Bioresource Technology 102 (2011) 8700–8706

Contents lists available at ScienceDirect

Bioresource Technology

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

Enhancement of bioenergy production from organic wastes by two-stage anaerobic hydrogen and methane production process

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ARTICLE INFO

Article history:
Received 21 November 2010
Received in revised form 31 January 2011
Accepted 1 February 2011
Available online 5 February 2011

Keywords:
Anaerobic digestion
Hydrogen
Methane
Two-stage process

ABSTRACT

The present study investigated a two-stage anaerobic hydrogen and methane process for increasing bioenergy production from organic wastes. A two-stage process with hydraulic retention time (HRT) 3 d for hydrogen reactor and 12 d for methane reactor, obtained 11% higher energy compared to a single-stage methanogenic process (HRT 15 d) under organic loading rate (OLR) 3 gVS/(L d). The two-stage process was still stable when the OLR was increased to 4.5 gVS/(L d), while the single-stage process failed. The study further revealed that by changing the $HRT_{hydrogen}$:$HRT_{methane}$ ratio of the two-stage process from 3:12 to 1:14, 6.7%, more energy could be obtained. Microbial community analysis indicated that the dominant bacterial species were different in the hydrogen reactors (*Thermoanaerobacterium thermosaccharolyticum*-like species) and methane reactors (*Clostridium thermocellum*-like species). The changes of substrates and HRT did not change the dominant species. The archaeal community structures in methane reactors were similar both in single- and two- stage reactors, with acetoclastic methanogens *Methanosarcina acetivorans*-like organisms as the dominant species.

1. Introduction

Hydrogen produced from biomass is a renewable energy carrier. Among the various hydrogen production methods, dark fermentation of organic wastes seems to be the most promising and environmentally friendly method. The feasibility of such method has been demonstrated in several studies (Cai et al., 2004; Liu et al., 2006). However, the main obstacles in such process are the lower hydrogen yield (<4 mol H_2/mol Glucose) and higher residual organic concentration in the effluent (Xie et al., 2008). The effluents of the dark fermentation process contain mainly acetate, propionate, butyrate, etc., which should be further utilized to increase the total energy recovery efficiency.

Combined hydrogen and methane production in a two-stage process is a concept which has been developed in recent years (Kyazze et al., 2007; Liu et al., 2006; Ueno et al., 2007). It is similar to the traditional two-phase process that separates hydrolysis/acidogenesis and methanogenesis, and optimizes each process separately, leading to a larger overall reaction rate and biogas yield (Fox and Pohland, 1994). The main difference is that hydrogen is retrieved in the first stage of the two-stage process for hydrogen and methane production. The co-production of hydrogen and methane is more promising from an energy perspective. Liu et al. (2006) have demonstrated that more methane could be obtained by two-stage hydrogen and methane process. Also, the mixture of hydrogen and methane has many advantages than methane alone, which could improve the efficiency of the methane combustion motors and decrease the emissions of CO_2 and CO (Akansu et al., 2004). Several studies have been conducted to investigate the hydrogen and methane production in the two-stage process. However, they mainly focused on the optimization of hydrogen and methane reactors individually (Antonopoulou et al., 2008; Venetsaneas et al., 2009). It is necessary to optimize the whole system for higher total energy production. In addition, the mechanisms involved in the two-stage process and the microbial community structures have not been investigated and clarified, which is crucial for better understanding of the process.

Concerns about instability of fossil fuels supply, limits on fossil fuel reserves and not the least environmental pollutions and climate changes have brought new insights into the utilization of biomass in biorefinery concepts, where biomass is used as feedstock instead of fossil fuels for production of bio-based fuels, chemicals, solvents, etc. by biological conversion processes. We have proposed a novel biorefinery concept based on rapeseed plant (Luo et al., 2010a), where the oil seed is used for biodiesel production and the straw is used for bioethanol production. From this process several effluent sub-streams are generated, which need to be utilized for full utilization of the organic matter. Rapeseed cake

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doi:10.1016/j.biortech.2011.02.012

and glycerol are the by-products in the biodiesel process, and the search for proper disposal methods is still going on (Thamsiriroj and Murphy, 2010). Stillage is the wastewater from bioethanol production process and it contains high concentrations of degradable organic pollutants. The utilization of the above three substreams for bioenergy production is necessary from environmental protection and sustainability viewpoints.

Therefore, in the present study we investigated and compared different configurations of two-stage process for hydrogen and methane production from the above organic streams and studied the role of the hydrogen reactor in the whole system. Single-stage process for methane production was operated as control. Finally, the microbial communities in different reactors and operation conditions were identified.

2. Methods

2.1. Feedstocks and inoculum

The stillage used in this study was obtained from an ethanol plant in Lithuania. Rapeseed cake and glycerol waste from the biodiesel production process were obtained from a local company (Emmelev). The samples were stored at −20 °C. The substrates were thawed and kept at 4 °C for 2–3 d before usage. Cake (24 g) and glycerol (2 ml) were added to 1 L stillage based on the biorefinery concept described in Luo et al. (2010a). The characteristics of the three wastes and their mixture are shown in Table 1. Thermophilic anaerobic digested manure (Biogas plant, Snertinge, Denmark) was used as inoculum for both hydrogen and methane production.

2.2. Reactor set-up and operations

Two-stage (hydrogen and methane) operation was compared with single-stage methane operation. The hydrogen reactor (H) was a 2 L continuously stirred tank reactor (CSTR) with working volume 1.2 L, while the methane reactors (M) was 4.5 L CSTR with working volume 3.5 L. The configurations of all the reactors were similar and described in Boe et al. (2009). All reactors were stirred four times (3 min for each time) per hour throughout the experiment by motor mixer with a timer. The substrates were fed to all the reactors four times per day using peristaltic pump with timer control. Before feeding, 8 g/L $NaHCO_3$ was added to the stillage or mixture to adjust the pH to around 6. The two-stage process was tested at two different distributions of HRT between hydrogen and methane reactors. The first HRT distribution tested was 3:12, i.e. the HRT for the hydrogen reactor was 3 d (H3) and the HRT for the methane reactor was 12 d (M12), while the second HRT distribution was 1:14, i.e. 1 d HRT for the hydrogen reactor (H1) and 14 d HRT for the methane reactor (M14). A single-stage methane reactor was operated at HRT of 15 d (M15). All experiments were conducted at 55 °C. The operation data of the reactors are shown in Table 2.

For the first two-stage experiment, the HRT distribution of 3:12 was tested. The reactor H3 was initially filled with 200 ml inoculum, 500 ml stillage and diluted by water to final volume 1.2 L. The initial pH of the mixture was adjusted to 6 by NaOH. After the hydrogen production ceased, the reactor was fed semi-continuously. For M12 and M15, the reactors were initially filled with 3.2 L inoculum and 300 ml stillage. After the methane production ceased, the reactors were also fed semi-continuously. The effluent of H3 was fed to M12. Initially, H3 and M15 were fed with only raw stillage to get a successful start-up at relatively low OLR. After steady-states were achieved, the mixture was fed to the reactors (from day 46 to day 118). The steady-state in this study was defined as a stable biogas production with daily variation of lower than 10%.

From day 75 to day 126, the second two-stage experiment with the same total HRT 15 d, but HRT distribution of 1:14 between hydrogen and methane reactors was started. The reactors were the same as those used in the experiment with HRT distribution of 3:12, but with different feeding flow rates. The inocula for H1 and M14 were from the effluents of H3 and M12, respectively. The reactors were directly fed with the mixture of stillage, cake and glycerol.

2.3. Specific methanogenic activity (SMA) tests

Batch experiments for estimation of the specific methanogenic activity (SMA) on a specific substrate were carried out when steady-states were achieved in the methane reactors. Forty milliliters of basal anaerobic (BA) medium (Karakashev et al., 2005) was dispensed anaerobically in 100 mL serum bottles. The media were supplemented with different substrates – acetate (20 mM), propionate (10 mM), butyrate (10 mM), hydrogen/carbon dioxide (80/20) under 1 atm, and glucose (10 mM). After addition of vitamin solution and $Na_2S{\cdot}9H_2O$ as a reducing agent the medium was inoculated with 10 mL fresh samples from each reactor and incubated in respective temperature of inoculums. Bottles with BA medium and inocula only, but without substrates, were used as controls (blanks). All the tests were prepared in duplicate. The SMA was calculated as the initial, linear methane accumulation rate divided by the biomass VS content in each series.

2.4. Microbial community analysis

Bacterial communities in both hydrogen and methane reactors at steady-states were analyzed. Genomic DNA extraction, PCR-DGGE and sequencing were made as previously described (Zhao et al., 2009). Archaeal communities in methane reactors at steady-states were also analyzed. The procedure was similar to bacterial community analysis. The only differences were the PCR primers and amplification procedures. For the first amplification,

Table 1
Characterization of substrates.

	Stillage	Cake	Glycerol	Mixture
pH	3.9 ± 0.1	/	7 ± 0.1	4.2 ± 0.1
TS (%)	4.75 ± 0.15	85.6 ± 1.55	/	6.85 ± 0.05
VS (%)	4.5 ± 0.11	79.6 ± 1.28	/	6.82 ± 0.03
COD (g/L)	61.9 ± 1.8	/	1638 ± 103	97.3 ± 2.1
SCOD (g/L)	20.8 ± 1.9	/	/	29.8 ± 1.2
VFA (g/L)	0.15 ± 0.08	/	/	0.08 ± 0.01
TSS(g/L)	35.4 ± 1.2	/	/	54.8 ± 1.3
VSS(g/L)	34 ± 1.6	/	/	54 ± 2.1
Total nitrogen (g/L)	1.44 ± 0.06	30.6 ± 0.85[a]	0.23 ± 0.01	2.16 ± 0.05
Ammonia (g/L)	0.27 ± 0.05	1.4 ± 0.05[a]	N.D	0.27 ± 0.03
Carbohydrate (g/L)	30	580[a]	/	48.9
Lipid (g/L)	7.5 ± 1.2	35 ± 1.2[a]	51 ± 2.5	8 ± 0.6
Protein (g/L)	7.2 ± 0.8	181 ± 5.02[a]	1.42 ± 0.03	11.3 ± 0.8

"/", not detected.
"N.D", not detectable.
[a] Value expressed in g/kg.

Table 2
Reactor operation data.

Parameter	Single-stage	Two-stage		Two-stage	
	M15	H3	M12	H1	M14
HRT, d	15	3	12	1	14
Working volume, mL	3.5	1.2	3.5	1.2	3.5
Feed rate, mL/d	233	400	292	1200	250