Bioresource Technology 146 (2013) 223–233

Contents lists available at ScienceDirect

Bioresource Technology

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

Microbial community shifts and biogas conversion computation during steady, inhibited and recovered stages of thermophilic methane fermentation on chicken manure with a wide variation of ammonia

Qigui Niu [a], Wei Qiao [a,c], Hong Qiang [b], Yu-You Li [a,d,e,*]

[a] Graduate School of Environmental Studies, Tohoku University, 6-6-06 Aza-Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8579, Japan
[b] College of Resources and Environment Northwest A&F University, Yangling 712100, China
[c] College of Chemical Engineering, China University of Petroleum, Beijing 102249, China
[d] Department of Civil and Environmental Engineering, Graduate School of Engineering Tohoku University, 6-6-06 Aza-Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8579, Japan
[e] Key Lab of Northwest Water Resource, Environment and Ecology, MOE, Xi'an University of Architecture and Technology, Xi'an, 710055, PR China

HIGHLIGHTS

- Microbial community shifts with inhibition was investigated in CH_4 fermentation (55 °C).
- TAN cased VFA accumulation and then synergistically inhibited process over 6 g/L.
- The unrecoverable Archaeal dynamic affected by ammonia makes the resilience weak.
- *Methanothermobacter* has higher tolerance than *Methanosarcina* on ammonia inhibition.
- Hydrolytic and acidogenic bacteria dynamic were significantly after inhibition.

ARTICLE INFO

Article history:
Received 6 June 2013
Received in revised form 5 July 2013
Accepted 10 July 2013
Available online 17 July 2013

Keywords:
Chicken manure
Methane fermentation
Thermophilic
Ammonia inhibition
Microbial community shift

ABSTRACT

The thermophilic methane fermentation of chicken manure (10% TS) was investigated within a wide range of ammonia. Microbiological analysis showed significant shifts in Archaeal and Bacterial proportions with VFA accmulation and CH_4 formation before and after inhibition. VFA accumulated sharply with lower methane production, 0.29 L/g VS, than during the steady stage, 0.32 L/g VS. Biogas production almost ceased with the synergy inhibition of TAN (8000 mg/L) and VFA (25,000 mg/L). Hydrogenotrophic *Methanothermobacter thermautotrophicus* str. was the dominate archaea with 95% in the inhibition stage and 100% after 40 days recovery compared to 9.3% in the steady stage. Aceticlastic *Methanosarcina* was not encountered with coincided phenomenal of high VFA in the inhibition stage as well as recovery stage. Evaluation of the microbial diversity and functional bacteria indicated the dominate phylum of *Firmicutes* were 94.74% and 84.4% with and without inhibition. The microbial community shifted significantly with elevated ammonia concentration affecting the performance.

1. Introduction

The methane fermentation of organic solid wastes, such as the organic fractions of municipal solid waste and agricultural residues have been investigated extensively worldwide. This process serves organic waste stabilization and simultaneously generates energy, thus responding to two of society's most urgent needs: the one for alternative clean energy and the one for more sustainable waste disposal. About 13 million tons of chicken manure (CM) are produced in Japan annually which is a typical agricultural waste well-suited to methane fermentation because it has a high fraction of biodegradable organic matter (Niu et al., 2013).

Compared to mesophilic fermentation (30–40 °C), thermophilic fermentation (50–60 °C) generally results in high methanogenic efficiency, is more economical to operate and eliminates pathogens with sanitizing effects. However, the thermophilic process is more sensitive to changes in the factor with affect operation, such as TS, pH, Volatile Fatty Acid (VFA), ammonia and toxic substrates (Abbassi-Guendouz et al., 2013). Free ammonia (FA) is pH and TAN (total ammonia nitrogen) depended in thermophilic fermentation, and is considered a key inhibitor in chicken manure methane fermentation (Niu et al., 2013). It has been widely accepted that FA is the cause of the inhibition with inactive enzymes and the ease of diffusivity into the cell membranes the NH_3 ionized to NH_4^+, with

* Corresponding author at: Department of Civil and Environmental Engineering, Graduate School of Engineering, Tohoku University, 6-6-06 Aza-Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8579, Japan. Tel.: +81 22 7957464; fax: +81 22 7957465.
E-mail address: yyli@epli.civil.tohoku.ac.jp (Y.-Y. Li).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.07.038

results in a pH imbalance between inside and outside of the cell. This pH change affects both the transportation of the materials and leads to lower enzyme activity (Kadam and Boone, 1996).

Anaerobic digestion usually involves several consequent degradation phases, such as hydrolysis, acidogenesis, and then methanogenesis (Gujer and Zehnder, 1983). High N-content makes CM fermentation easily inhibited by ammonia. Hashimoto (1986) reported that both thermophilic and mesophilic processes are inhibited at a TAN of 2500 mg/L. Compared to the bacteria responsible for hydrolysis and acidogenesis, the inherently low growth rate of methanogen archaea makes the anaerobic systems sensitive to environmental changes (Xing et al., 1997). Different trophic level affects the entire community and thus reducing the efficiency of the process. As functionally establish the tolerance of microbial community for the environment press, it is important to investigate the microbial community in the reactor when characterizing the entire sequential metabolic process as a guide to operation conditions. A previous comparison of the diversity in different anaerobic bioreactors reveals that variations in reactor design, operating conditions and substrate composition have a strong impact on the development of microbial communities (Leclerc et al., 2004).

Although there are studies on microbial communities under thermophilic wet digestion conditions, only a few reports have focus on the characteristic microorganisms and microbial communities in thermophilic methane fermentation on manure (Yabu et al., 2011). Previous researches reported the microbial community mainly focuses on chicken manure composts (He et al., 2013). As such, more information is required about the microbial community in thermophilic CM fermentation in order to understand how to operate the process effectively. Therefore, in this work, a long-term methane fermentation process feeding with 10% TS of CM using a continuously stirred tank reactor (CSTR) was performed to investigate the performance and microbial community in the stable, inhibited and recovery stages of the reactor. The main inhibited factors of the process were also evaluated by principal components analysis (PCA).

2. Methods

2.1. CM properties

Original CM with a TS of 44.3% was kept in the refrigerator at 4 °C. Raw CM was diluted to 10 ± 2% TS content with tap water. The diluted CM was shredded into slurry using a heavy duty laboratory blender and was provided for the CSTR reactor. The shredded CM was stored in a substrate tank with 4 °C cooling water circulation to avoid microbial activity. The raw CM was pretreated to reduce nitrogen through ammonia fermentation and ammonia stripping. The ammonia stripped CM, hereafter referred to as pretreated CM, and had a lower nitrogen substrate than the Raw CM. Both the pretreated CM and raw CM were used in the experiments respectively. The total solid (TS), total volatile solid (TVS), total COD (TCOD), $NH_4^+ - N$ and total nitrogen (TN) were analyzed to ascertain the stability of the substrate. The characteristics of CM are given in Table 1.

2.2. CSTR operation procedure

A lab-scale continuous stirred tank reactor (CSTR) with a working volume of 12 L (total 15 L) was operated under thermophilic (55 ± 1 °C) conditions. The reactor was warmed by water circulation and agitated with a motor (200–300 rpm). A wet gas meter was used to measure the amount of daily biogas. The substrate tank was stirred with (200–300 rpm) to keep the CM in a uniform state. The TS content of the feedstock was adjusted to about 10% with the HRT set at 30 days. After serious inhibition, the feeding was cased and recovery strategy were investigated. After the recovery strategy the process was feed again with 5% TS based on the Food/Microorganism (F/M) ratio. The daily feedstock amount was 0.4 L with OLR approximately 0.21 kg/L/d. The peristaltic influent pump with a timer was used to control the feeding at 12 times per day to reduce feeding shock. Each feed was lower than 1% of the reactor working volume. The seed sludge was taken from anaerobic digestion of the municipal sewage treatment plant.

Table 1
Characteristics of raw CM and ammonia stripping CM.

	Constituent	Unit	Average (n = 6)	SD (±)
Raw CM	TS	(%)	11.2	0.53
	VS	(%)	8.27	0.83
	SS	(%)	10.1	0.11
	VSS	(%)	7.55	0.67
	T-COD	(mg/l)	102600	3200
	TN	(mg/l)	6450	810
	TAN	(mg/l)	3850	200
	C	%	35.2	0.45
	H	%	4.83	0.05
	N	%	5.44	0.24
	O	%	30.12	0.18
	S	%	0.84	0.10
Ammonia stripping CM	TS	(%)	8.93	0.144
	VS	(%)	6.13	0.197
	SS	(%)	0.79	0.049
	VSS	(%)	5.59	0.046
	T-COD	(mg/l)	94400	8230
	TN	(mg/l)	3590	570
	TAN	(mg/l)	2500	100

T-COD: total COD, TN: total nitrogen, TAN: total ammonia nitrogen, C:C element in dry CM.

2.3. Analytical methods

The analyses of pH, alkalinity, COD, $NH_4^+ - N$, TS and TVS were performed according to Japan standard methods (JSWA, 1997). The VFA concentration was determined by gas chromatography (Agilent-6890) equipped with a DB-WAXetr capillary column (30 m 0.53 mm 1 lm) and an FID detector. Biogas was calibrated to that under standard conditions (0 °C; 1.013 bar). The biogas composition was measured by a gas chromatograph (SHIMADZU GC-8A) equipped with a thermal conductivity detector (TCD). An elemental analyzer (Nario EL III CHNS) analyzed the elemental composition of C, H, O, N, and S. FA is pH and TAN concentration dependent and can be calculated according to the following equilibrium equation (Hansen et al., 1998).

$$\frac{NH_3}{TAN} = \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(k)}\right)}}\right)^{-1}$$

Similarly to ammonia, VFA also exist in solution as unionized. The unionized volatile fatty acids were calculated based on the total VFA as HAc concentration by the following equilibrium expression:

$$\text{Free VFA} = \frac{VFA \times 10^{(pk_a - pH)}}{1 + 10^{(pk_a - pH)}}.$$

The effects of TAN and FA on methanogenesis was described and calculated using the extended Boltzmann equation: $y = A_2 + \{(A_1 + A_2)/[1 + \exp[(x - x_0)/d_x]\}$. Where A_1 is initial value (left horizontal asymptote), A_2 is final value (right horizontal