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Characterization of methanogenesis, acidogenesis and hydrolysis in thermophilic methane fermentation of chicken manure

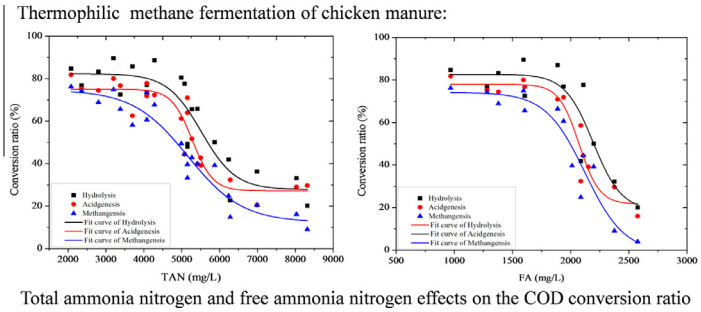
Qigui Niu [a], Toshimasa Hojo [a], Wei Qiao [a,b], Hong Qiang [c], Yu-You Li [a,d,*]

[a] Graduate School of Environmental Studies, Tohoku University, 6-6-06 Aza-Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8579, Japan
[b] State Key Laboratory of Heavy Oil Processing, China University of Petroleum, PR China
[c] College of Resources and Environment, Northwest A&F University, Yangling 712100, China
[d] Department of Civil and Environmental Engineering, Graduate School of Engineering, Tohoku University, Japan

HIGHLIGHTS

- Thermophilic methane fermentation of chicken manure was studied at 10% TS.
- Ammonia inhibition was evaluated by long time operation of continuous experiment.
- Methanogenesis was more sensitive to ammonia than acidogenesis and hydrolysis.
- Microbial community shifts were characterized by 16S rDNA cloning library.

GRAPHICAL ABSTRACT



Total ammonia nitrogen and free ammonia nitrogen effects on the COD conversion ratio

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ABSTRACT

The thermophilic methane fermentation of chicken manure (CM) with a total solids (TS) of approximately 10% was investigated with regard to ammonia inhibition. A gradual increase in total ammonia nitrogen to 6000 mg/L was observed in the case of raw CM fermentation. A distinct rise in VFA accumulation combined with a low methane production of 0.29 L/gVS occurred at a TAN of 4000 ~ 5000 mg/L. Biogas production completely ceased when TAN reached 8000 mg/L after the addition of NH_4HCO_3. The high sensitivity of methanogenesis to TAN and free ammonia (FA) was quantitatively confirmed. The IC_{50} of TAN for methanogenesis, acidogenesis and hydrolysis was 5058, 5305 and 5707 mg/L at a pH value of 8.1 ± 0.2. Similar results but with a lower IC_{50} were obtained for FA inhibition during fermentation. The microbial community analysis revealed significant differences in hydrogenotrophic methanogens and aceticlastic methanogens before and after inhibition.

1. Introduction

Chicken manure (CM) is a typical agriculture waste with a high fraction of biodegradable organic matter. In 2008, about 13 million tons of CM was produced in Japan [1]. This huge amount of CM is mainly treated by incineration and composting. However, due to secondary pollutant emissions, both of these methods are controversial [2].

Methane fermentation, a widely used technology to stabilize organic waste and simultaneously generate energy in the form of biogas [3], has been proposed as a method for treating CM. Thermophilic fermentation in particular has been attracting more and

* Corresponding author. Address: Graduate School of Engineering, Tohoku University, 6-6-06 Aza-Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8579, Japan. Tel.: +81 227957464; fax: +81 227957465.
E-mail address: yyli@epl1.civil.tohoku.ac.jp (Yu-You Li).

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more attention due to the high rate of biogas production, the short hydraulic retention time (HRT) and improved pathogen destruction. Thermophilic fermentation has been applied to many cases, such as sludge, municipal solid waste, cattle manure, poultry manure and corn grain ethanol industry waste treatment [4–6]. Compared with the mesophilic process, however, the thermophilic process is more sensitive to changes in pH, VFA, ammonia and toxic substrates. Nitrogen overloading has been shown to have a significant negative influence on methanogenic activity, and can render a reactor unstable. The TAN concentration of 3000–4000 mg/L is regarded to be at the edge of inhibition. Even at a low TAN of 1700 mg/L, inhibition occurrence has been reported [7]. Hashimoto reported that both thermophilic and mesophilic processes are inhibited at a TAN of 2500 mg/L [8]. Thermophilic fermentation has been reported to be unsuccessful for the relatively low nitrogen content in pig waste [9]. The fermentation of swine manure as the sole substrate has been shown in earlier reports to be unsuccessful, mainly due to the high content of ammonia in the waste [8]. It has been shown that FA is a crucial fraction of TAN for inhibition [10].

Individual volatile fatty acids (VFAs) can be used as process stability indicators in methane fermentation. Among them, butyrate and isobutyrate acid have been shown to provide especially sensitive results [11]. Acetic and propionic acid were the most abundant VFAs in manure digestion. VFA accumulated as Ammonia inhibition occurred, in effect indicating the extent of the inhibition.

It should be noted that CM is a high nitrogen content organic waste with nitrogen levels much higher than those of cow manure, food waste, pig manure and waste active sludge [12]. Hydrolysis in thermophilic is faster than mesophilic which in itself makes CM thermophilic fermentation a higher risk process [13]. More importantly, nitrogen inhibition is the result of chronic accumulation during the operation of the reactor. The high concentration of ammonium and volatile fatty acids (VFAs) tend to occur simultaneously, resulting in a stable pH. It was decided that the only way to get convincing results on the chronic inhibition effect is to operate the reactor in a long term.

Moreover, neither the whole performance nor the mechanism of ammonium inhibition of thermophilic CM fermentation has yet to be revealed in detail. Alternative methods of reducing the nitrogen loading are by diluting CM to a low TS of 0.5–3% [14] or mixing the CM with other livestock manure or sludge [15]. These methods tend to increase the amount of final discharged waste water and result in a more complex process system. The effectiveness of high solid and sole CM fermentation needs further investigation.

In the present research, a 240 days' thermophilic fermentation process using a continuous stirred tank reactor (CSTR) fed with high solid CM was performed to investigate the followings: (1) the methane yields from raw CM and ammonia stripping CM, (2) the inhibition predisposing factor of high solid and high nitrogen CM fermentation, (3) the effect of nitrogen inhibition on methanogenesis, acidogenesis and hydrolysis, (4) the microorganism community evolution before and after inhibition.

2. Material and methods

2.1. CM properties

Original CM with 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 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 stability. The characteristics of CM are given in Table 1.

Table 1
Characteristics of raw CM and ammonia stripping CM.

Constituent	Unit	Average (n = 6)	SD (±)
Raw CM element analysis			
TS	(%)	11.2	0.53
VS	(%)	8.27	0.83
SS	(%)	10.1	0.11
VSS	(%)	7.55	0.67
T-COD	(mg/L)	102,600	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.

2.2. CSTR operation procedure

A schematic diagram of the experimental device is provided in Fig. 1. 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. A wet gas meter was used to measure the daily biogas amount. The substrate tank was stirred with 200–300 RPM to keep the CM at a uniform semisolid state. The TS content of the feedstock was adjusted to about 10% by adding tap water. The HRT was set at 30 days. The daily feedstock amount was 0.4 L with OLR 0.21 kg/gd. 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. Seed sludge with TS of 3.1% was sampled from thermophilic anaerobic digestion of municipal sewage treatment plant.

2.3. Analytical methods

The analyses of pH, alkalinity, COD, total ammonia nitrogen (TAN), TS and TVS were performed according to Japan standard methods [16]. 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. The carrier gas was Helium, with a pressure of 30.4 kPa, injected at a rate of 399 mL min^{-1}. The temperature of the injection port and detector were 250 °C and 250 °C, respectively. The oven temperature was set at 125–180°Cat a speed of 15 °C min^{-1}.

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