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Comparing mesophilic and thermophilic anaerobic digestion of chicken manure: Microbial community dynamics and process resilience

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ABSTRACT

While methane fermentation is considered as the most successful bioenergy treatment for chicken manure, the relationship between operational performance and the dynamic transition of archaeal and bacterial communities remains poorly understood. Two continuous stirred-tank reactors were investigated under thermophilic and mesophilic conditions feeding with 10%TS. The tolerance of thermophilic reactor on total ammonia nitrogen (TAN) was found to be 8000 mg/L with free ammonia (FA) 2000 mg/L compared to 16,000 mg/L (FA1500 mg/L) of mesophilic reactor. Biomethane production was 0.29 L/gV S_{in} in the steady stage and decreased following TAN increase. After serious inhibition, the mesophilic reactor was recovered successfully by dilution and washing stratagem compared to the unrecoverable of thermophilic reactor. The relationship between the microbial community structure, the bioreactor performance and inhibitors such as TAN, FA, and volatile fatty acid was evaluated by canonical correspondence analysis. The performance of methanogenic activity and substrate removal efficiency were changed significantly correlating with the community evenness and phylogenetic structure. The resilient archaeal community was found even after serious inhibition in both reactors. Obvious dynamics of bacterial communities were observed in acidogenic and hydrolytic functional bacteria following TAN variation in the different stages.

1. Introduction

The treatment of chicken manure (CM) by methane fermentation has become increasingly common, as an essential component in the global quest for sustainable energy sources which stabilize waste and minimize the impact on the environment and ecological system (Angenent et al., 2004; Niu et al., 2013b). Generally, mesophilic and thermophilic reactors were the most chosen for the engineering application. In practice, even with a better reduction of volatile solids and the deactivation of pathogens, thermophilic methane fermentation is much more sensitive to environmental perturbations than mesophilic methane fermentation (Pender et al., 2004).

It must be remembered that CM has high nitrogen content due to uric acid and protein, both of which are degraded to ammonia. The total ammonia (TAN) contents are NH_4^+ and free ammonia (FA). The FA has been shown to be pH, temperature and TAN dependent and is known to be a significant cause of inhibition due to its inactive enzymes and affects the transportation of the materials (Kadam and Boone, 1996).

The efficient and stable operation of methane fermentation relies on syntrophic relationships among a community of microbes, including fermenting bacteria, specialized acidogenic and acetogenic syntrophs, and methanogenic archaeas with diverse and parallel pathways for substrate metabolism (Briones and Raskin, 2003). Different microorganisms have different tolerance on the inhibitors, methane fermentation reactors are maintained by the relationships between performance and operating parameters. However, differences underlying bioreactors that perform well and bioreactors that perform inadequately under inhibitors are often poorly understood. Understanding both the community structure and ecology, feeding with high ammonia content substrate is essential to the control of the reactor. Moreover, the dynamic transition of microbial communities with functional resilience has been seldom investigated. Previous researchers have observed stable community structures in functionally stable (LaPara et al., 2002) and unstable (Goberna et al., 2009) bioreactors with microbial community shifts that could be

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explained by reactor conditions (McMahon et al., 2001). The reactor conditions can explain the community structures in functionally stable (LaPara et al., 2002) and unstable (Goberna et al., 2009) bioreactors. A functional diverse microbial community provides a suite of parallel pathways for each metabolism step, as has been reported by Hashsham et al. (2000). Resistance, resilience and redundancy are the three main factors (Allison and Martiny, 2008) shown in the population dynamics: with resistance a population maintains an abundance over time, with resilience a population rebounds following a disturbance, and with redundancy a disturbed population is replaced by a new population whose function makes the original population redundant. The chaotic community shifts were proved in functionally stable bioreactors (Fernandez et al., 1999), and the full-scale community by sampling over a long period of time was established with proving that the resilience of reactor is more important than the community structure (Werner et al., 2011).

Indeed, ammonia overloaded of methane fermentation and its recovered strategies only have a few reported (Niu et al., 2014; Rajagopal et al., 2013). To be useful in the process operation, a microbial community must have a stable metabolic function over time. The objective of this study was to evaluate the relationships between the biomethane production, COD removal efficiency and the impact factors of VFA, TAN, and FA. The microbial communities associated with the functional diversity, evenness in the structure of microbial diversity were profiled. The two CSTRs were compared by evaluating the resilience of both process and functional microbial communities under a wide range of ammonia concentration.

2. Material and methods

2.1. CM properties

Two kinds of CM were used in this experiment, one is ammonia stripping CM which was pretreated to reduce nitrogen through ammonia fermentation and ammonia stripping and the other is raw CM which was diluted to 10 ± 2% TS from the original CM (44.3%). Both of the substrates were kept at 4 °C and feeding to the reactors with the characteristics is shown in Table S1.

2.2. CSTR operation procedure

Two laboratory-scale continuous stirred tank reactors (CSTR) with a working volume of 12 L (total 15 L) were operated under thermophilic (55 ± 1 °C) and mesophilic (35 ± 1 °C) conditions. The substrate tank was stirred to keep the CM in a uniform state. A substrate tank was set at 4 °C with a cooling circulation system to keep the substrate stable. A peristaltic influent pump with a timer was used to control the feeding at 12 times totally 0.4 L per day to reduce feeding shock. Each feed was lower than 1% of the reactor working volume. The HRT was set at 30 days with seed sludge taken from anaerobic digestion of the municipal sewage treatment plant. Both reactors were subject to a recovery strategy involving dilution and washing after exposing in high ammonia concentration.

2.3. Analytical methods

The analyses of pH, alkalinity, COD, NH_4^+–N, TS and TVS were performed according to the Japan standard methods (JSWA, 1997). The VFA concentration was determined by gas chromatography (Agilent-6890). Biogas was calibrated to the standard conditions (0 °C; 1.013 bar). The biogas composition was measured by a gas chromatograph (SHIMADZU GC-8A). FA is calculated according to the following equation (Hansen et al., 1998).

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

The continuous experimental data of VFA were simulated by the modified Gompertz model:

$$C_V = C_{V_{max}} * \exp\left\{-exp\left[\frac{K_{max} * e * (C_{A,0} - C_A) + C_{V_{max}}}{C_{V_{max}}}\right]\right\}$$

where

C_A: Ammonia concentration (mg/L)
C_V: VFA accumulation concentration (mg/L)
K_{max}: The maximum VFA accumulation rate; K_{max} is defined as the tangent in the inflection point
$C_{A,0}$: The TAN concentration of the initiate accumulation under inhibition; C_0, is defined as the x-axis intercept of this tangent (mg/L)
$C_{V_{max}}$: The maximal value of VFA concentration (mg/L)

The microbial community structure was analyzed by 16S rRNA gene cloning and sequencing. Genomic DNA was extracted from samples with an Ultra Clean Soil DNA Isolation Kit (MO-BIO). The amplification of 16S rRNA gene was performed with the primers EUB8F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and Univ1500R (5′-GGT TAC CTT GTT ACG ACT T-3′) for *Bacteria* and A109F (5′-ACK GCT CAGTAACACGT-3′) and 1059R (5′-GCC ATG CAC CWC CTC T-3′) for *Archaea*. Thermal cycling of PCR consisted initial denaturation at 95 °C for 2 min following 30 cycles of each denaturation at 95 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 1.5 min with final extension at 72 °C for 7 min for archaea. With initial denaturation at 94 °C for 30 s, 23 cycles of each denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min were conducted for bacteria. The PCR products were purified by Micro Spin™ S-400 HR (Amersham Pharmacia GE, USA). The purified DNA was cloned with the TOPO TA Cloning®Kit (Invitrogen, USA) and transformed into Escherichia coli DH5$_\alpha$ competent cells. Cloned DNA fragments were obtained and spread on plates. After an incubation period of 24 h at 37 °C, the white ones were randomly picked out and transferred to LB with another 6 h of continuous incubation. An insert check was performed using vector of M13 primers. The successful ones were subjected for sequencing with the 907r primer (supplied at TaKaRa Bio sequencing service) and the ARC1059r primer for *Bacteria* and *Archaea*, respectively. The NCBI Blast search program (http://www.ncbi.nlm.nih.gov/blast/) was used to find close-relatives. The partial sequences of 16S rRNA gene received in this work were deposited in the DDBJ/GenBank/EMBL database (Accession No. AB849977–AB850513). For all of the samples, the Archaeal communities were performed by the 16S rRNA gene-based terminal-restriction fragment length polymorphism (T-RFLP) according to Kobayashi (Kobayashi et al., 2009). The primer of Cy5-labeled ARC1059R (5′-GCC ATG CAC CWC CTC T-3′) and ARC109F (5′-ACK GCT CAGTAACACGT-3′) was used for PCR (the same conditions for clone library construction). The purified PCR products were digested with restriction nucleases Taq I. After ethanol precipitation, the digested PCR products were electrophoresed using a CEQ8000 sequencer (BECKMAN) at 60 °C for 65 min with a CEQ size standard 600 (BECKMAN). T-RF peaks were detected and analyzed by Microbial Community Analysis III (http://mica.ibest.uidaho.edu/trflp.php) and confirmed based on the sequencing date of 16S rRNA gene cloning. All the sequences were grouped into operational taxonomic units (OTUs) calculated by MOTHUR (Schloss et al., 2009). The regression lines of richness index of Chao, Simpsoneven and Shannoneven were also calculated to assaying the cloning library and microbial diversity.