Waste Management 81 (2018) 117–127

Contents lists available at ScienceDirect

Waste Management

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

Effect of inoculum and substrate/inoculum ratio on the performance and methanogenic archaeal community structure in solid state anaerobic co-digestion of tomato residues with dairy manure and corn stover

Yangyang Li [a,1], Yanqin Wang [a,b,1], Zihan Yu [a], Jiaxin Lu [a], Danyang Li [a], Guoying Wang [a], Yu Li [a], Yue Wu [c], Shuyan Li [a], Fuqing Xu [d], Guoxue Li [a], Xiaoyan Gong [a,*]

[a] Beijing Key Laboratory of Farmland Soil Pollution Prevention and Remediation, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China
[b] Institute of Agricultural Resource and Environment, Shandong Academy of Agricultural Sciences, Jinan 250100, China
[c] Department of Mechanical Engineering, Marquette University, 53233, USA
[d] Department of Food, Agricultural, and Biological Engineering, The Ohio State University, 44691, USA

ARTICLE INFO

Article history:
Received 27 March 2018
Revised 14 August 2018
Accepted 26 September 2018

Keywords:
Solid state anaerobic digestion
Inoculum
Substrate/inoculum ratio
Microbial community structure
On farm organic waste

ABSTRACT

Effects of methanogenic community of inoculum (liquid anaerobic digestion effluent (L-AD effluent), waste activated sludge (WAS), and anaerobic granular sludge (AGS)) and substrate/inoculum ratio (S/I) on reactor performance in solid state anaerobic digestion (SS-AD) were investigated. L-AD effluent, which can provide sufficient microbes and enough buffering capacity to the reactor at an S/I ratio of 6, was found to quickly initiate SS-AD processes. The highest methane production was obtained in reactor inoculated with WAS at an S/I ratio of 2. Higher volatile fatty acids and total ammonia nitrogen concentrations were found when AGS was used as inoculum, which led to a low methane production. *Methanosaetaceae*, *Methanosaetaceae*, and *Methanosaetaceae* together with *Methanobacteriaceae* were the dominant methanogens in reactors inoculated with L-AD effluent, WAS, and AGS, respectively. These findings suggest among the three inoculum, L-AD effluent is the most effective inoculum for SS-AD of on farm organic waste.

1. Introduction

Anaerobic digestion (AD) is a promising technology for organic material management with renewable energy production, nutrient recycle and reduction of waste streams. Much effort has been made in the implementation of AD to treat on-farm organic waste such as livestock manure, agricultural byproduct, and fruit and vegetable residues. Traditionally, AD process of biogas production uses different biomass as mono-substrates. However, mono-substrates usually reduce economic viability of AD plants due to low methane production caused by nutritional imbalance, lack of diversified microorganisms, rapid acidification, and the presence of inhibiting compounds (Li et al., 2016; Hagos et al., 2017). Thus, recently various AD systems and designs are available for anaerobic co-digestion (AcoD). Solid-state AD (SS-AD) generally operates at total solid (TS) contents of more than 15% (Li et al., 2011; Ge et al., 2016). Solid state anaerobic co-digestion has been commercialized in Europe for treating animal manure and other solid organic materials, such as energy crops (Xu et al., 2016). During the last decade, numerous garage-type dry fermentation batch process plants were built and approved for operation in Germany and other European countries (i.e., BEKON Energy Technologies GmbH & Co. KG) (Qian et al., 2015). However, an inadequate mass transfer, high organic loading and recalcitrance of lignocellulosic biomass are the common problems which usually occur in SS-AD and result in reduced methane yields and a high tendency to accumulate inhibitors such as ammonia and volatile fatty acids (VFAs) (Yang et al., 2015). These obstacles have caused decision makers to be hesitant about developing large-scale SS-AD systems, while SS-AD systems are more commonly found in European countries but most commercial scale AD processes are operated under liquid-AD (L-AD) conditions in the U.S. and China (Ge et al., 2016).

The start-up phase of an SS-AD system is considered the most critical step especially in batch digestion (Brown and Li, 2013). The substrate/inoculum (S/I) ratio (based on volatile solids (VS)) is one of the most important factors for the start of a balanced microbial population in anaerobic system (Zhu et al., 2014). It was reported that in commercial SS-AD digesters, about 50–70%

* Corresponding author.
E-mail address: beixiaoxi@cau.edu.cn (X. Gong).
[1] Author contribution. These authors contributed equally to this work.

https://doi.org/10.1016/j.wasman.2018.09.042
0956-053X/© 2018 Published by Elsevier Ltd.

(wet basis) of the finished solid materials (digestate) need to be recycled as inoculum. The positive effects of a larger inoculation size on SS-AD are increased microbial populations (especially methanogens), higher buffering capacity, and in some cases balanced C/N ratios, while the negative effects is that excessive inoculum takes space and thus reduces the reactor utilization efficiency (Li et al., 2011; Xu et al., 2013; Shi et al., 2014). Yang et al. (2015) reported that low S/I ratio is usually preferred to provide more methanogens for the fast and successful startup of SS-AD. Li et al. (2016) found that SS-AD of the ternary mixtures with dairy manure, tomato residues (less than 40%), and corn stover were performed successfully with S/I ratio of 6. However, Lesteur et al. (2010) indicated that each feedstock has its suitable S/I ratio because of the amount of volatile fatty acids (VFA) and the capacity to buffer the VFA cumulation in AD process. Similar results were also reported by Zhu et al. (2014) during the SS-AD of soybean processing waste.

The source (microbial activities) and chemical composition of the inoculum have been found to significantly influence the methane yield, startup time, and greatly affect SS-AD performance, especially during start-up (Motte et al., 2013). AD sludge contains highly complex microbial communities, which has the potential to be used as a inoculum (Lin et al., 2017). Inoculating SS-AD with AD sludge has the potential to reduce treatment costs for AD sludge, make the system more self-sustainable, and provide a microbial community acclimated to the feedstock and operating conditions (Xu et al., 2016).

Several types of sludge materials have high potential to be used as SS-AD inoculum due to their biological and physico-chemical properties. Researches have reported that the finished material (effluent) from L-AD can be a good inoculum for SS-AD due to L-AD effluent can supplement sufficient microbes, moisture, micronutrients, trace elements, alkalinity, and nitrogen to SS-AD system (Wang et al., 2013; Yang et al., 2015; Ge et al., 2016). Anaerobic granular sludge (AGS) is another type of AD-sludge that has been generated in treating several types of high-concentration organic wastewater in up-flow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactors (Mu et al., 2012). AGS can be considered as a spherical biofilm consisting of billions of anaerobic microorganisms, and has been used to enhance the methane production in the second phase of waste activated sludge AD (Mu et al., 2012; Lim and Fox, 2013; Zhang et al., 2014). Waste activated sludge (WAS) is a byproduct of the wastewater treatment process, which has become a serious environmental issue because of its huge production, potential environmental risk and high cost for disposal (Feng et al., 2014). AD has been widely applied to treat WAS, and has been one of the most efficient solutions to both energy crisis and environmental pollution challenges (Guo et al., 2015). The most abundant bacterial populations in AD sludge from a full-scale municipal wastewater treatment plant were found to be *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*. The dominant proliferation of *Methanosaeta* and *Methanosarcina* suggested that the acetoclastic methanogenesis is the dominant methanogenesis pathway in the full-scale anaerobic digester (Guo et al., 2015). However, currently very limited information can be found for the optimal S/I ratio and methanogenic archaeal community population using recycled AD sludge especially AGS and WAS as inoculum for SS-AD.

Previous studies have demonstrated that solid state anaerobic co-digestion of dairy manure with corn stover and tomato residues has a great potential to improve methane yield and net energy production (Li et al., 2016, 2018b). Co-digestion of dairy manure, corn stover, and tomato residues at an optimal ratio of 54:33:13 obtained the highest methane yield of 415.4 L/kg VS $_{feed}$ which led to a 0.5–10.2-fold increase than that of individual feedstock (Li et al., 2016). Li et al. (2018) reported that higher net energy production were achieved with the mixture of 24% corn stover, 36% dairy manure, and 40% tomato residues, which had a 135% increase in net energy production compared to SS-AD of 100% dairy manure, and 88% higher compare with SS-AD of binary mixture with 60% dairy manure and 40% corn stover. However, there is limited information available on the effects of different inoculum and S/I ratio on reactor performance for SS-AD of tomato residues with dairy manure and corn stover. The objectives of the present study were to determine the effects of inoculum and S/I ratio on reactor performance using recycled AD sludge (L-AD effluent, AGS, and WAS) as an inoculum for SS-AD of tomato residues with dairy manure and corn stover. The effect of inoculum and S/I ratio on microbial population community in terms of relative abundance and diversity during SS-AD was also investigated, and was correlated with reactor performance.

2. Methods

2.1. Substrate and inoculum

Dairy manure was obtained from a dairy farm located in Daxing district of Beijing, China. The collected dairy manure was mixed in laboratory using a blender (Braun-MQ705, Braun Inc., Poland) and was stored at 4 °C for further use in AD. Tomato residues were collected from a farm located in Fangshan district of Beijing, China. The tomato residues mainly contained stalks, leaves and residual tomatoes. After collection, the tomato residues were shredded using an electrical blender, thoroughly mixed, and frozen at −20 °C to prevent biological decomposition during storage until experiments (Li et al., 2016). Corn stover was collected from a farm operated by China Agricultural University, Beijing, China. After delivery, the corn stover was air dried to a moisture content of less than 15% and then ground to pass through a 40 mm sieve (Huafeng Inc., Zhejiang, China).

Three different inocula were selected. Inoculum A was effluent from a mesophilic L-AD system (operated by Beilangzhong pig farm, Beijing, China) fed with pig manure. Inoculum B was WAS from a municipal wastewater treatment plant in Beijing. Inoculum C was AGS obtained from a brewery located in Shunyi district of Beijing, China. Inoculum A and C were collected and stored at 4 °C for less than 3 days before being used. For enrichment of anaerobic microorganisms, prior to use, inoculum B was degassed and anaerobically cultivated at 35 °C for 56 days. The average values of three measurements are presented in Table 1.

2.2. Batch digestion experiment

Dairy manure, corn stover, and tomato resides were weighted and mixed to obtain the designed mixing ratio of 48:32:20 (on a VS basis), which was reported to be a preferred mixing ratio for co-digestion of dairy manure, tomato residues and corn stover (Li et al., 2018). For each treatment, deionized water (DI) and inoculum were mixed with feedstock using a hand-mixer (Braun-MQ705, Braun Inc., Poland) to achieve a mixture with S/I ratio of 2, 4, and 6 (VS based). Since we controlled the reactor volume to be the same, reactors with lower S/I ratios received more inoculum. The inoculum and S/I ratio of treatments are shown in Table 2. The mixture was loaded into 1 L glass reactors, which were tightly closed with screw caps, and N_2 gas was purged into each reactor for at least 3 min to ensure an anaerobic environment. The reactors were incubated for up to 45 days in an incubator at 35 ± 1 °C. Triplicate reactors were set for each condition. Each reactor was manually mixed twice a day. Inoculum without any feedstock addition was used as a control.

Biogas was collected in 1-L Tedlar gas bags (Safe-laboratory Inc., Beijing, China), and the biogas composition and volume were