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The role of hydrogenotrophic methanogens in an acidogenic reactor

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HIGHLIGHTS

- pH reduction has negative impacts on hydrogenotrophic methanogen in acidogenic phase.
- Hydrogenotrophic methanogen is essential in regulating hydrogen content in biogas.
- Hydrogen can affect the production of volatile fatty acids in acidogenic phase.
- VFA production was recovered after recovery of hydrogenotrophic methanogen population.
- Methane was mainly produced from hydrogenotrophic methanogenesis in acidogenic phase.

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ABSTRACT

A laboratory-scale acidogenic anaerobic sequencing batch reactor was set up to test the effect of pH change on microbial community structure of the reactor biomass and process performance. No immediate performance change on acidogenesis was observed after the pH change. However, as the hydrogenotrophic methanogen population decreased, hydrogen content in biogas increased followed by a sharp decrease in volatile fatty acids (VFAs) with acetic acid (HAc) in particular. Recovery of reactor performance following pH correction was only apparent after recovery of hydrogenotrophic methanogen population. These suggested hydrogenotrophic methanogens played a very important role in performance of the acidogenic process.

1. Introduction

High-strength organic and biodegradable wastewaters, such as food processing wastewater and young landfill leachate, are often treated anaerobically (Speece, 1983; Luostarinen et al., 2007; Onodera et al., 2007; Abu Ghunmi et al., 2010; Bialek et al., 2011). Without large energy requirement, anaerobic treatment is an efficient process to achieve substantial reductions in pollutant content, and the production of methane – an energy source. As energy cost rises, the anaerobic treatment process, as a pretreatment for high-strength organic wastewaters, becomes increasingly favored. Since multiple biological processes (i.e., hydrolysis, acidogenesis, acetogenesis and methanogenesis) are involved in the anaerobic process and these different processes require various optimal operating conditions, the idea of the two-phase (i.e., acidogenic phase and methanogenic phase) anaerobic process had been proposed (Pohland and Ghosh, 1971). Through phase separation, it was argued that optimum growth conditions could be developed for the acidogens and methanogens respectively. The substrate turnover rate therefore could be increased, which consequently would improve treatment efficiency, overall process stability and methane production (Cooney et al., 2007; Rincon et al., 2009).

As the lead phase, the acidogenic phase would not only produce the necessary substrates for the next phase, it was also anticipated to help maintain stability of the overall anaerobic process (Ince, 1998). Various microorganisms, such as acidogens, acetogens, homoacetogens and methanogens, and complex biological conversions between acetate, other volatile fatty acids (VFAs), hydrogen and carbon dioxide are involved in this phase (Mosey, 1983). Acidogens, as one of the key microbial groups in the acidogenic phase, have been extensively investigated. Intuitively it would seem reasonable that only a few studies have investigated methanogens in the acidogenic phase (Mizuno et al., 1998; Shimada et al., 2011). However, methanogens, such as hydrogenotrophic methanogens, do exist and may also play an important role in the acidogenic

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phase. Results of Shimada (2011) showed that hydrogenotrophic methanogens constitute the major archaeal microbes in the acidogenic reactors of two-phase anaerobic digesters, and the methane generation did not necessarily result in lower production of acetic acids (HAc).

Hydrogen, which is an inevitable byproduct during the acidogenic process, has a major impact on acid fermentation as it plays an essential role in keeping the redox balance within the acidogenic phase and so affect composition of the acid products generated from fermentation of organic matters (Mosey, 1983; Madigan and Brock, 2009). Mosey (1983) suggested increased concentration of hydrogen slows down glycolysis but speeds up conversion from pyruvate to butyrate and propionate in acidogenesis reactions by lowering the ratio of $[NAD^+]/[NADH]$. It may also inhibit acetogenesis of butyrate and propionate to acetate by acetogenic bacteria due to thermodynamic reasons. So for a healthy acidogenic phase, production of hydrogen need to be kept as low as possible.

Hydrogenotrophic methanogen can use hydrogen and carbon dioxide as substrate to produce methane. Its role in single-phase anaerobic digestion has never been underestimated and has been suggested to contribute to 28–34% of methane production in single-phase anaerobic digestion (Conrad, 1999). However, its role in the acidogenic phase, where pH is usually less than 6.0, is yet to be studied. It should be noted, due to the heterogeneous nature of acidogens, acidogens are perceived as being more robust and adaptable than methanogens; methanogens in the acidogenic phase where conditions favor the acidogens, are therefore likely more vulnerable and thus potentially more sensitive to environmental changes in the reactor. Environmental condition changes, e.g. pH, may therefore have a larger impact on resident methanogens than acidogens in the acidogenic reactor. A change in the microbial structure in terms of the methanogenic community may then go on to affect the acidogenic process performance. Therefore, research on methanogens therein is essential for a better understanding of acidogenic reactor performance.

In the present study, a laboratory-scale acidogenic anaerobic system was set-up to study the effects of pH changes on the performance of the acidogenic reactor. Microbial community structure was analyzed by quantitative real-time PCR during these periods of stress to understand the role of methanogens in the acidogenic phase.

2. Materials and methods

2.1. AnSBR setup and substrate

A laboratory-scale acidogenic anSBR system (R_A) was operated using a programmable logic controller (PLC) system (Fig. 1). The seed sludge for R_A was obtained from an anaerobic digester at a municipal wastewater treatment plant. The raw seed sludge was filtered through a 600 μm sieve and stored at 4 °C before seeding in the reactors. The anSBR system was fed with synthetic feed simulating high strength organic wastewater. The composition of the synthetic feed is shown in Table 1. pH, feeding, mixing, desludging, settling and decanting processes were controlled with the PLC. Detailed operating parameters and the operating schedule are shown in Tables 2 and 3.

2.2. pH change test on R_A

In order to suppress the methanogenic population and investigate performance change of R_A, on Day 180, the pH was decreased from 5.5 ± 0.2 to 4.5 ± 0.2 and the culture was incubated under low pH for 10 d. Then the pH was changed back to 5.5 ± 0.2 to allow R_A to recover. Other operation details were the same as normal during the pH change test.

2.3. Analytical methods

Influent and effluent samples were collected from feed tanks and reactors routinely for chemical analysis. Volatile fatty acids (VFAs, C_2–C_8) were measured using gas chromatography (Agilent Technologies 7890A GC system, US) with Zebron ZB-FFAP 30 m × 320 μm × 0.5 μm column and a flame ionization detector (FID). Prior to analysis, 0.1 ml of 10% formic acid was added to each 0.9 ml of samples and standards for better dissolution. COD, MLSS, and MLVSS were determined in accordance with Standard Methods (APHA, 1998). MLSS and MLVSS inside the reactors and in the discharge were tracked in order to monitor the SRT.

Biogas was collected using a 3 L or 10 L gasbag (TEDLAR, US), and the volume of biogas generated each day was estimated from the gas bag. Methane, carbon dioxide and hydrogen in the biogas was analyzed using gas chromatography (Agilent Technologies 7890A GC system, US) with (1) an Agilent HayeSep R 0.9 m × 1/8″ × 2.0 mm packed column, (2) an Agilent HayeSep C 3.0 m × 1/8″ × 2.0 mm packed column, (3) an Agilent MolSieve 5A 3.0 m × 1/8″ × 2.0 mm packed column, (4) an Agilent HayeSep Q 0.9 m × 1/8″ × 2.0 mm packed column, and (5) an Agilent MolSieve 13 × 3.0 m × 1/8″ × 2.0 mm packed column with two thermal conductivity detectors (TCD, a front detector for measuring methane and carbon dioxide, and a back detector for measuring hydrogen). Helium was the reference gas for Column 1–3 for detection of methane and carbon dioxide and argon was the reference gas for Column 4–5 for detection of hydrogen.

2.4. DNA extraction

0.5 mL sludge samples were collected in 2 mL plastic tubes, centrifuged at 10000 rpm for 30 s, followed by decantation of the supernatant. The sludge was then washed twice with 1 mL phosphate buffer solution (PBS 1X). The pellets were stored at 4 °C before DNA extraction. Before extraction, the sludge samples were diluted 5 times to reach cell concentration of around 10^{10} mL^{-1}. Total DNA was then extracted from samples using an automated nucleic acid extractor (MagNA Pure Compact, Roche, Germany). The purified DNA was then stored at −20 °C before analysis.

2.5. 16S ribosomal RNA gene real-time quantitative PCR (qPCR)

16S rRNA gene quantifications of the DNA samples were performed on LightCycler 480 II (Roche, Germany). The primer and probe sets specific for two domains: Bacteria (BAC) and Archaea (ARC); two order-level Archaea: Methanomicrobiales (MMB) and Methanobacteriales (MBT); and two family-level Archaea: *Methanosarcinaceae* (MSC) and *Methanosaetaceae* (MST) were used (Yu et al., 2005; Bialek et al., 2011). Most bacteria and methanogens in anaerobic reactors were expected to be covered by these primer and probe sets. MMB and MBT are hydrogenotrophic methanogens, which utilize only H_2 and CO_2 or formate to produce methane; MST only utilize acetate, and MSC utilize acetate as well as various other methyl compounds and hydrogen (Madigan and Brock, 2009). The reaction was performed with a total volume of 20 μL mixture: 10 μL of 2 X LightCycler 480 Probes Master, 4 μL of PCR-grade water, 2 μL of TaqMan probe (final concentration 200 nM), 1 μL of each forward and reverse primer (final concentration 500 nM), and 2 μL of template DNA. The operation processes consisted of a predenaturation step of 10 min at 95 °C, amplification of 55 cycles (10 s) at 95 °C and 30 s at 60 °C, and cooling for 10 s at 40 °C. Standard curves were constructed using those strains corresponding to primer and probe sets used in this experiment