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Vertical distribution of microbial community and metabolic pathway in a methanogenic propionate degradation bioreactor

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ABSTRACT

The methanogenic propionate degradation consortia were enriched in a propionate-fed semi-continuous bioreactor. The microbial community shift with depth, the microbial network and its correlation with metabolic pathway were also investigated. The results demonstrated that the maximum organic loading rate (OLR) of the reactor was 2.5 g propionic acid (HPr) L^{-1} d^{-1} with approximately 1.20 LL^{-1} d^{-1} of volumetric methane production (VMP). The organisms in the enrichment were spanning 36 bacterial phyla and 7 archaeal orders. *Syntrophobacter*, the main Hpr oxidizer in the digester, dominated bacteria with relative abundance changing from 63% to 37% with depth. The predominant methanogens shift from hydrogenotrophic *Methanoculleus* (~60%) at the upper liquid layer to acetoclastic *Methanothrix* (~51%) at the lower sediment layer in the bioreactor. These methanogens syntrophically support *Syntrophobacter* by degrading HPr catabolism by-products (H_2 and acetate). Other bacteria could scavenge anabolic products (carbohydrate and protein) presumably derived from detrital biomass produced by the HPr-degrading community.

1. Introduction

Propionate is a major intermediate of organic matter in the anaerobic digestion, which accounts for 35% of the precursor for methane production (Koch et al., 1983). The syntrophic propionic acid degradation to acetic acid and hydrogen by acetogens coupled with acetic acid and hydrogen removal via methanogenesis is the major route for its degradation in digesters (Ariesyady et al., 2007). This dominant propionic acid oxidation pathway, however, is the most thermodynamic unfavourable reaction among the volatile fatty acids (VFA) degradation, and occurs only when the partial pressure of hydrogen is low enough (Boone and Xun (1987)). Propionate was often found accumulated in anaerobic digesters (Regueiro et al., 2015; Zhang and Banks (2012)), which even causes a failure of stable methane production.

Degradation of propionate and limiting its accumulation are important for improving performance of an anaerobic digester. Bioaugmentation could be one of the approaches to meet this challenge, which is the practice of adding specific microorganisms to a system to enhance a desired activity (Schauer-Gimenez et al., 2010). As the previous studies suggested, adding propionate-utilizing cultures (Li et al., 2017; Schauer-Gimenez et al., 2010; Tale et al., 2015) or VFA-degrading culture (Acharya et al., 2015) could reduce propionate accumulation and improve digestion.

Since the pure propionate-oxidizing bacteria are often difficult to be isolated and cultured, several studies enriched propionate-utilizing cultures in the completely stirred tank reactor (Shigematsu et al., 2006) or the upflow anaerobic sludge bed reactors (Ma et al., 2009) fed with synthetic wastewater containing propionate as the sole carbon source. For the propionate-utilizing enrichment, nevertheless, knowledge of the whole microbial community structure has not been well characterized because of past technological limitations. Second-generation sequencing techniques have revolutionized the microbiome study by producing huge amount of data leading to increased coverage depth further permitting identification of even the less abundant community members (Nathani et al., 2015), which provides a powerful tool for dissecting microbial community structure.

In an attempt to obtain the bioaugmentation consortia, this study enriched propionate-degrading cultures, which can enhance digestion by accelerating the conversion of acetate and propionate to methane. The microbial community structure and its distribution shift with the depth of the reactor were characterized at taxonomic level as well as to

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establish the database for the similar AD system. A more complete understanding of the microbial network and its correlation with metabolic pathway of nutrient utilization was also provided.

2. Materials and methods

2.1. Propionate-degrading consortia enrichment

Inoculum: The inoculum for propionate-degrading consortia enrichment was taken from an anaerobic digester fed with energy crop (70 L, Laboratory of biomass bio-chemical conversion, GuangZhou Institute of Energy Conversion, Chinese Academy of Sciences, China).

Bioreactor: The propionate-degrading consortia were enriched in a BioReactor Simulator (2 L, Bioprocess Control AB, Sweden). The bioreactor is equipped with a funnel-shaped inlet port and an bend outlet port (Fig. 1). The pressure from the fresh feed makes the same volume of automatic discharge. There are two gas outlets through the stopper. One is used for biogas sampling, the other one collects to the gas flow meter for biogas production measurement. The biogas production can be real-time recorded by the computer.

Digestion procedure: The reactor was maintained at 35 ± 1 °C in an water bath and operated in daily fill-and-draw mode with identical hydraulic retention time (HRT) of 20 days by removing appropriate volume of reactor content and replacing it same volume of feed once per day. The bioreactor was running without stir during the whole enrichment process. The feed comprised a certain amount of sodium propionate and the volume was made up with nutrient medium. The nutrient medium contained the following [mg/L]: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]; $CoCl_2{\cdot}6H_2O$ [0.5]; $NiCl_2{\cdot}6H_2O$ [0.5] the trace metal salts $MnCl_2{\cdot}4H_2O$, $CuCl_2{\cdot}2H_2O$, $AlCl_3{\cdot}6H_2O$, $Na_2WO_4{\cdot}2H_2O$, H_3BO_3, Na_2SeO_3 and $ZnCl_2$ [each at 0.5] (Tale et al., 2011). OLR started at 0.5 g Hpr L^{-1} d^{-1} in HRT1 and was then step-wise rising to 3.0 g Hpr L^{-1} d^{-1} by adding the appropriate amount of sodium propionate.

2.2. Sampling for the analyses of digestion performance and microbial community

The bioreactor was running without stir during the whole enrichment process, therefore the solid from the inoculum (mainly straw residue) sediment at the bottom of the reactor lead to two layers present.

The samples for determination of pH, VFA and alkalinity were taken from the effusion of liquid layer of the bioreactor at certain intervals.

For a better understanding of the microbial community distribution with the depth, three samples were taken from the bioreactor on day 260 (relative stable and high digestion performance) at the upper (U), middle (M), and bottom (B) of the digester with the distance from the top of the digestate surface of 3 cm, 8 cm and 13 cm, respectively (Fig. 1), representing the liquid layer, the sediment layer and the junction of both layers.

2.3. Analytical methods for the digestion performance

Biogas was automatically recorded by the computer. pH was determined using a FE28-Standard meter (Mettler – Toledo, Switzerland) with a combination glass electrode calibrated in buffers at pH 7.0 and 9.2. Alkalinity was measured by a Titroline 5000 titrator (Julabo, German) with 0.25 N H_2SO_4 to endpoints of pH 5.7 and 4.3, allowing calculation of total alkalinity (TA), partial alkalinity (PA) and intermediate alkalinity (IA). VFA were quantified in a Waters e2698 High Performance Liquid Chromatography (Waters, USA) with a Bio-RAD column. Biogas composition (CH_4 and CO_2) was determined using a GC-2014 Gas Chromatograph (Shimadzu, Japan) calibrated with 65% (v/v) CH4 and 35% (v/v) CO_2.

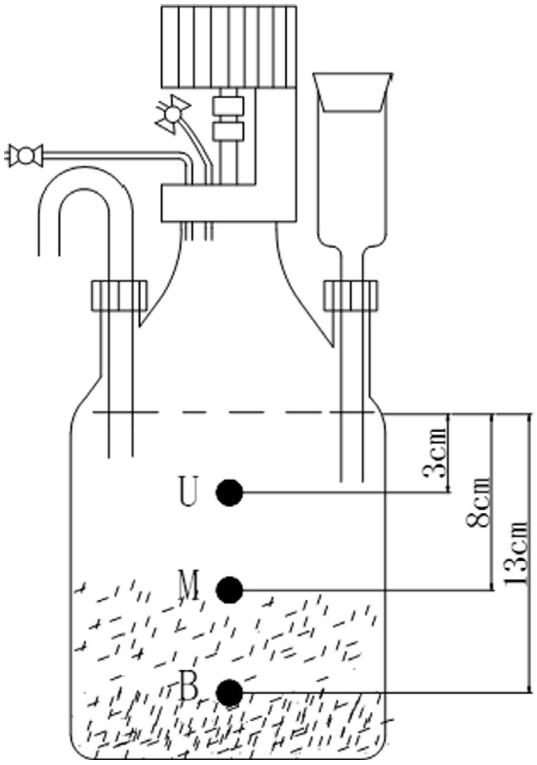

Fig. 1. Schematic diagram of sampling points for microbial community analysis of propionate-degrading enrichment (the depth of the upper (U), middle (M), and bottom (B) sampling points were 3 cm, 8 cm and 13 cm, respectively. The sediment in the bottom was the straw residue from the initial inoculum).

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 Miseq2 × 300 instructions. For Archaea, the 16S rRNA genes were amplified through three rounds of PCR. The primers for the first round were 340F (5′-CCCTAYGGGGYGCA SCAG-3′) and 1000R (5′-GGCCATGCACYWCYTCTC-3′). Then the PCR products were used as templates for a second PCR with 349F (5′-CCCTACACGACGCTCTTCCGATCTN (barcode) GYGCASCAGKCGMGAA W-3′) and 806R (5′-GACTGGAGTTCCTTGGCACCCGAGAATTCCAGGA CTA 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′-CCCTACAC GACGCTCTTCCGATCTG (barcode) CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTGGAGTTCCTTGGCACCCGAGAATTCCAGACTACHVGG GTATCTAATCC-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