Chinese Journal of Chemical Engineering 23 (2015) 847–852

Contents lists available at ScienceDirect

Chinese Journal of Chemical Engineering

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

Energy, Resources and Environmental Technology

Biogas by two-stage microbial anaerobic and semi-continuous digestion of Chinese cabbage waste☆

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ARTICLE INFO

Article history:
Received 3 September 2014
Received in revised form 24 December 2014
Accepted 14 January 2015
Available online 18 March 2015

Keywords:
Anaerobic digestion
Semi-continuous
Two-stage
Biogas
Chinese cabbage waste
Microbial ecology

ABSTRACT

Anaerobic digestion of Chinese cabbage waste was investigated through a pilot-scale two-stage digester at a mesophilic temperature of 37 °C. In the acidification digester, the main product was acetic acid, with the maximum concentration of 4289 mg·L^{-1} on the fourth day, accounting for 50.32% of total volatile fatty acids. The oxidation reduction potential (ORP) and NH_4^+-N level decreased gradually with hydraulic retention time (HRT) of acidification. In the second digestion phase, the maximum methanogenic bacterial concentration reached 9.6×10^{10} ml^{-1} at the organic loading rate (OLR) of 3.5–4 kg VS·m^{-3}, with corresponding HRT of 12–16 days. Accordingly, the optimal biogas production was 0.62 m^3·(kg VS)$^{-1}$, with methane content of 65%–68%. ORP and NH_4^+-N levels in the methanizer remained between −500 and −560 mV and 2000–4500 mg·L^{-1}, respectively. *Methanococcus* and *Methanosarcina* served as the main methanogens in the anaerobic digester.

1. Introduction

Chinese cabbage, a staple vegetable of Northern China in winter, has a giant annual production of 500 million tons in Liaoning Province [1]. Due to excess production, low market price or other reasons, large amounts of cabbage are discarded, causing economic loss and environmental problems. Biogas produced by biomass fermentation is regarded as a substitute for natural gas and anaerobic digestion of this biodegradable waste will provide a solution to recycle value-added products [2,3]. Anaerobic digester (AD) is very promising due to low cost and ease of operation in absence of light and inorganic electron acceptors, such as oxygen [4]. Two-stage AD has more applications in biogas productivity and organic matter removal compared with conventional one-phase digesters. Because the phase separation in two-stage AD provides the optimal conditions for acidogens and methanogens in turn, acidifying and methanizing organisms in their separate environs are assured. The production and quality of biogas can be substantially improved. By means of sufficient acidification-phase regulation, an efficient methane-producing effect has been realized, a key technology for improving overall efficiency of two-stage anaerobic digestion [5–8]. One of our subjects, *Methanosarcina*, belongs to archaea, which is difficult to isolate and culture. Compared to the traditional method, molecular biology provides method to determine the composition of microbial community during AD processes. 16S rDNA library-based analyses provide an accurate overview of the diversity within microbial biocoenoses because of greater sequence divergences between different species at 16S rDNA level [9]. Real-time polymerase chain reaction (RT-PCR) and 454 pyrosequencing are usually used in the detection of microbial diversity, but 454 pyrosequencing is especially expensive. Next-generation sequencing has revolutionized genome sciences [10]. We have performed our sequencing by improving the method using MiSeq sequencer, designated here as quantitative MiSeq (qMiSeq), for accurate quantification of libraries on large-scale sequencing projects, based on the Illumina platform with the advantages of speed and low cost [11]. However, little work has been reported on application of MiSeq on mtGenome analysis. Several reports have provided quality metrics supporting the strength of Illumina MiSeq as a candidate for mtDNA analysis [12].

In this study, a pilot-scale two-stage AD is adopted in a semi-continuous feeding mode to treat Chinese cabbage waste (CCW) and biogas production is continuous. We present an investigation on optimal organic loading rate, biogas production and methane content. We will analyze methanizing archaea and general bacteria during co-production by novel molecular method (miseq) to investigate predominant methanogens and related functional bacteria.

☆ Supported by the National Science-Technology Support Plan of China (2014BAD02B04).
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http://dx.doi.org/10.1016/j.cjche.2015.03.001
1004-9541/

2. Materials and Methods

2.1. Anaerobic digestion reactor

To investigate the two-stage anaerobic digestion for CCW, a 10 L acidification digester and a 100 L methanization digester were used, as shown in Fig. 1. Both digesters was heated to (37 ± 1)°C in a water bath system (24 h). A screw pump fed 100 $L \cdot h^{-1}$ of the material from the acidification digester once a day to the methanization digester, and then the same volume of effluents was discharged to the return tank. 3–5 L of effluents in return tank was used for reflux to adjust pH in the methanizer after periodic checks. A wet gas flow meter was used to measure the daily biogas production.

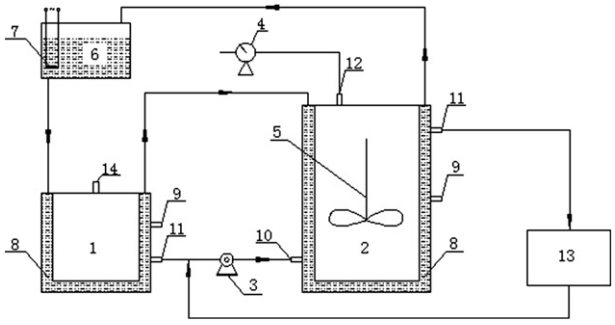

Fig. 1. Semi-continuous two-stage digester. 1 — acidifier; 2 — methanizer; 3 — screw pump; 4 — gas flow meter; 5 — agitator; 6 — water bath; 7 — heating rod; 8 — interlayer; 9 — sampling mouth; 10 — feed port; 11 — overflow port; 12 — gas outlet port; 13 — return tank; 14 — outlet.

2.2. Substrate composition

The composition of substrates is listed in Table 1.

Table 1
Average chemical composition of raw materials

Parameters	Units	CCW	Inocula	
			Pig manure	Sludge water
Total solids (TS)	%	7.44	23.14	45.23
Volatile solids (VS)	%	95.26	74.23	33.56
Total carbon	%	15.17	48.13	20.14
Total nitrogen	%	1.64	2.58	1.23
C/N	–	9.25:1	18.6:1	16.3:1

2.3. Experimental procedures

CCW was collected from vegetable market and shredded by machine. To investigate the optimal acidification parameters, the slurry of CCW (0.2–0.7 kg $VS \cdot d^{-1}$) was introduced into the first digester for acidification. With 40% (by volume) fresh pig manure and 20% (by volume) sludge water, the methanogenesis phase was inculcated, left about one month to culture methanogens. When the concentration of methanogens was stable, the effluent from acidifiers was fed to the second digester daily at the feed rate of 3–11 kg CCW for more than two months. The feed was increased at a constant rate and each feed rate was employed for one week.

2.4. Chemical analysis

The liquid samples of total volatile fatty acids (VFAs) in the acidification and methane phase were centrifuged at 10000 $r \cdot min^{-1}$ for 10 min, then filtered through a 0.45 μm membrane, and finally assessed using a capillary DB-WAX column (30 m × 0.53 mm × 1 μm) in a gas chromatograph (GC-7820A) with nitrogen as the carrier gas. Temperatures of injector and detector were 250 °C. The temperature of oven was 100–220 °C, heating at a rate of 15 $°C \cdot min^{-1}$. Sample volumes of 0.4 μl were injected *via* the detector (TCD: 250 °C). An oven was used to dry TS and VS and K_2Cr_2O method was applied to soluble chemical oxygen demand (SCOD) [13]. The biogas production was tracked with a wet gas meter. Using a PHS-25 meter (Shanghai Leichi Instrumentation Factory), the pH value and ORP were measured. Ammonia nitrogen (NH_4^+-N) level was determined by pay reagent luminosity law [13] and methanogen concentration was determined by fluoroscope TYU-30C (Shanghai Yuguang Factory).

2.5. Microbial community analysis

2.5.1. DNA extraction

When the system was operated under steady conditions, the anaerobic digested sludge in the methanizer was sampled. After extracting total bacterial DNA from the sample by DNA isolation kit (Takara, Dalian, China), a PCR amplifier (Flexcycler, German) was applied according to the conditions described by Jonathan *et al.* [11] and the product was stored at −20 °C.

2.5.2. High-throughput sequencing

A library was constructed on the combined V4 region of 16S rDNA district based on Illumina Miseq technology. For the archaea, primer pairs 5′-CAGYMGCCRCGGKAAHACC-3′ and 3′-GGACTACNSGGGTMTC TAAT-5′ were used. For the bacteria, primer pairs 5′-GTGCCAGCMGC CGCGGTAA-3′ and 3′-GGACTACHVGGGTWTCTAAT-5′ were employed. The design of primers and sequencing were provided by Novogene Co., Ltd. in Beijing.

3. Results and Discussion

3.1. Acidification

The four VFAs (acetic, propionic, isobutyric and butyric acid) increased with acidification HRT to 8058 $mg \cdot L^{-1}$ in the first five days [Fig. 2(a)]. Acetic acid (HAc) reached 4289 $mg \cdot L^{-1}$ on day 4, accounting for 53.2% of total VFAs. Isobutyric (HIBu) and butyric (HBu) acid were high, reaching 1894 and 2015 $mg \cdot L^{-1}$ on days 5 and 6, respectively. These three VFAs composed the main substrate for biogas production. Unlike other studies, the production of HIBu in this experiment was relatively high, possibly due to the properties of cabbage. The specific metabolic mechanism of HIBu, however, needs further study. Accumulated at a certain concentration, HPa is usually an inhibitory substrate, with highest content at 721 $mg \cdot L^{-1}$ on the 7th day. All VFAs were degraded by microbes to HAc before converting to CH_4, with their conversion sequence being HAc > HEt > HBu > HPa [14]. Other VFAs, valeric and caproic acids, were also detected, accounting for 2%–4% and 7%–9% of total volatile fatty acids (tVFAs), respectively. The tVFAs in the acidifiers attained a concentration of 9750 $mg \cdot L^{-1}$. Dinsdale *et al.* performed acidification tests with fruit and vegetable waste and achieved tVFAs of 6100 $mg \cdot L^{-1}$ [15]. Siegert and Banks found that VFA concentrations above 2000 $mg \cdot L^{-1}$ inhibited cellulose degradation, while those above 4000 $mg \cdot L^{-1}$ feebly inhibited glucose degradation [16]. Most VFAs remain in the solution, while a small amount of VFAs convert to gases such as H_2, H_2S, CH_4, and escape from the reactor [17,18]. VFAs produced in the separate acidification digester have the advantage in obtaining a high yield while avoiding the inhibition of methanogen growth. The initial pH of acidifier is 5.65, decreases to a low point of 3.72 on day 4, and increases after the production of VFAs reduces. Acidifying bacteria exhibit some tolerance of pH variation and the limit for acidifying bacterial enzyme activity is pH 5.0–6.0 [19]. The pH value between 5 and 7 is beneficial for the hydrolysis of particulate organic matter. ORP is used to indicate oxygen content in anaerobic environment.