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Effects of dairy manure and corn stover co-digestion on anaerobic microbes and corresponding digestion performance

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HIGHLIGHTS

- Both HRT and corn stover had significant impacts on the anaerobic digestion.
- Bacteroidetes, Clostridia and methanogens fully influenced the biogas production.
- Adding corn stover did not change the chemical composition of solid digestate.
- Hydrolyzibility of solid digestate was associated to microbial composition.

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ABSTRACT

This study investigated the effects of corn stover as a supplemental feed on anaerobic digestion of dairy manure under different hydraulic retention times (HRT). The results elucidated that both HRT and corn stover supplement significantly influenced microbial community and corresponding anaerobic digestion performance. The highest biogas production of 497 mL per gram total solid loading per day was observed at a HRT of 40 days from digestion of manure supplemented with corn stover. Biogas production was closely correlated with the populations of Bacteroidetes, Clostridia and methanogens. Composition of the solid digestate (AD fiber) from the co-digestion of corn stover and dairy manure was similar to the digestion of dairy manure. However, the hydrolysis of AD fiber was significantly ($P < 0.05$) different among the different digestions. Both HRT and feed composition influenced the hydrolyzability of AD fiber via shifting the composition of microbial community.

1. Introduction

USDA has recently estimated that a total of about 120 billion dry kilograms of cattle manure are produced annually from 67,000 dairy farms and 956,500 beef cattle producers in the United States (USDA Economic Research Service, 1997; USDA National Agricultural Statistics Service, 2009). Compared to other practices of manure management, anaerobic digestion (AD) is the biological treatment process that is capable of simultaneously generating a renewable energy, biogas, from the wastes as well as alleviating the associated environmental concerns such as odor, greenhouse gas (GHG) emissions, and groundwater contamination (Speece, 1996).

To improve the system efficiency, co-digestion of manure and crop residues has been considered as one of the potential strategies to enhance biogas generation (Kavacik and Topaloglu, 2010; Molinuevo-salces et al., 2010; Nizami et al., 2009). It has been reported that the improvement in biogas production using co-digestion system is due to the better balanced nutritional composition which would support microbial growth for efficient digestion and increase buffering capacity that helps maintain the stability of the AD system (Kaparaju et al., 2008). Recent studies also demonstrated new applications of both liquid AD effluent and solid digestate (AD fiber) for biofuels production. For instances, various techniques have been developed using liquid AD effluent to culture algae as a non-edible feedstock for biorefineries (Chen et al., 2012; Wilkie and Mulbry, 2002), and the AD fiber has been demonstrated as a good feedstock for ethanol production (Teater et al., 2011; Yue et al., 2010).

Microbes as the processor in anaerobic digestion play a key role influencing the amount of gas production, and corresponding quality of effluent streams. The most common microbes in AD can be roughly assigned into three categories: the polymer degraders, the acetogens, and the methanogens (Bagi et al., 2007). The polymer degrading bacteria (such as Bacteroidetes and Clostridia) function in sensing, regulating, and degrading polymers (such as

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polysaccharides) into monomers or oligomers (Gross 2007). The acetogens consume the simple sugars produced by the polymer degraders and then generate organic acids (such as acetate), or hydrogen gas and carbon dioxide. These products are eventually utilized by methanogens to produce methane. Although many investigations have been conducted to identify microbial populations in anaerobic digestion (Bonin and Boone, 2006; Bryant and Boone, 1987; Kendall and Boone, 2006; McHugh et al., 2003; Nettmann et al., 2008; Sekiguchi et al., 2001), detail relationships among microbial populations, digestion performance and effluent quality has not yet been well established.

This study investigated influences of hydraulic retention time (HRT) and feedstock composition on microbial community in the digester and the quality of AD fiber as a biorefining feedstock. A co-digestion process of dairy manure and corn stover in a lab-scale continuous stirred-tank reactor (CSTR) at different HRTs was compared with the digestion of dairy manure. The AD fiber obtained at each HRT was consequently pretreated and hydrolyzed in the enzymatic process. The quality of such AD fiber was evaluated based on the glucose production and conversion efficiency. The predominated microbial community for the digestion was quantified using 454 pyrosequencing, clone libraries, and 16S rRNA analysis. The effects of corn stover addition and HRT on the evolution of the microbial community and AD fiber quality were explored.

2. Methods

2.1. Feedings for anaerobic digesters

Fresh dairy manure was collected from Michigan State University dairy farm (located at 42.698207, −84.485729). The animal feed of the dairy farm was alfalfa and corn silage blended based on the standard Total Mixed Rations (TMRs) for dairy cattle (National Research Council, 2001). The corn stover of Yellow Dent Corn (*Zea mays indentata*) was collected from a private farm in Muir, Michigan (located at 43.005965, −84.975343) in October, 2009. The corn stover was air-dried and ground using a grinder (U.S. Machineries HMCS500) equipped with a sieve of 2 mm diameter circular openings at the Crop and Soil Science Teaching and Research Field Facility of Michigan State University. The characteristics of both dairy manure and corn stover, including total solids (TS), neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicelluloses, lignin, and alkalinity were listed in Table 1.

The feeding for manure (M) digesters contained 5% of dry matter (DM) and 95% of moisture. The feeding for manure and corn stover (M+CS) digesters was prepared by mixing dairy manure and corn stover in the ratio of 4:1 (wt), and diluted to 5% DM using water. Both feeds were homogenized using a blender prior to feeding.

2.2. Anaerobic digestion

Ten-liter fermenters (Bioflo, New Brunswick, Co.) were used to carry on the digestion. Both M and M+CS digestions were operated under anaerobic condition at 35 °C. A completely randomized design (CRD) with two feedstocks (M and M+CS) and three hydraulic retention times (HRT) (30, 40, and 50 days) was used to study the effects of co-digestion on anaerobic digestion performance. The digesters were fed and discharged manually every day. The amount of feeding was based on different HRTs. Biogas production of each digester was monitored using an inverted tipping bucket gas meter. The AD effluent from each digestion was separated into solid digestate (AD fiber) and liquid effluent using a filtration through 8-layer cheese cloth. The hydrolysis of the AD fiber was evaluated by a combination of alkali pretreatment and enzymatic hydrolysis. A small amount of digestion effluent from each digestion was also stored at −20 °C for microbial community analysis.

Biogas productivity was calculated by daily gas production (mL) divided by dairy organic loading (g TS), and cellulose and hemicellulose reduction were calculated by cellulose and hemicellulose consumed during the digestion divided by the cellulose and hemicellulose in the feed, respectively.

Table 1
Characteristic of dairy manure and corn stover.[a]

	Dairy manure	Corn stover
TS (%)	10.7 ± 0.7	92.1 ± 0.1
Cellulose (% TS)	26.6 ± 1.5	39.7 ± 1.0
Hemicellulose (% TS)	18.8 ± 1.9	29.9 ± 3.4
Lignin (% TS)	14.2 ± 0.5	8.9 ± 1.2
Alkalinity (mg $CaCO_3$ g^{-1} TS)	93.5 ± 7.8	3.0 ± 0.1

[a] Data are the average of two replicates with standard errors.

2.3. Alkali pretreatment

The AD fiber obtained from previous step was pretreated using 0.5% (wt) sodium hydroxide solution at 130 °C for a reaction time of 2 h. AD fiber in the pretreatment was fixed at 6% DM. Pretreated mixture solutions were neutralized to pH 4–5 using 20% (wt) sulfuric acid solution, then filtered by an 8-layer cheese cloth and rinsed using de-ionized water. Wet pretreated AD fiber was used to carry on enzymatic hydrolysis of sugar conversion. A small amount of the pretreated AD fiber was analyzed for dry matter and fiber contents. All analyses were duplicated and the average data with standard error are reported.

2.4. Enzymatic hydrolysis

Wet pretreated AD fiber (2 g DM) and 50 mM citrate buffer solution (pH = 5.0) were mixed to a total mass of 40 g, which makes the solid concentration of 5% DM. All mixed samples were autoclaved and cooled to room temperature. Cellulase enzyme complex (ACCELLERASE™ 1500, Genencor, Rochester, NY, USA) was then added into the mixed sample at a loading of 26 FPU g^{-1} DM. The flasks were placed on an incubated shaker rotating at 150 rpm under the reaction temperature of 50 °C. After 72 h of hydrolysis, the reaction solutions were immediately placed in a boiling water bath for 5 min to deactivate the enzyme and filtered using Millex-GS 0.22 μm membrane for analysis of monosaccharides in the hydrolysate using high-performance liquid chromatography (HPLC).

The carbohydrate conversion from AD fiber after pretreatment and enzymatic hydrolysis was used to compare the effects of anaerobic digestion on AD fiber quality. The calculation for the cellulose conversion [%] is: cellulose conversion [%] = ((substrate dry matter after pretreatment [g] × glucose concentration after enzymatic hydrolysis [g L^{-1}] × volume of enzymatic hydrolysate [L])/(substrate dry matter before pretreatment [g] × 50 [g] × cellulose content in AD fiber [%] × 1.11)) × 100%. The calculation for the xylan conversion [%] is: xylan conversion [%] = ((substrate dry matter after pretreatment [g] × xylose concentration after enzymatic hydrolysis [g L^{-1}] × volume of enzymatic hydrolysate [L])/(substrate dry matter before pretreatment [g] × 50 [g] × hemicellulose content in AD fiber [%] × 1.05)) × 100%.

2.5. Analytical methods

Fiber compositions of both digestion effluent (before liquid/solid separation) and AD fiber were determined by Van Soest Fiber Analysis System (Goering and Van Soest, 1970). NDF, ADF and