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Acclimation of acid-tolerant methanogenic propionate-utilizing culture and microbial community dissecting

Ying Li[a,1], Yongming Sun[a,b,1], Lianhua Li[a,c,*], Zhenhong Yuan[a,b,c]

[a] Laboratory of Biomass Bio-chemical Conversion, GuangZhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, PR China
[b] Key Laboratory of Renewable Energy, Chinese Academy of Sciences, Guangzhou 510640, PR China
[c] Guangdong Provincial Key Laboratory of New and Renewable Energy Research and Development, Guangzhou 510640, PR China

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ABSTRACT

The acid-tolerant methanogenic propionate degradation culture was acclimated in a propionate-fed semi-continuous bioreactor by daily adjusting the digestate pH. The performance of propionate fermentation, the respond of microbial community structure to the acidic environment, and the microbial network for propionate degradation in the acid-tolerant culture was investigated. The results demonstrated that after long term of acclimation to low pH, the digester could produce methane from propionate at pH 4.8–5.5 with 0.3–0.4 L g^{-1} propionic acid (HPr) d^{-1} of the volatile solids (VS) methane production. The predominant methanogens shifted from acetoclastic methanogens (~87%) to hydrogenotrophic methanogens (~67%) in the bioreactor with the dropping pH, indicating that hydrogenotrophic methanogens were more acid-tolerant than acetoclastic methanogens. *Smithella* (~11%), *Syntrophobacter* (~7%) and *Pelotomaculum* (~3%) were the main propionate oxidizers in the acid-tolerant propionate-utilizing culture. *Methanothrix* dominant acetoclastic methanogens, while *Methanolinea* and *Methanospirillum* were the major H_2 scavengers to support *Syntrophobacter* and *Pelotomaculum* syntrophic propionate degradation.

1. Introduction

Volatile fatty acid (VFA) accumulation, a common problem encountered in anaerobic digestion (AD), often leads to pH drop, which would inhibit methanogenesis and result in methane production losses (Hu et al., 2017; Voelklein et al., 2017; Zhang et al., 2017). The accumulation of VFA, especially of propionate in anaerobic digesters has been recorded repeatedly (Felchner-Zwirello et al., 2013; Gallert and Winter, 2008; Inanc et al., 1996; Zhang and Banks, 2012), which even causes a failure of stable methane fermentation (Ariesyady et al., 2007).

Three possible pathways for anaerobic propionate degradation has been described: syntrophic degradation by acetogens associated with H_2-consuming microbes such as hydrogenotrophic methanogens (Kato and Watanabe, 2010; Worm et al., 2014), dismutation by propionate-oxidizing bacteria (POB) to acetate and butyrate via a six-carbon intermediate (de Bok et al., 2001), degradation by propionate-utilizing sulfate-reducing bacteria with sulfate as electron acceptor (El Houari et al., 2017; Kharrat et al., 2017). Since the limited amount of sulfate in the most of the anaerobic digesters, syntrophic degradation coupled with H_2 removal via methanogenesis would be the major route for propionate degradation (Ariesyady et al., 2007). This dominant propionic acid oxidation pathway, however, is the most thermodynamic unfavourable reaction among the VFA degradation, and occurs only when the partial pressure of hydrogen is low enough (Boone and Xun, 1987). Thus, propionate was often found accumulated in anaerobic digesters.

Utilizing of propionate timely and limiting its accumulation are important for improving anaerobic digestion performance. Bioaugmentation, a practice of introducing specific microorganisms to a system to enhance a desired activity (Schauer-Gimenez et al., 2010), could be one of the approaches to meet this challenge. As the previous studied suggested, bioaugmentation with some specific enriched consortia could reduce VFA accumulation and enhance anaerobic digestion, such as propionate-utilizing culture (Li et al., 2017b; Ma et al., 2009a; Tale et al., 2015), VFA-degrading enrichment (Acharya et al., 2015; Amani et al., 2011), and acetate utilizing consortia (Town and Dumonceaux, 2016).

Due to the pure POB are often difficult to be isolated and cultured, several studies enriched methanogenic propionate-utilizing cultures in anaerobic reactors by feeding with synthetic wastewater containing propionate as the sole carbon source (Li et al., 2017a; Ma et al., 2009b; Shigematsu et al., 2006). For the methanogenic propionate-utilizing

* Corresponding author at: No. 2 Nengyuanlu, Tianhe District, Guangzhou 510640, PR China.
E-mail address: lilh@ms.giec.ac.cn (L. Li).
[1] The first two authors contributed equally to this paper.

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consortia, nevertheless, enrichment conducted under acidic condition and the effect of pH on the shift of microbial community structure has not been well addressed. In our previous study, propionate-utilizing culture was enriched under neutral pH condition (Li et al., 2017a). Considering VFA accumulation is often companied with pH dropping, so the methanogenic propionate-utilizing consortia with high resistance to low pH might play important role in recovering the sick digesters caused by VFA acidosis.

In order to obtain bioaugmentation seed which could be further used to recover the overloaded anaerobic digester or improve the performance of the VFA-accumulated digesters, this study enriched acid-tolerant propionate-utilizing culture by adjusting the digestate pH, which can accelerate the conversion of propionate and acetate to methane under acidic conditions. The microbial community structure and its response to the decreasing pH were characterized through second-generation sequencing techniques. A deeper understanding of the relationships between propionate-oxidizing bacteria and methanogens, as well as the microbial community and substrate catabolism in acidic environment were also revealed.

2. Materials and methods

2.1. Experimental set-up

The inoculum was taken from an anaerobic digester treating crops (Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, China), which performed well for enrichment of propionate-degrading culture in our pervious study (Li et al., 2017a). Before use it was sieved through a 1 mm mesh to remove grit and other solids.

The experiment was carried out in a mesophilic semi-continuous CSTR-type reactor with 1.0 L of working volume, which was initially inoculated with sieved digestate, with headspace flushed with a N_2:CO_2 gas mixture (mixed in 80:20 ratio v/v). An identical hydraulic retention time (HRT) of 20 days was maintained by removing 50 mL of reactor content and replacing it with 50 mL of feed once per day under anaerobic condition.

Sodium propionate was chosen as the sole substrate. The volume of the feed was made up by nutrient medium. The nutrient medium contained the following [mg L^{-1}]: NH_4Cl [400]; $MgSO_4{\cdot}6H_2O$ [250]; KCl [400]; $CaCl_2{\cdot}2H_2O$ [120]; $(NH_4)_2HPO_4$ [80]; $FeCl_3{\cdot}6H_2O$ [55]; and the trace element salts (*i.e.* $CoCl_2{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, $MnCl_2{\cdot}4H_2O$, $CuCl_2{\cdot}2H_2O$, $AlCl_3{\cdot}6H_2O$, $ZnCl_2$, $Na_2WO_4{\cdot}2H_2O$, H_3BO_3, Na_2SeO_3 and $Ma_2MoO_4{\cdot}2H_2O$) [each at 0.5] (Li et al., 2017a).

2.2. Acclimation strategy

The acid-tolerant culture was acclimated by adjusting the daily initial pH of the digestate. The set of daily initial pH of the digestate during each HRT shown as Table 1. HCl was used for pH adjustment.

To avoid the shock of low pH on AD system, the daily initial pH of the bioreactor was started at pH 7, and then step-wise decreased from7.0–6.5–6.0 during the first 3 HRTs (0–60 d). Considering the digester may not adapt to pH of 6 well after first 3 HRTs of running at pH 7.0–6.5–6.0, the following 6 HRTs (60–180 d) repeated same pH change (7.0–6.5–6.0) twice, and then kept adjusting the daily initial pH at 6.0 for another 5 HRTs (180–280 d). For the last 4 HRTs (280–360 d) the pH was set further lower from 5.5–5.0–4.8. The loading rate was set at 0.53 g propionic acid L^{-1} d^{-1}druing the whole experimental period.

2.3. Analytical methods for digestion performance

Methane (CH_4) and carbon dioxide (CO_2) contents were analyzed by GC-2014 gas chromatograph (Shimadzu, Japan) which equipped with TCD detector (120 °C) Porapak Q column (70 °C) and argon as carried gas (20 mL min^{-1}). pH was determined using a FE28-Standard meter (Mettler – Toledo, Switzerland) with a combination glass electrode calibrated in buffers at pH 7.0 and 4.0. VFA were quantified by HPLC system (Waters e2698, USA), equipped with Bio-RAD column at 50 °C and 0.005 mM H_2SO_4 as the mobile phase at the flow rate of 0.5 mL/min.

2.4. Metagenomic DNA extraction and amplification

DNA extraction was performed using the fast DNA spin kit for soil (QBIOgene Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. DNA quality was assessed using gel electrophoresis (1% agarose) and DNA concentrations were determined using a Qubit Fluorometer (Thermo, USA).

The extracted DNA sample was then handled according to the protocol of genomic DNA sample preparation kit (Illumina). The DNA fragmentation was firstly performed using Covaris S2 Ultrasonicator, and the DNA fragments were then processed by end reparation, A-tailing, adapter ligation, DNA size-selection. PCR reaction and products purification based on Illumina Miseq2x300 instructions. For Archaea, the 16S rRNA genes were amplified through three rounds of PCR. The primers for the first round were 340F (5′-CCCTAYGGGGYGCASCAG-3′) and 1000R (5′-GGCCATGCACYWCYTCTC-3′). Then the PCR products were used as templates for a second PCR with 349F (5′-CCCTACACGACGCTCTTCCGATCTN(barcode)GYGCASCAGKCGMG-AAW-3′) and 806R (5′-GACTGGAGTTCCTTGGCACCCGAGAATTCCA-GGACTA CVSGGGTATCTAAT-3′), and the third round PCR amplified with Illumina nested primers. The bacteria 16S rRNA genes were amplified through two rounds of PCR. Firstly, using 341F (5′-CCCTACACGACGCTCTTCCGATCTG (barcode) CCTACGGGNGGCWG-CAG-3′) and 805R (5′-GACTGGAGTTCCTTGGCACCCGAGAA-TTCCAGACTACHVGGGTATCTAATCC-3′).

The PCR products were then used as templates for a second PCR with Illumina Nested primers.

2.5. Whole genome pyrosequencing analysis

Before sequencing, PCR products of different samples were normalized in equimolar amounts in the final mixture, which was used to construct the PCR amplicon libraries. Sequencing was carried out on an Illumina HiSeq 2000. The obtained sequences were phylogenetically allocated down to the phylum, class, and genus level with the RDP classifier (http://rdp.cme.msu.edu/misc/resources). To define the relative abundance of a given phylogenetic group, the number of sequences affiliated to that group was divided by the total number of obtained sequences. The results were used for the analysis and comparison of microbial community structure differences.

3. Results and discussion

3.1. Digestion performance

The digestion performance in terms of volumetric biogas production

Table 1
Daily target pH of AD system during each HRT.

HRT	1	2	3	4	5	6	7	8	9–14	15	16	17–18
Period/d	0–20	20–40	40–60	60–80	80–100	100–120	120–140	140–160	160–280	280–300	300–320	320–360
Target pH	7.0	6.5	6.0	7.0	6.5	6.0	7.0	6.5	6.0	5.5	5.0	4.8