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Effects of co-digestion of cucumber residues to corn stover and pig manure ratio on methane production in solid state anaerobic digestion

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ABSTRACT

This study investigated the performance of co-digesting cucumber residues, corn stover, and pig manure at different ratios. Microbial community structure was analyzed to elucidate functional microorganism contributing to methane production during co-digestion. Results show that mixing cucumber residues with pig manure and corn stover could significantly improved methane yields 1.27–3.46 times higher than mono-feedstock. The methane yields decreased with the cucumber residues increasing when the pig manure ratio was fixed at 4 and 3, and was opposite at ratio 5. The optimal mixture ratio was T2 with the highest methane yield (305.4 mL/g VS) and co-digestion performance index (1.97). The main microbiological community in T2 was bacteria of *Firmicutes* (44.6%), *Bacteroidetes* (32.5%), *Synergistetes* (3.8%) and archaea of *Methanosaeta* (37.1%), *Methanospirillum* (18.2%). The mixture ratios changed the microbial community structures. The adding proportion of cucumber residues changed the community composition of the archaea, especially the proportion of *Methanosaeta*.

1. Introduction

China as one of the largest agricultural country is abundant of biomass resources such as agricultural residues and animal manure (Li et al., 2015). Cucumber is a main vegetable in China produced a total of over 73 million tons of residues every year (FAO 2014), and has been a source of nuisance in municipal landfills for acidifying easily (Lin et al., 2011). China, which ranks first in the world in crop residue production, is estimated to produce a total of over 800 t million per year of crop residues and corn stalks (CS), rice straw (RS), and wheat straw (WS) account for 80.5% of the total output (40.6%, 24.2%, and 15.7%, respectively) (Jiang et al., 2012). The total production of animal manure from large-scale centralized farms is about 837 million tons of which 208 million tons (accounting for 24.9%) are pig manure (PM) and most of the manure produced has not been harmlessly disposed, which poses a serious threat to soil, water, and air as well as livestock and poultry (Tian, 2012; Jiang et al., 2011).

Agricultural residues have been successfully converted to methane-rich biogas in anaerobic digestion (AD) which have presents a double advantage of producing biogas and simultaneously treating the residues, reducing their disposal in sanitary landfills (Wang et al., 2012; Li et al., 2011; Jacqueline et al., 2016). In China, biogas plants have been rapidly developed, and most of them were liquid anaerobic digestion (L-AD, < 15% TS) fed by a single raw material especially animal manure (Maraseni and Maroulis, 2008; Zhang et al., 2009). Compared to conventional L-AD, solid state anaerobic digestion (SS-AD, > 15% TS) features high organic loading rate, small digester volume, low energy demand for heating, and high volumetric methane production (Li et al., 2011; Yang and Li, 2014). Mono-substrate in AD has problems of insufficiency of imbalanced nutrients such as carbon and nitrogen and the low buffering capacity, also periodic shortage of material caused by the market changes in the breeding industry (Wang et al., 2017).

Co-digestion by utilizing various types of raw materials may be a more cost effective way to balance nutrients (C/N ratio and macro-and micronutrients) and reduced inhibitors/toxic compounds accumulation so that improving biogas production (Abouelenien et al., 2010; Lar et al., 2010), also can treat the wastes in the same area or nearby.

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Livestock manure and poultry manure are well-studied co-substrates for its high buffering capacity and high nitrogen content which could enhance the digestion of lignocelluloses, and rich in micronutrients necessary for optimum bacterial growth (Callaghan et al., 2002). Many studies was about the co-digestion of manure and crop residues (Panichnumsin et al., 2010; Li et al., 2013a,b; Ye et al., 2013). And the use of manure and crop residues as a co-substrate to vegetables residues may reduce the environmental impact while simultaneously enhancing energy production for their complementary properties.

However, two or more different types of substrates involved in AD requires careful selection of feedstocks and mixing ratios in order to ensure and even improve the efficiency (Álvarez et al., 2010; Fonoll et al., 2015). Ye et al. (2013) reported that co-digestion of kitchen waste, pig manure, and rice straw at an optimal ratio of 0.4:1.6:1 obtained the highest biogas yield of 674.4 L/kg VS which was 71.67% and 10.41% higher than that of the solo-digestion of rice straw and pig manure, respectively. Vincenzo et al. (2015) found stable biogas production was obtained of about 400 l/kg Volatile Solids at a Hydraulic Retention Time of 40 days in a mixture containing 85% cow slurry, 10% olive pomace and 5% apple pulp (% by volume). The methane yield (415.4 L/kg VS feed) were achieved with the ternary mixtures of 33% corn stover, 54% dairy manure, and 13% tomato residues, lead to a 0.5–10.2-fold higher than that of individual feedstocks (Li et al., 2016). So different types of wastes have its own properties and needs carefully selected mixing ratios.

Few studies on co-digestion of ternary agricultural wastes (especially adding vegetables residues) in SS-AD had been reported. Also, there is limited information available on composition of microorganism in ternary mixtures system in SS-AD. To date, no investigation on regularity between the methane production and microbial community structure of ternary mixtures in SS-AD has been reported. The aim of this study was to determine the influence of different mixing ratios (pig manure PM: corn stover CS: cucumber residues CR), in comparison to PM (10:0:0), CS (0:10:0) and CR (0:0:10) alone, on anaerobic digestion performance in batch tests, then evaluate the synergistic effects of ternary mixtures in terms of methane yield and system stability of SS-AD. Also the community of bacteria and archaea in co-substrates at different ratios were investigated. Finally, the regularity and relationship of the mixing ratio, microbial composition and process performance in SS-AD were assessed.

2. Materials and methods

2.1. Feedstocks and inoculum

Pig manure was collected from a local pig farm (Yanqing of Beijing, China). Cucumber residues (all parts above-ground including stalks, leaves, and residual cucumber) were obtained from a local vegetable greenhouse (Daxing of Beijing, China). Cucumber residues were crushed to 5-cm pieces once collected. Corn stover was collected from a experimental Station of China Agricultural University. Inoculum was obtained from an industrial AD system (Beilangzhong of Beijing, China), which was operated at a mesophilic condition.

2.2. Batch anaerobic digestion tests

Two sets of AD batch tests (Table 1) were conducted in this study. In the first set of tests, the three substrates (i.e., pig manure, corn stover, and cucumber residues) were individually inoculated with anaerobic digesters to achieve 22% TS. Their feedstock to inoculum (F/I) ratios were 0.3 (VS based).

In the second set of tests, the three substrates (i.e., pig manure, corn stover, and cucumber residues) were completely mixed at a ratio of 5:1:4, 5:2:3, 5:3:2, 5:4:1; 4:1:5, 4:2:4, 4:3:3, 4:4:2; 3:1:6, 3:2:5, 3:3:4, 3:4:3, respectively. These mixtures were then inoculated with anaerobic digesters to obtain a TS of 29% and the F/I ratio of 0.6 (VS based). In this study, all digestion substrates were filled in 1 L glass reactors and then placed in an incubator for 65 days with temperature constant at 35 °C. All treatments were conducted in triplicates. Inoculum without any feedstock was used as a control. Biogas generated from each reactor was collected using Tedlar gas bags for the production and composition analyses on a daily basis.

Table 1
Summary of batch tests conditions.

Setups	Treatments	PM: CS: CR (wet basis)
Series 1	CK1	10:0:0
	CK2	0:10:0
	CK3	0:0:10
Series 2	1	5:1:4
	2	5:2:3
	3	5:3:2
	4	5:4:1
Series 3	5	4:1:5
	6	4:2:4
	7	4:3:3
	8	4:4:2
Series 4	9	3:1:6
	10	3:2:5
	11	3:3:4
	12	3:4:3

2.3. Analytical methods

The production and composition of biogas were measured by a portable biogas meter (BIOGAS5000, Geotech, England). TS and VS contents were analyzed based on standard methods (APHA, 2005). The pH of samples was determined with a pH meter. The concentrations of VFAs (i.e., acetic, propionic, and lactic acids) were analyzed using a Gas Chromatograph (GCMS-2010, Shimadzu, Japan) based on the method previously reported by Zheng et al. (2015). The content of NH_4^+-N was measured by a flow injection analyzer (AA3, SEAL, Germany). TN and TC content were measured by the Elemental Analyzer (Hanau, Germany).

2.4. Co-digestion performance index (CPI)

The combination of substrates can result in synergistic effects which may be the dilution of inhibitory intermediaries, addition of valuable nutrients that result in increased biodegradability, and/or a change in the microbiome that results in an enhanced metabolism. Pages-Díaz et al. (2014) investigated optimal mixture composition between cattle slaughterhouse wastes, municipal solid waste, manure and various crops, and assessed the synergistic effect solely by specific methane production rate. Similarly, Astals et al. (2014) identified the synergistic effect during anaerobic co-digestion of pure and slaughterhouse carbohydrate, protein, and lipid substrates as an improvement of process kinetics, rather than an increase in ultimate biodegradability. Labatut et al. (2017) suggested comparing the bio-methane potential of a co-digested substrate with the weighted sum of the single substrate bio-methane potentials as a measure of synergistic or antagonistic interactions. A co-digestion performance index (CPI) was calculated as (Jacqueline et al., 2016):

$$CPI_{i,n} = \frac{B_{i,n}}{\overline{B}_{oi,n}} = \frac{B_{i,n}}{\sum_i^n \%VS_i B_{o,i}}$$

where $B_{i,n}$ is the bio-methane potential of the co-digestion blend, $\overline{B}_{oi,n}$ is the weighted average based upon VS content (%VS) of the individual substrate bio-methane potentials ($B_{o,i}$); substrates i through n are co-digested such that $\sum_i^n \%VS_i = 1$. Thus, a CPI > 1 indicates a synergistic