Renewable Energy 118 (2018) 335–342

Contents lists available at ScienceDirect

Renewable Energy

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

Anaerobic co-digestion of animal manures with corn stover or apple pulp for enhanced biogas production

Kun Li [a, b], Ronghou Liu [a, b, *], Shaofeng Cui [a, b], Qiong Yu [a, b], Ruijie Ma [a, b]

[a] *Biomass Energy Engineering Research Centre, School of Agriculture and Biology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, PR China*

[b] *Key Laboratory of Urban Agriculture (South), Ministry of Agriculture, 800 Dongchuan Road, Shanghai 200240, PR China*

ARTICLE INFO

Article history:
Received 21 December 2016
Received in revised form
27 July 2017
Accepted 11 November 2017
Available online 11 November 2017

Keywords:
Anaerobic co-digestion
Stability
Microbial community
Fluorescent *in situ* hybridization (FISH)

ABSTRACT

Corn stover (CS) or apple pulp (AP) were used to improve the anaerobic digestion performance of chicken manure (CM) or pig manure (PM), with the aim of adjusting carbon/nitrogen ratio and increasing the system stability compared to animal manures alone. This study was conducted in batch and semi-continuously fed digester at laboratory scale. The results of batch tests showed that the optimal mixture ratios for CM/CS, CM/AP, PM/CS and PM/AP were 4:1, 2:1, 4:1, 4:1, respectively. In the semi-continuous mode, inhibition to methane generation occurred when organic loading rate (OLR) of manures mono-digestion was higher than 2.4 g VS $L^{-1}d^{-1}$. However, the co-digestion of chicken manure with apple pulp at ratio 2 allowed operation at OLR of 4.8 g VS $L^{-1}d^{-1}$ and obtained the highest specific methane production (0.34 L g^{-1} VS_{added}), due to its enhanced buffer capacity and nutrient balance. Fluorescence *in situ* hybridization revealed that the microbial community in digester fed with CM/AP mix was dominated by *Methanosarcina* and the remaining microorganisms mainly belonged to *Methanobacteriales*, both of which reflected the tolerance of inhibitors in this system. However, in digesters with other mixture (CM/CS, PM/CS, PM/AP), the negative impact of high levels ammonia and volatile fatty acids on sensitive Methanogenic Archaea resulted in serious decrease in the dominant species and finally caused the failure of anaerobic digestion.

1. Introduction

In China, the biogas industry has grown quickly in recent years and has undoubtedly become one of the biggest biomass energy industry. Small rural biogas digesters were dominant in China especially from 2002 to the end of 2010 [1]. As large-scale livestock farms become more and more common, medium and large-scale biogas plants are needed to dispose large amounts of intensive animal manures. On the other hand, incentive policies were introduced by the Chinese government with the aim of supplying a sustainable alternative energy source, which significantly promoted the development of medium and large-scale biogas plants [2]. Scaled production of biogas requires a stable feedstock supply, and the shortage of single material therefore became constraints in mono-digestion. In addition, manures can hardly be digested as mono-feedstock because the ammonia inhibition and even failure in some cases may happen [3]. Li et al. [4] reported operating limits of organic loading rate when livestock manures were digested as single wastes. Drawbacks of manure mono-digestion can be solved by co-digestion with low nitrogen biomass so as to increase the carbon/nitrogen ratio (C/N) as an approach of overcoming process limitations originated from ammonia [5]. But unwise selection of co-substrate would also cause problems in digestion process [6]. Thus, this study focuses on two types of co-substrate, corn stover (CS) or apple pulp (AP), for anaerobic co-digestion by using chicken manure (CM) or pig manure (PM) as based feedstock.

China is rich in resource of corn stover which annually generated an amount of approximately 216 million metric tons [7]. Due to its abundance and high carbohydrate content, corn stover has a great potential for scaled biogas generation [8]. However, owing to the inherent limitations of lignocellulosic substrates (high C/N ratio), their digestion were greatly hindered. Studies showed that the problem associated with nitrogen deficiency in crop residues was liable to produce poor buffering capacity as well as unbuffered

* Corresponding author. Biomass Energy Engineering Research Centre, School of Agriculture and Biology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, PR China.
E-mail address: liurhou@sjtu.edu.cn (R. Liu).

https://doi.org/10.1016/j.renene.2017.11.023
0960-1481/

Nomenclature

AD	Anaerobic digestion
BMP	Biochemical methane potential
COD	Chemical oxygen demand
C/N	Carbon/nitrogen ratio
Cy3	Fluorochrome of Cy3
DAPI	DAPI staining solution
FA	Free ammonia
FISH	Fluorescent *in situ* hybridization
FW	Fresh weight
HRT	Hydraulic retention time
IA	Intermediate alkalinity
OLR	Organic loading rate
PA	Partial alkalinity
PVC	Polyvinyl chloride resin
SMP	The specific methane productions
TA	Total alkalinity
TAN	Total ammoniacal nitrogen
TS	Total solids
VFA	Volatile fatty acid
VMPR	Volumetric methane production rate
VS	Volatile solid

volatile fatty acid (VFA) accumulation in the digestion process [9,10]. Then, activity of methanogens was inhibited, and the conversion of organic carbon was decreased [11].

An alternative co-substrate concerns rotten apple which was easily collected from market. Apple waste has been targeted for biogas production because of its high biodegradable organic matter content and moisture. However, this carbohydrate-rich waste is rapidly acidified to VFA when used as mono-feedstock [12]. Alkali addition has been suggested to correct the lower pH of apple waste. Without any measures, the pH quickly decreased and tended to inhibit methanogenesis [13].

The cause of the process disruption in mono-digestion of the above two feedstocks is considered to be excessive production of VFA and inadequate alkalinity levels [14]. Anaerobic co-digestion refers to the digestion of mixed substrates [15]. To prevent the drop in pH, these wastes could be buffered by co-digesting with a buffering substrate. Manures are high nitrogen substrates, and have wide range of nutrients and high microbial activity [16]. Co-digestion of manure with corn stover or apple waste could not only achieve high buffer capacity and nutrient-balance, but balance the C/N ratio of each feedstock, thereby decreasing the risk of ammonia inhibition which likely occurred when manures are used as sole-substrate. This is also a cost-effective method for nutritional regulation compared with addition of nitrogen-rich substrates like urea or ammonium bicarbonate to corn stover.

Although many co-digestion trials have been conducted by using livestock and poultry manure mixed with other crop-residues, studies on manures co-digested with corn stover which is a promising renewable feedstock for methane production are limited. And the optimum ratio for biogas production varied widely. For example, Li and Zhang et al. [17] found that chicken manure and corn stover only mixed at ratio 1:3 could acquire a methane yield of 298.2 mL g^{-1} VS which is higher than that of single chicken manure (291.1 mL g^{-1} VS), while in another batch tests, synergism could be seen in co-digestion of chicken manure and corn stover with a ratio reported as 1:1 [18]. On the other hand, rapid acidification had been found in mono-digestion of cellulose-poor wastes like apple waste, consequently inhibited the activity of methanogenic organisms. The feasibility of co-digestion of animal manures and apple waste still needs to be deeply explored.

This paper presents the results of batch and semi-continuous fed digestion trials carried out with mixture of animal manure (chicken manure, pig manure) and corn stover or apple waste at ratios of 4:1, 2:1, 1:1, 1:2 and 1:4, with the purpose of optimizing feed ratio between CM/PM and CS/AW as well as investigating the co-fermentation performance of continuous digester with feedstock of CM/CS (4:1), CM/AP (2:1), PM/CS (4:1) and PM/AP (4:1) under increasing organic loading rate. This study also focused on microbial community analyses for the co-digestion process by fluorescent *in situ* hybridization. Insights gained from this work would enhance the comprehension of microorganisms involved in the anaerobic co-digestion of manures and other substrates as well as the factors that impact the performance and stability of anaerobic digester.

2. Methods and materials

2.1. Materials and digesters

2.1.1. Animal manures

Chicken manure (CM) was obtained from a chicken farm in Heilongjiang province, China. Pig manure (PM) was collected from farm located in the Fengxian county of Shanghai city, China. Contaminants like stones and grass were picked away from manures and then they were homogenized, packed into 3-L plastic storage boxes, and frozen at −20 °C. Manures were thawed before using, and stored at 4 °C for no more than two weeks. Table 1 shows the properties and characteristics of inoculums, manures, corn stover and apple waste.

2.1.2. Corn stover and apple pulp

The CS was obtained from experimental field of Agriculture and Biology School in Shanghai Jiao Tong University, Shanghai, China. The collected corn stover was ground into particles less than 1 mm. Rotten apples were collected from a market in Minhang District of Shanghai, China. Apples were firstly crushed with a blender, then homogenized and pass through 2.0 mm screen. Both CS and AP were stored in the same way as for manure.

2.1.3. Inoculum

The inoculum for all digesters was obtained from a commercial biogas plant treating livestock manure under mesophilic conditions (continuous stirred tank reactor, operated by Sennong Company, Pudong District of Shanghai, China). Before use, the digestate was mixed thoroughly and sieved through 0.85 mm screen to remove large particles.

2.1.4. Digesters

The batch digestion tests were carried out in 250 mL conical flasks each connected to a polyvinyl chloride resin (PVC) tube which was sealed one end using for biogas collection and volume determination. The temperature of flasks was kept at 37 °C by a water-bath.

The semi-continuous digester had a 2.5-L capacity with a 2-L working volume and was constructed of PVC pipe with airtight top and bottom. The top was sealed by water and fitted a feed port as well as a gas outlet. Drainage port was incorporated on the bottom. A stirrer was inserted through the top plate to allow mixing