Bioresource Technology 238 (2017) 542–551

Contents lists available at ScienceDirect

Bioresource Technology

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

The effect of mixing intensity on the performance and microbial dynamics of a single vertical reactor integrating acidogenic and methanogenic phases in lignocellulosic biomass digestion

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HIGHLIGHTS

- A pilot reactor integrating acidogenic and methanogenic phases was constructed.
- The reactor was designed specifically for digestion of lignocellulosic biomass.
- The effect of mixing intensity on methane yield was determined.
- The microbial communities in the acidogenic and methanogenic phases were analyzed.

ARTICLE INFO

Article history:
Received 10 March 2017
Received in revised form 19 April 2017
Accepted 20 April 2017
Available online 23 April 2017

Keywords:
Novel reactor
Integrated two-phases
Maize stover
Mixing intensity
Microbial dynamics

ABSTRACT

The ready formation of scum in vertical reactors has been a bottleneck in the digestion of lignocellulosic materials for biogas production. This study describes a single vertical reactor that integrates the acidogenic and methanogenic phases of this process. The effects of two types of maize stover feedstock (fresh and silage) and two mixing intensities (20 and 70 rpm) on methane yield were orthogonally determined. Fresh maize stover yielded approximately 14% more methane than silage maize stover. Mixing at 20 rpm contributed to methane yield, while mixing at 70 rpm blurred the phase boundary, resulting in accumulation of volatile fatty acids and loss of methanogens. The upper and lower phases clearly constituted a two-phase fermentation system. *Clostridiales* occupied the acidogenic phase, while the predominant bacteria in the methanogenic phase were *Bacteroidetes*, *Chloroflexi*, and *Synergistetes*. The absolute predominance of *Methanosaetaceae* clearly demonstrated that aceticlastic methanogenesis was the main route of methane production.

1. Introduction

Crop straw is a promising fermentation substrate for biogas plants due to its low price, easy collection, and high total yield, and the ease of its mechanized handling and integration into farm operations (Amon et al., 2007; Zhao et al., 2016; Shi, 2011). Straw is mainly comprised of cellulose, hemicellulose, and soluble carbohydrates that produce high yields of methane (Hamelinck et al., 2005), especially from maize stover. However, straw's high lignocellulose content and tendency to form scum in reactors have been major barriers to improving the organic loading rate and decomposition efficiency in its digestion for biogas.

Data from the literature on the best configuration for digesters using solid waste as feedstock are inconclusive (Nizami and Murphy, 2010). Various researchers have reviewed and compared different digester types suitable for digesting solid wastes. These studies used one-stage or two-stage digesters, wet or dry/semi-dry digesters, batch or continuous digesters, attached or non-attached digesters, high-rate digesters, and combined approaches (Nizami and Murphy, 2010; Vandevivere et al., 2003; De Baere and Mattheuws, 2008). However, digesters that are optimized for other solid waste, for example the organic fraction of municipal solid waste (OFMSW), may not be ideal for straw because the volatile solid content of OFMSW is of the order of about 60% whereas it may be as high as 90% for straw (Murphy and Power, 2009). Therefore, a digester optimized specifically for straw is needed.

A digester's functionality is dependent on its design, including the number of tanks and the mixing efficiency, along with operational factors such as the properties of the feedstock, the hydraulic

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http://dx.doi.org/10.1016/j.biortech.2017.04.080

retention time/organic loading rate, and the temperature (Nizami and Murphy, 2010). Considering that the vertical, continuously stirred tank reactor (CSTR) configuration is the configuration used in 90% of the newly erected digesters in recent years (Weiland, 2006), and that two-phase anaerobic fermentation technology may allow both hydrolysis and acidification and acetogenesis and methanogenesis to go to completion through microbial processes (Nizami and Murphy, 2010; Shah et al., 2015). Thus, it seems that a two-phase CSTR system, with a small acid-producing tank followed by a several-fold larger methanogenic tank, should be widely used. However, the single-stage system has been more popular at industrial scale because of its simplicity of operation and reduced costs. The single tank and relatively low energy demand for pumping and mixing (Bal and Dhagat, 2001) result in fewer technical problems. There are some potential drawbacks when using the above system to treat straw, such as the ready formation of scum, channeling of leachate, and restricted reactor heights when operating in batch mode (Mumme et al., 2010).

It follows that a single system combining a tall reactor equipped with a low-power mixer and continuous operation would have several advantages. With continuous feeding, a tall reactor would provide enough space to let straw float at the top of the reactor, in an acidogenic phase. Simultaneously, the digested residue would sink to the bottom to form a methanogenic phase. The transfer of low-molecular-weight substances from the upper, acidogenic phase to the lower, methanogenic phase could be achieved by mixing, completing methanogenesis. In order to test this hypothesis, an experimental study was conducted focusing on a novel, integrated two-phase (acidogenic and methanogenic) vertical reactor (ITPVR) specifically designed for lignocellulose biomass, with the following objectives:

- To determine and evaluate the performance of a mesophilic ITPVR under different mixing intensities with respect to methane production and volatile fatty acid concentrations and liquor pH in the two phases;
- To analyze the microbial communities in the two phases of the mesophilic ITPVR through high-throughput sequencing and determine how mixing intensity influences them;
- To analyze the effect of mixing intensity on the quantity of methanogens and total bacteria in the two phases of the mesophilic ITPVR using real-time quantitative polymerase chain reaction (Q-PCR) analysis of 16S rDNA.

2. Materials and methods

2.1. Raw materials and inoculum

Maize stover (fresh maize stover, FM; and silage maize stover, SM) was selected as a typical lignocellulosic biomass for the test, and was supplied by the ShangZhuang Experimental Station, China Agricultural University, Beijing. Fresh maize stover, still green and fully ripe, was harvested, separated from corn kernels, chopped into 2-cm pieces and then divided into two parts. One part was immediately frozen at −20 °C and used as FM. The rest was ensiled in a semi-underground silo for 30 days, to make SM, and then packaged into zip-lock bags and stored frozen at −20 °C. The FM and SM were crushed in a fruit-and-vegetable crushing machine as soon as they were removed from the freezer when used as feedstock and for analysis (Table 1). The water soluble carbohydrate (WSC), crude protein, and crude lipid content were all higher in FM than in SM, while the lignocellulose content was higher in SM. Inoculum, with total solid (TS) and volatile solid (VS) contents of 7.66% and 3.96%, was obtained from a 3-m^3 continuous stirred-tank reactor (CSTR) system with a long-term feeding regimen of maize stover and cow manure for microbial acclimation. The inoculum was kept for one week at room temperature without feeding before use, in order to exhaust any biodegradable chemical oxygen demand.

Table 1
Chemical composition of FM and SM.

Composition	FM	SM
TS (% of wet weight)	28.15 ± 0.48	26.42 ± 0.73
VS (% of wet weight)	25.92 ± 0.29	24.31 ± 0.73
pH	6.54 ± 0.39	4.13 ± 0.51
WSC (% TS)	10.10 ± 0.41	7.86 ± 0.31
Cellulose (% TS)	25.16 ± 0.71	27.59 ± 0.56
Hemicellulose (% TS)	22.64 ± 0.39	24.49 ± 0.37
Lignin (% TS)	1.39 ± 0.21	1.59 ± 0.29
Crude protein (% TS)	11.19 ± 1.44	10.13 ± 2.38
Crude lipid (% TS)	2.43 ± 0.11	1.78 ± 0.15
Total carbon (% TS)	55.17 ± 0.28	53.06 ± 0.12
Total nitrogen (% TS)	1.79 ± 0.23	1.62 ± 0.38
C/N ratio	30.82 ± 1.22	32.75 ± 0.32

2.2. Experimental design and operations

Fig. 1 shows the structure of the reactor, with a 56 L working volume. A 100 cm tall and 28.5 cm internal diameter cylindrical Plexiglas barrel was fixed between a stainless steel headcover and base. The base was hollow and allowed injected water to maintain a stable fermentation temperature of 35 ± 1 °C. An electric blanket was wrapped around the outer surface of the barrel. Three pairs of "S" shaped vanes were aligned on the top, middle, and bottom of a mixing shaft controlled by an electric motor to promote mass transfer from the top to the bottom and from the center to the periphery. The top, middle, and bottom vanes were 75 cm, 50 cm, and 25 cm above the base, respectively, and each two adjacent pairs of vanes were angled at 90° relative to each other. A feeding hole, which also served as a top sampling port, and biogas outlet were mounted on the headcover. A discharge outlet and bottom sampling port were fixed to the base. The former extended to a height of 30 cm, which was higher than the bottom vanes, to avoid loss of activated sludge.

The experiments were carried out in two parallel reactors, R1 and R2. The two reactors were injected with 45 L inoculum and then fed daily with small amount of maize stover until biogas production and the composition and pH of the fermentation liquid were stable. Subsequent experiments were divided into two stages. The first stages were fed with FM and the second with SM. The hydraulic retention time was set to 20 days, and the crushed FM and SM were diluted with tap water to reach 6% TS. R1 and R2 differed only in mixing intensity: R1 was mixed for only 5 min twice daily, at 20 rpm (rpm); R2 was stirred vigorously for 10 min five times daily, at 70 rpm. With time, an acidogenic phase gradually formed around the floating maize stover. Most of the digestive residue moved gradually downward and was discharged through the discharge outlet. The rest of the residue sank to the bottom and formed activated sludge that constituted the methanogenic phase. The acidogenic and methanogenic phases co-existed in the vertical reactors and had no obvious interface.

2.3. Sampling and analysis

2.3.1. Chemical analysis

At the end of each experimental stage, digestate samples from the top (the middle section of the acidogenic phase) and bottom (methanogenic phase) of R1 and R2, eight in total, were collected as samples for physicochemical and microbial analysis. The TS and VS were analyzed according to the modified standard method (Zhao et al., 2016). The neutral detergent fiber (NDF), acid deter-