. The initial characterizations of cow manure and corn straw were shown in Table 1. Corn straw was chopped to a length of less than 5 mm. Fresh cow manure was obtained from Sanyuan Farm, Beijing. As different substrate ratios caused by various materials and conditions were reported in previous studies (Cavinato et al., 2010; Hinken et al., 2008; Mata-Alvarez et al., 2014), an experiment on the optimal ratio of cow manure to corn straw was conducted. Finally, a cow manure to corn straw ratio of 10:3 was determined as the optimal mixture in this study (Valipour, 2012). The C/N of the co-substrates was about 25. The inoculums were obtained from the anaerobic digestion of cow manure.

2.2. Enzymes

Amylase, protease, and cellulase were used in this study. All the enzymes were in liquid form and obtained from Novozymes. Cellulase is composed of *exo*-1,4-β-D-glucanases, cellobiohydrolases, *endo*-1,4-β-D-glucanases, and beta-glucosidases. Amylase is alpha-amylase. Table 2 shows the working conditions of the enzymes. The cellulase pretreatment of substrates was conducted at 55 °C (heated in a water bath for 18 h), and the substrates used in the other sets were also utilized at 55 °C (heated in a water bath for 18 h). Amylase and protease were added into their respective digesters at the start of the experiment.

2.3. Biochemical methane potential (BMP) tests

The effects of enzymes on methane production in AcoD were analyzed through BMP tests, which were carried out in the Automatic Methane Potential Test System (AMPTS) II (Bioprocess Control AB, Sweden). The substrates were settled in serum bottles with a total volume of 600 ml. The substrate to inoculum ratio was 0.5 g·oTSs/g·oTSi. In this case, 500 g of inoculum (2.89 g·oTS/100 g), 50.0 g of cow manure (10.29 g·oTS/100 g), 2.5 g of corn straw (83.45 g·oTS/100 g), and corresponding enzyme or without enzyme were added to different bottles. The five trials were named CON1, CON2, CEL, AMY, and PRO. In CEL, cellulase was dissolved in 30 ml of distilled water and mixed with corn straw. To recognize the effect of cellulase, a control experiment (CON2) was set with the pretreatment of corn straw using 30 ml of distilled water (without enzyme) under the same condition. In CON1, AcoD without pretreatment or enzyme was set as the control trial. In all trials, TS was adjusted to 7% ± 0.5%. Details are shown in Table 3.

Subsequently, bottles were flushed with helium gas, sealed with butyl rubber stoppers, and incubated at 37 ± 0.5 °C during the fermentation period for 30 days. A blank treatment was used to quantify the amount of methane produced by the inoculums. All trials were performed in triplicate.

2.4. Analytical methods

The substrates were characterized by the concentrations of TS, VS, starch, and protein, which were measured according to Standard Methods (APHA, 2012). The cellulose concentration was determined according to Van Soest Methods (Soest et al., 1991). pH was analyzed with a Hach pH meter (HQ30d). C and N were analyzed with a Vario EI Elemental Analyzer (Germany). Methane