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Effects of microbial and non-microbial factors of liquid anaerobic digestion effluent as inoculum on solid-state anaerobic digestion of corn stover

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HIGHLIGHTS

- Solid state anaerobic digestion (SS-AD) using liquid AD effluent as inoculum.
- Effect of microbial and non-microbial factors of effluent on performance of SS-AD.
- Non-microbial factor of effluent was more influential on methane yield of SS-AD.
- Both bacterial and archaeal communities underwent considerable successions.

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ABSTRACT

The microbial activity of the inoculum (liquid anaerobic digestion effluent) was altered by autoclaving part of the effluent to study the effect of feedstock to active effluent ratio (F/Ea, 2.2–6.6) and the feedstock to total effluent ratio (F/Et, 2.2 and 4.4) on reactor performance in solid state anaerobic digestion (SS-AD) of corn stover. When the F/Ea ratio was increased from 2.2 to 6.6, methane yield was not significantly reduced; however, reactors became acidified when the F/Et ratio was increased from 2.2 to 4.4. It was concluded that F/Et had a greater effect on methane yields than F/Ea for the range studied in this paper. As analyzed by denaturing gradient gel electrophoresis using PCR amplified 16S rRNA genes, the microbial community underwent dynamic shifts under acidified conditions over 38 days of SS-AD. These shifts reflected the acclimation, both adaptive selection and diversification, of the initial inoculated microbial consortia.

1. Introduction

According to the "3Rs" (reduce, reuse and recycle) hierarchy, the aim of waste management is to generate the minimum amount of waste, while extracting the maximum uses and benefits from it (EPA, 2013). Anaerobic digestion (AD) is one of the most attractive waste treatment technologies for organic wastes as it both stabilizes it and produces energy (biogas) (Karthikeyan and Visvanathan, 2013). Although the dominant type of AD facilities are those fed with liquid waste streams, the need for treating solid materials, including municipal and agricultural wastes, has fostered the development of solid state AD (SS-AD) systems that operate at more than 20% solids (Li et al., 2011a). Over the past few decades, SS-AD systems have been used to treat the organic fraction of municipal solid waste (OFMSW), diverting it from landfills (Fantozzi and Buratti, 2011), and has recently gained attention due to its potential application to process lignocellulosic biomass for energy production (Li et al., 2011a).

More recent studies demonstrated that inoculating SS-AD with effluent from liquid AD (L-AD) systems can successfully initiate biogas production from a variety of lignocellulosic biomass wastes (Li et al., 2011a; Liew et al., 2011; Zhu et al., 2010). L-AD effluent can provide sufficient microbes, moisture, micronutrients and nitrogen for the SS-AD process (Li et al., 2011b; Wang et al., 2013). However, SS-AD of lignocellulosic biomass faces challenges such as slow acclimation of inoculum to lignocellulosic feedstocks and acidification of the reactor at high organic loading (Xu et al., 2013). A few key factors have been identified that affect SS-AD

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performance: inoculum size, carbon/nitrogen (C/N) ratio, type of feedstocks, organic loading, operating temperature, and total solids (TS) content (Karthikeyan and Visvanathan, 2013; Motte et al., 2013; Shi et al., 2013). Recent studies showed that microbial activities and the chemical composition of the AD effluent/digestate as inoculum can greatly affect SS-AD performance, especially during start-up of SS-AD of lignocellulosic biomass (Griffin et al., 1998; Ma et al., 2013; Motte et al., 2013; Xu et al., 2013). Furthermore, reactors fed with cellulosic biomass sometimes become acidified ("sour") due to the accumulation of volatile fatty acids (VFAs) as a result of imbalanced C/N ratios and inhibited microbial activities. However, the operating parameters and microbial community structures that relate to the "sour" phenomena during SS-AD are not well understood.

It has been shown that decreasing the feedstock-to-inoculum ratio can effectively reduce startup time and increase methane yield in SS-AD (Forster-Carneiro et al., 2007; Motte et al., 2013). The positive effects of a larger inoculation size on SS-AD are believed to be a combined influence from increased microbial populations (especially methanogens), higher buffering capacity, and in some cases balanced C/N ratios (Griffin et al., 1998; Xu et al., 2013). The effect of microbial factors of L-AD effluent (inoculum) on performance and microbial communities of the SS-AD process have been reported recently (Motte et al., 2013; Shi et al., 2013); however, the non-microbial factors of the liquid AD effluent the performance of SS-AD has not been investigated.

Microbial community dynamics can provide valuable information for understanding the effect of operating conditions on AD system performance. Culture independent molecular analysis of the microbial communities in environmental samples is the preferred methodology for the investigation of AD systems. These methods are generally based on analysis of certain well characterized marker genes, the most common of which is the ribosomal RNA (rRNA) gene. Acclimation of archaeal and bacterial communities under various AD conditions and with different feedstocks has been studied in L-AD systems (Lee et al., 2010). The effect of temperature variations (ranging from mesophilic to thermophilic) and temperature shocks on microbial community structures in L-AD systems were also studied (Gao et al., 2011). However, limited information can be found in the literature for microbial dynamics in SS-AD systems, especially for those fed with lignocellulosic biomass as the major feedstock (Shi et al., 2013). In this study, the effects of inoculation on reactor performance, such as methane yield, pH, VFA production, and reduction of TS, volatile solids (VS, wet based in this paper), cellulose, and hemicelluloses, were studied by controlling the feedstock to active effluent ratio (F/Ea) and feedstock to total effluent ratio (F/Et). The effect of F/Et and F/Ea on the successions of the initial microbial community during SS-AD of corn stover was also studied. The shift of archaeal and bacterial communities in both "healthy" and "acidified" SS-AD reactors were investigated using denaturing gradient gel electrophoresis (DGGE) analysis of the archaeal and bacterial communities following PCR amplification of 16S rRNA.

2. Methods

2.1. Feedstock and effluent

Corn stover was collected from a farm operated by the Ohio Agriculture Research and Development Center (OARDC) in Wooster, OH, USA in October 2009. Upon receipt, corn stover was air dried to a moisture content of less than 10% and then ground to pass a 9 mm sieve (Mighty Mac, MacKissic Inc., Parker Ford, PA, USA). The TS, VS, and C/N ratio of the corn stover were 91.8 ± 0.0%, 88.1 ± 0.2%, and 79.7 ± 3.7%, respectively. On a dry matter basis, the corn stover contained 18.6 ± 0.6% lignin, 38.0 ± 0.4% cellulose, and 17.2 ± 0.3% hemi-celluloses. AD effluent was obtained from a mesophilic liquid anaerobic digester fed with municipal sewage sludge and food wastes (operated by *quasar energy group*, Cleveland, OH, USA). Effluent was dewatered by centrifugation to increase its TS content from 6.3% to 9.6%, and was acclimated at 36 ± 1 °C for 1 week prior to inoculation. The characteristics of the concentrated effluent were TS of 9.6 ± 0.1%; VS of 5.5 ± 0.1%; C/N ratio of 6.6 ± 0.3; pH of 8.3 ± 0.1; alkalinity of 14.5 ± 1.2 g $CaCO_3$/kg; total VFA of 3.6 ± 0.6 g/kg; and total ammonia nitrogen of 3.8 ± 0.3 g N/kg.

2.2. Solid-state anaerobic digestion

SS-AD reactors (1 L) were loaded with a mixture of corn stover and L-AD effluent (inoculum). The experimental design and related initial parameters are shown in Table 1. Three F/Ea ratios (2.2, 4.4, and 6.6) and two F/Et ratios (2.2 and 4.4) were selected based on the range of typical F/Et ratios for SS-AD of corn stover in literature (Li et al., 2011b; Xu et al., 2013). In one set of reactors, the F/Et ratio was kept at 2.2, and the F/Ea ratio was adjusted to 2.2, 4.4, and 6.6 by replacing a portion of the microbial active effluent with autoclaved (at 121 °C for 60 min) effluent. As a result, these reactors had similar non-microbial properties such as initial C/N ratio and alkalinity, but proportionally decreased microbial populations. The other set of reactors, which were run at an F/Et ratio of 4.4, did not receive autoclaved L-AD effluent, thus the F/Ea ratio was also 4.4. Reactors with the same F/Ea ratio of 4.4 but different F/Et ratios (4.4 and 2.2) had the same microbial populations per feedstock VS, but different non-microbial properties, such as pH, alkalinity, C/N ratio, and micronutrients. Water was added if necessary to obtain a TS content of 20% for all reactors.

After loading with well mixed feedstock and L-AD effluent, each reactor was sealed by a rubber stopper and connected to a 5-L gas bag (CEL Scientific Tedlar gas bag, Santa Fe Springs, CA, USA) for biogas collection. The reactor was incubated at 36 ± 1 °C for 38 days. Every 2–3 days, biogas collected in the gas bag was measured for composition and volume. At predetermined times (day 0, 2, 4, 6, 8, 10, 12, and 38), two of the reactors were terminated as two replicates and all the contents were taken out of the reactor, mixed thoroughly by a hand-held mixer, and sampled for chemical and microbial analyses. All tests were conducted in duplicates.

2.3. Analytical methods

Samples of feedstock, effluent, and digested material collected as described in Section 2.2 were analyzed as described below. TS and VS contents were analyzed according to the Standard Methods for the Examination of Water and Wastewater (American Public Health Association., 2005). Total carbon and nitrogen contents were determined by an elemental analyzer (Vario Max CNS, Elementar Americas, Mt. Laurel, NJ, USA) and were used to calculate the C/N ratio. For the pH and VFA determination, 10 g solid sample was mixed with 15 mL deionized water and homogenized by a vortex mixer for 20 s, then centrifuged at 10,000 rpm for 10 min by a Sorvall Legend T Plus Centrifuge (Thermo Scientific, Waltham, MA, USA). Alkalinity was measured following a titration procedure (McGhee, 1968) using an auto-titrator (Mettler Toledo, DL22 Food & Beverage Analyzer, Columbus, OH, USA). Samples for titration were prepared by diluting five grams of digestate with 50 mL of deionized water, mixing for 2 min, then filtrated with four layers of cheese cloth. The pH of the supernatant was measured and then adjusted to a pH of 3–4 with 2 M HCl for analysis of VFAs, including acetic, propionic, butyric, iso-butyric, and valeric acid, using a Shimadzu GC-2010 Plus (Shimadzu, Columbia, MD, USA). The GC was equipped with a 25 m × 0.32 mm × 0.5 μm Stabiwax-DA column