Bioresource Technology 144 (2013) 281–287

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effects of total ammonia nitrogen concentration on solid-state anaerobic digestion of corn stover

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HIGHLIGHTS

- Solid-state anaerobic digestion (SS-AD) of corn stover.
- Effect of total ammonia nitrogen (TAN) concentration on SS-AD of corn stover.
- Effluent of liquid anaerobic digester provides enough nitrogen for SS-AD.
- Methane yield decreased with the supplementation of TAN.
- Reduced microbial activities for hydrolysis and methanogenesis with increased TAN.

ARTICLE INFO

Article history:
Received 23 April 2013
Received in revised form 25 June 2013
Accepted 26 June 2013
Available online 2 July 2013

Keywords:
Solid-state anaerobic digestion
Biogas
Methane
Corn stover
Ammonia inhibition

ABSTRACT

The inhibitive effect of total ammonia nitrogen (TAN) (including NH_3 and NH_4^+) on solid-state anaerobic digestion of corn stover was investigated in batch reactors at 37 °C. The highest methane yield of 107.0 L/kg VS_{feed} was obtained at a TAN concentration of 2.5 g/kg (based on total weight). TAN concentrations greater than 2.5 g/kg resulted in decreased methane yields, with a 50% reduction observed at a concentration of 6.0 g/kg. Reduced reaction rates and microbial activities for hydrolysis of cellulose and methanogenesis from acetate were observed at TAN concentrations higher than 4.3 g/kg. Strong ammonia stress was indicated at butyrate concentrations higher than 300 mg/kg. Result showed that the effluent of liquid anaerobic digestion can provide enough nitrogen for solid-state anaerobic digestion of corn stover.

1. Introduction

Solid-state anaerobic digestion (SS-AD) has been extensively studied in recent years in the US as a method to convert various high-solid organic wastes to biogas (about 40–70% CH_4 and 30–60% CO_2) and produce renewable energy (De Baere et al., 2010; Yabu et al., 2011). Compared with traditional liquid-AD (L-AD), SS-AD operates at 15–40% total solids (TS) and has much higher volumetric methane productivity (Brown et al., 2012; Duan et al., 2012). An important potential feedstock for SS-AD is lignocellulosic biomass (e.g., crop residues, energy crops, and forestry residues), which is expected to be a major source for bioenergy production, due to its large-scale availability, low cost, low water content, and various environmental benefits such as reducing greenhouse gas emissions (US Department of Energy, 2011).

Nitrogen supplementation, in the form of organic nitrogen or ammonia nitrogen, is essential for the SS-AD of lignocellulosic biomass, which is typically rich in carbon and low in nitrogen (Giuliano et al., 2013). Deficiencies in ammonia nitrogen (0.5 g/L) have been shown to cause low methane yields due to low microbial activity and buffering capacity (Prochazka et al., 2012), while excessive ammonia nitrogen may inhibit biogas production (Chen et al., 2008; Kayhanian, 1999). A common method for nitrogen supplementation of lignocellulosic biomass is to add nitrogen-rich co-substrates, such as manure, sewage sludge, food waste, and other protein-rich materials (Brown and Li, 2013; Giuliano et al., 2013). Another method is to use the waste effluent from L-AD as a nitrogen source and microbial-rich inoculum, which has been previously studied by our group (Li et al., 2011; Shi et al., 2013; Xu et al., 2013). However, studies on the efficiency of using L-AD effluent as a nitrogen supplement to lignocellulosic biomass are

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http://dx.doi.org/10.1016/j.biortech.2013.06.106

limited. Thus, it is important to investigate the inhibitory effects of ammonia nitrogen supplementation for SS-AD of lignocellulosic biomass.

Currently, most of the studies on ammonia inhibition have been conducted for traditional L-AD. The optimal TAN concentration in mesophilic L-AD was reported to be about 1–3 g/L (Prochazka et al., 2012). Ammonia inhibition is more likely to happen in SS-AD, due to the higher organic loading and lower water content which affects dilution. However, only limited information can be found on the inhibitive TAN levels in SS-AD, especially for lignocellulosic biomass at high TS concentrations. Ammonia inhibition was reported in a few studies on the digestion of chicken manure at TS content of about 20% (Abouelenien et al., 2009; Bujoczek et al., 2000). However, due to the differences in systems and feedstocks, the results obtained from L-AD and other SS-AD systems cannot be directly used to determine optimal nitrogen supplementation for SS-AD of lignocellulosic biomass.

Thus, the purpose of this study was to evaluate the effect of nitrogen supplementation on the SS-AD of lignocellulosic biomass. In addition, a systematic investigation was conducted on the influence of excessive ammonia on critical steps of SS-AD, including hydrolysis and methanogenesis.

2. Methods

2.1. Feedstock and inoculum

Corn stover was collected from a farm operated by the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH, USA (40°48′33″N, 81°56′14″W) in October, 2009. Upon receipt, the corn stover was 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). Effluent from a mesophilic liquid anaerobic digester fed with municipal sewage sludge (operated by *quasar energy group*, Cleveland, OH, USA) was used as inoculum. The TS, volatile solids (VS) and carbon-to-nitrogen (C/N) ratio of corn stover (wet basis) were 92.5%, 90.8%, and 68.3 respectively. The TS, VS, C/N ratio, and ammonia concentration of the effluent were 8.6%, 3.5%, 7.0, and 3.7 g/kg, respectively.

2.2. Solid-state anaerobic digestion

A series of SS-AD reactors (1 L) were loaded with a mixture of corn stover and L-AD effluent, at a feedstock-to-effluent (F/E) ratio of 3.4 (based on VS) and TS content of 18.3%. The initial TAN concentration of reactors was 2.5 g/kg. To obtain the designed TAN concentrations of 4.3 and 6.0 g/kg, urea was added during the mixing process to represent organic nitrogen supplementation. Eighteen identical reactors were prepared for each TAN concentration. At predetermined times (day 2, 4, 6, 8, 10, 12, 20, 30, and 35), two reactors were terminated and all digested material was taken out, mixed thoroughly by a hand-held blender, and sampled for composition and microbial analyses. Biogas samples were collected every 2–3 days from the two reactors that were terminated at day 35. Each reactor was sealed with a rubber stopper with a gas outlet, and placed in a 37 ± 1 °C walk-in incubator. A 5-L gas bag (CEL Scientific Tedlar gas bag, Santa Fe Springs, CA, USA) was attached to the gas outlet of the reactor to collect biogas for all reactors. Composition and volume of the biogas were measured every 2–3 days.

2.3. Analytical methods

The volume of biogas collected in the Tedlar bags was measured by a drum-type gas meter (Ritter, TG 5, Germany) and the composition of the biogas (CO_2, CH_4, N_2, and O_2) was analyzed by a gas chromatograph (GC) (Agilent Technologies, HP 6890, Wilmington, DE, USA) equipped with a 30 m × 0.53 mm × 10 μm alumina/KCl deactivation column and a Thermal Conductivity Detector (TCD) using helium as carrier gas at a flow rate of 5.2 mL/min. The temperatures of the injector and detector were set at 150 and 200 °C, respectively.

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. TAN was determined by a modified distillation and titration method (ISO 5564, 1984). Distillation and titration were performed using a B-324 distillation unit (Büchi Labortechnik AG, Flawil, Switzerland) combined with a DL 22 titrator (Mettler-Toledo Inc., Columbus, OH, USA) filled with 0.01 M hydrochloric acid. Fifty milliliters of 33% NaOH (w/v) was added to the sample to convert ammonium to ammonia. In the titration process, 4% boric acid (w/v) with pH adjusted to 4.65 was used as the receiving solution. Fifteen grams of solid sample were mixed with 15 mL of deionized water and homogenized by a Mini Fixed Speed Vortex Mixer (Fisher Scientific, Pittsburg, PA, USA) for 20 s, then centrifuged at 10,000 rpm for 10 min in a Sorvall Legend T Plus Centrifuge (Thermo Scientific, Waltham, MA, USA). The pH of the supernatant was measured and then adjusted to a pH of 3–4 with 2 M HCl to convert the ionized volatile fatty acid (VFA) salts to acid molecules for VFA analysis, including acetic, propionic, butyric, iso-butyric, and valeric acids 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 (Restek, Bellefonte, PA, USA) and flame ionization detector (FID). Helium was used as carrier gas at a flow rate of 10.8 mL/min. The temperatures of the column and detector were 150 and 250 °C, respectively. Cellulose and xylan contents were determined by a two-step acid hydrolysis method in accordance with the NREL Laboratory Analytical Procedure (Sluiter et al., 2010). Monomeric sugars (glucose, xylose, galactose, arabinose and mannose) and cellobiose were measured by HPLC (Shimadzu LC-20AB, MD, USA) equipped with a Biorad Aminex HPX-87P column and a refractive index detector (RID). Deionized water was used as the mobile phase at a flow rate of 0.6 ml/min. The temperatures of the column and detector were maintained at 80 and 55 °C, respectively.

2.4. Microbial analysis

Populations of microbial functional groups in SS-AD were quantified by the most-probable-number (MPN) method, since PCR based methods usually target on individual species or total bacteria and archaea, which are either too specific or too general. In addition, both the MPN method and the PCR-based DGGE analysis have been shown to be comparable in reflecting the dynamic change of microbes in the SS-AD process (Shi et al., 2013). The key functional groups in this research included cellulolytic microbes (CM), xylanolytic microbes (XM), and acetotrophic methanogens (AM) since in SS-AD, hydrolysis and methanogenesis are commonly believed to be the two major rate limiting steps, and methane produced from acetate accounts for 70% of the total methane production (White, 2000). The medium, developed by Champion et al. (1988), was prepared under anaerobic conditions and was used to dilute samples taken from SS-AD reactors. The medium contains (in 1 L): 2.0 g trypticase, 1.0 g yeast extract; 25 mL mineral solution #1 (8.92 g/L K_2HPO_4); 25 mL mineral solution #2 (9.6 g/L KH_2PO_4, 19.2 g/L $(NH_4)_2SO_4$, 19.6 g/L NaCl and