Response of Syntrophic Propionate Degradation to pH Decrease and Microbial Community Shifts in an UASB Reactor

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Introduction

Anaerobic digestion technology is widely used for treating all kinds of organic wastes, including municipal sewage, organic wastewater, animal waste, and agricultural wastes, and simultaneously generates methane as an energy source [2, 6, 48, 52]. During the anaerobic degradation of these organic matters, many intermediates, including propionate, are formed [32, 40]. The main sources of propionate in bioreactors are odd-numbered fatty acids from fat, oil, and carbohydrate [7]. Propionate is easily accumulated when the anaerobic digestion system is disturbed by temperature fluctuation, toxic substances, or high organic load rate [32, 40]. The accumulation of propionate would decrease the system pH and can lead to process failure and instability [13, 15, 42]. In addition, a high concentration of propionate (>2,500 mg/l) may inhibit the activity of propionate-oxidizing bacteria and methanogens, even if pH is maintained near neutral [8]. Therefore, the degradation of propionate is considered as a rate-limiting step in anaerobic digestion systems [1].

Under methanogenic conditions, propionate is converted by cooperation between propionate-oxidizing bacteria and methanogens [32]. Propionate-oxidizing bacteria decompose the propionate into acetate and H₂/CO₂ to supply substrate for methanogens. In return, the methanogens relieve feedback inhibition for the propionate-oxidizing bacteria by transforming acetate and H₂/CO₂ into methane. Propionate-oxidizing bacteria are often difficult to isolate and culture owing to their stringent metabolism requirements [32]. So far, only a few slow-growing propionate-oxidizing bacteria were identified [32]. These bacteria can degrade...
propionate to acetate, CO₂, and hydrogen in the absence of electron acceptors and belong to four genera: Syntrophobacter, Smithella, Pelotomaculum, and Desulfotomaculum. Syntrophobacter and Smithella are grouped into the class 8-proteobacteria in the phylum Proteobacteria, whereas Desulfotomaculum and Pelotomaculum belong to the phylum Firmicutes. Propionate-oxidizing bacteria are extensively present in several anaerobic ecosystems, including flooded soils, freshwater sediments, tundra, wet-wood of trees, landfills, anaerobic granular sludge, and sewage digesters [23, 35, 39, 43, 44]. Their activities and distributions can be influenced by various factors, including temperature, hydraulic retention time (HRT), and hydrogen partial pressure [9, 31, 32]. For instance, the activities of propionate-oxidizing bacteria were significantly inhibited at temperature less than 20°C in an UASB reactor in which P. schinkii was the dominant propionate-oxidizing bacteria [9, 11]. A large amount of P. thermopropionicum is distributed in the internal layers of the thermophilic (55°C) granule [26]. In addition, propionate degradation ability was increased when the HRT was decreased to 4 h from 10 h in an anaerobic reactor, in which the genus Syntrophobacter was the dominant propionate-oxidizing bacteria [12]. However, Ariesyady et al. [4] found high proportions of Smithella spp. and lower proportions of Syntrophobacter spp. in anaerobic sludge, but they did not determine Pelotomaculum spp. Thus, the activities and species distributions of propionate-oxidizing bacteria vary in different systems.

Although some studies of propionate degradation have been performed, there is little information about the effects of pH on the activity and distribution of propionate-oxidizing bacteria. It is worth to investigate the effect of pH decrease on bacterial activity and determine whether it can cause a shift of microorganisms. The current study investigated the response pattern of propionate degradation on pH decrease in an upflow anaerobic sludge blanket (UASB) reactor containing propionate as a sole carbon source. In addition, changes in the population distributions of propionate-oxidizing bacteria and methanogens with pH decrease were analyzed using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE).

Materials and Methods

Bioreactor System

The study was performed in a lab-scale UASB reactor made of transparent plexiglass. The effective volume was 4 L. As shown in Fig. 1, three evenly distributed sampling ports and a solid outlet were installed over the height of the column. The feed was pumped to the reactor by a peristaltic pump. The reactor was wrapped by an electrothermal wire and was maintained at 35°C by a temperature controller. The evolved biogas was collected separately from the upper part of the reactor and entered a water lock. All the water locks and wet gas meters were filled with water of pH 3.0 to prevent dissolution of the biogas.

Feed Stock and Operation

Synthetic wastewater containing propionate as a sole carbon source was fed into a UASB reactor from startup. The propionate concentration in the influent was maintained at 2,000 mg/l. The basic medium used in the reactor was as described previously [9]. The UASB reactor was used for treating propionate synthetic wastewater for 6 months under different temperature conditions [9]. Then the operational temperature was gradually adjusted to 35 ± 1°C. The pH of the reactor was maintained at around 7.5 ± 0.5. The HRT was controlled to 6 h using a peristaltic pump. Since anaerobic sludge is focused on the sludge blanket in an UASB reactor, the pH in the sludge blanket was considered as a control parameter. The pH was measured three times daily and was adjusted constantly by adding NaHCO₃ (pH > 7.0) or HCl (pH < 7.0). The pH in the sludge blanket was gradually decreased in eight stages from 7.1 to 6.8, 6.5, 6.0, 5.5, 5.0, 4.5, and finally 4.0. The pH at each stage was maintained until a stable propionate removal was achieved. At each stable state, sludge samples were collected for microbial community analysis.

Analysis

The biogas volume was measured daily by wet gas meters (LML-1; Changchun Filter Co., Ltd., China). The fraction of CH₄ was measured by gas chromatograph (SP-6800A; Shandong Lunan Instrument Factory, China) equipped with a thermal conductivity detector [9]. The liquid samples from the influent, sampling ports, and effluent were centrifuged at 9,000 × g for 5 min and then treated by 6 M HCl acidification, before assaying for volatile fatty
acids (VFAs) using a gas chromatograph (SP6890; Shandong Lunan Instrument Factory, China) equipped with a flame ionization detector [9].

The pH and MLVSS (mixed liquor volatile suspended solid) were measured according to the procedures described in the standard methods [3].

Chemical oxygen demand (COD) mass balance was calculated with the equation below, where COD$_{\text{final}}$ (mg COD/l) and COD$_{\text{initial}}$ (mg COD/l) are the COD in the effluent and influent, respectively, and CH$_4$ (mg COD/l) and MLVSS$_{\text{increased}}$ (mg COD/l) represent equivalent COD of the produced methane and increased MLVSS for treating 1 L of propionate synthetic wastewater.

\[
\text{COD balance} (\%) = \frac{[\text{COD}_{\text{final}} (\text{mg} \text{ COD/l}) + \text{MLVSS}_{\text{increased}} (\text{mg} \text{ COD/l}) + \text{CH}_4 (\text{mg} \text{ COD/l})]}{\text{COD}_{\text{initial}} (\text{mg} \text{ COD/l})} \times 100
\]

### DNA Extraction, PCR, and DGGE

Genomic DNA was extracted using a DNA extraction kit (MO Bio Laboratories, USA) according to the manufacturer’s instructions. The universal bacterial primers used to perform the polymerase chain reaction are as follows: for bacteria, 341F (5'-CCTACGGGAGGCAGCAG-3', with a GC clamp) and 907R (5'-CCGTCAATTCMTTTGAGTTT-3'); for archaea, 344F (5'-ACGGGGYGCAGCAGGCGCGA-3', with a GC clamp) and 915R (5'-GTGCTCCCCCGCCAATTCCT-3'). The PCR amplification was conducted in a 50 μl volume containing 5 μl 10× ExTaq buffer, 4 μl dNTP mixture (2.5 mM), 1 μl forward primer (20 μM) and 1 μl reverse primer (20 μM), 2.5 ng DNA template, and 0.15 U ExTaq DNA polymerase (Takara, China). The amplification conditions started with a predenaturation of DNA for 10 min at 94°C, followed by 30 cycles for 1 min at 94°C, 1 min at 55°C, and 45 sec at 72°C, followed by a final extension of 10 min at 72°C.

The PCR products were separated using the DCode system (Bio-Rad Laboratories, USA). Polyacrylamide gels with 40%–60% vertical denaturing gradient were prepared. The 15 μl PCR products after making to the same concentration were loaded and electrophoresed at 120 V and 60°C for 10 h. Gels were silver stained as described previously [14]. Denaturing gradient gel electrophoresis bands were excised and dissolved in 30 μl of 1× TE at 4°C for 3 h, and then centrifuged at 12,000 rpm for 3 min. The 3 μl supernatant was used as the template for PCR amplification under the conditions as above described using the same primers without the GC clamp. These PCR products were purified by a Gel extraction mini kit (Watson Biotechnologies, China), ligated into the pMD18-T vector (Takara, China), and then cloned into E. coli DH5α. Three white colonies from each sample were randomly selected for PCR detection, and two positive colonies were selected for sequencing by Sangon Biotech., Ltd. (China), and partial 16S rRNA gene sequences were analyzed using the BLAST program of NCBI. After analysis, 20 partial 16S rRNA gene sequences were submitted to GenBank under the accession numbers KT896487–KT896493 and KT960984–KT960994.

### Results and Discussion

#### Effects of pH Decrease on the Propionate Degradation

Under an organic loading rate of 12 kg COD/m$^3$/day and 35°C conditions, the effects of pH decrease on propionate degradation were investigated in an UASB reactor. The UASB reactor was continuously operated for 240 days with a HRT of 6 h. Fig. 2 shows the daily variations in pH, propionate removal, methane content, biogas production, and acetate content. As the only independent variable, the pH was reduced gradually with decreased influent pH. However, the pH was increased from bottom to top in the same operational stage. In addition, the pH in the three sampling ports was similar when the pH of the sludge

**Fig. 2.** Performance data (pH, propionate removal, methane content, biogas production, acetate content) of the UASB reactor.
blanket was higher than 6.5. However, when the pH of the sludge blanket was lower than 6.0, the pH in sampling ports 2 and 3 was always higher than the pH in sampling port 1.

The total propionate removal reached 93.6% at pH 7.5–6.8 and most of the propionate (81.5%–90%) was degraded by the sludge blanket (Table 1 and Fig. 3). This is the previously reported favorable pH range for most propionate-oxidizing bacteria [32]. When the pH in the sludge blanket was swiftly lowered to 6.5, the total propionate removal was rapidly reduced to 75.9% and the propionate removal of the sludge blanket was only 60.6% (Figs. 2 and 3). After 20 days of operation, the propionate removal reached 67.6% in the sludge blanket and the total propionate removal stabilized at 88.5% (Table 1 and Fig. 3). When the pH was further decreased to 6.0, 5.5, and 5.0, the propionate removal of the sludge blanket declined dramatically to 49.1%, 33.8%, and 16.7%, respectively. These results indicated that the activities of propionate-oxidizing bacteria were markedly inhibited by decreasing pH. After a certain time of operation, propionate removal in the sludge blanket was gradually increased and reached a steady state with propionate removal levels of 56.9%, 45.5%, and 26.2% for the pH conditions of 6.0, 5.5, and 5.0. During the steady operation at pH 6.0, 5.5, and 5.0, the contribution rate of propionate degradation in the suspended layer was 31.6%, 40.6%, and 54.1%, respectively (Fig. 3). The higher level of propionate degradation was likely related to the higher pH in the suspended layer (sampling 2 and 3) of about 6.0–5.0, a pH range that most propionate-oxidizing bacteria can endure [32]. Under the cooperation between the sludge blanket and the outside of the sludge blanket, the total propionate removal of the UASB reactor at pH 6.0, 5.5, and 5.0 was 86.1%, 82.1%, and 79.3%, respectively. When pH was decreased from 5.0 to 4.5, the propionate removal of the sludge blanket was quickly reduced and reached to a steady state with propionate removal of 12.3% (Table 1). Propionate degradation mainly occurred in the suspended layer at pH 4.5, composing 75.2% of total propionate removal (Fig. 3). The final pH decrease from 4.5 to 4.0 resulted in barely detectable propionate decomposition (Fig. 2).

The results in Figs. 2 and 3 indicate that propionate degradation is closely related to pH, and a low pH of ≤6.5 significantly inhibited the metabolic ability of propionate-oxidizing bacteria. Similarly, Dhaked et al. [19] also reported faster propionate anaerobic conversion at neutral or weak alkaline pH (7.0 to 8.0) compared with conversion

<table>
<thead>
<tr>
<th>Stage</th>
<th>Propionate removal (%)</th>
<th>Acetate content in effluent (mg/l)</th>
<th>Biomass (gMLVSS/l)</th>
<th>Specific methane production rate (L CH₄/kg COD removed · day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.5</td>
<td>98.6 ± 1.5</td>
<td>55.6 ± 6.9</td>
<td>9.2 ± 0.6</td>
<td>312.8 ± 18.5</td>
</tr>
<tr>
<td>pH 7.1</td>
<td>95.1 ± 2.3</td>
<td>69.4 ± 10.5</td>
<td>9.5 ± 0.5</td>
<td>317.7 ± 15.6</td>
</tr>
<tr>
<td>pH 6.8</td>
<td>93.6 ± 1.6</td>
<td>25.4 ± 4.3</td>
<td>10.0 ± 0.7</td>
<td>330.9 ± 30.7</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>88.5 ± 1.3</td>
<td>94.6 ± 12.6</td>
<td>10.3 ± 0.1</td>
<td>307.6 ± 20.5</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>86.1 ± 2.5</td>
<td>58.3 ± 11.5</td>
<td>9.6 ± 0.4</td>
<td>283.0 ± 16.8</td>
</tr>
<tr>
<td>pH 5.5</td>
<td>82.1 ± 0.9</td>
<td>42.3 ± 7.2</td>
<td>7.3 ± 0.7</td>
<td>267.9 ± 15.9</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>79.3 ± 2.7</td>
<td>43.7 ± 9.1</td>
<td>6.5 ± 0.8</td>
<td>257.6 ± 19.6</td>
</tr>
<tr>
<td>pH 4.5</td>
<td>70.1 ± 1.8</td>
<td>43.1 ± 8.6</td>
<td>5.6 ± 0.3</td>
<td>190.7 ± 20.1</td>
</tr>
<tr>
<td>pH 4.0</td>
<td>0</td>
<td>0</td>
<td>4.9 ± 0.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 3. The contribution rate of propionate removal in each stage.
at weak acid (pH 6.0).

**Methane Production Response to pH Decrease**

The data in Fig. 2 show that the effects of pH also decreased methane production. Methane content and biogas production were similar at pH 6.8–7.5, a favorable pH range for most methanogens [34]. Methane content and biogas production at pH 6.8–P H 7.5 were maintained at 55.2%–69.3% and 22.8–26.7 l/day, respectively. When the pH in the sludge blanket was decreased to 6.5 from 6.8, the methane content was hardly changed but the biogas production was reduced by 27.7%. The biogas production gradually increased and reached to 23.0 l/day at steady state. Two pH decreases, from 6.5 to 6.0 and then 5.5, led to reductions in methane contents by 5.1% and 33.7%, respectively. The biogas production was decreased to 15.3 l/day with the pH decreased to 6.0 from 6.5 and the biogas production was reduced to 19.0 l/day after 9 days of operation at pH 5.5. After a long-term operation, the methane contents at pH 6.0 and 5.5 were recovered to 51.4% and 46.2%, respectively. The biogas production similarly gradually increased to the previous level. Although hydrogen and acetate did not markedly accumulate in this study, some studies reported that the feasible pH range for acetotrophic methanogens is 6.6–7.3 and methanogenesis is obviously inhibited when the pH is lower than 6.2 [18]. The explanation for this requires further investigation.

When the pH in the sludge blanket was further decreased from 5.5 to 5.0 and then 4.5, the methane content hardly changed but the biogas production was reduced by 22.7% and 32.8%, respectively. After about 20 days running, the biogas production reached a steady state with 18.1 l/day at pH 5.0 and 14.5 l/day at pH 4.0. During the final operation stage (pH 4.0), there was little detectable production of biogas.

It is obvious from Figs. 2 and 3 and Table 1 that both propionate-oxidizing bacteria and methanogens exhibited high activity at neutral (6.8 to 7.5) conditions. At acidic (pH 6.5–4.5) conditions, methanogens still showed relatively higher metabolic ability with a specific methane production rate of 190.7–307.6 L CH₄/(kgCOD removed·day). However, propionate removal was significantly inhibited at pH 6.5–4.0. This indicates that propionate-oxidizing bacteria are more sensitive to acid environment than methanogens in this experimental reactor.

Investigation of carbon mass balance enables monitoring and distribution of the metabolic products involved in an anaerobic fermentation process [16]. The amount of metabolic products formed from propionate during the steady-state periods of COD mass balance are listed in Table 2. The closure of the COD balances at 68.3%–111.7% verifies the reliability of the data. The data in Table 2 show that COD balance was decreased as pH decreased from pH 6.0, likely due to sludge loss. During the experiment, the suspended substances in the effluent increased with pH decrease.

**Microbial Community Shift with pH Decrease**

**Bacteria.** The performance of an anaerobic biological treatment system is primarily linked to the structure of the microbial community in the reactor. To investigate the bacterial composition in anaerobic sludge at each operational

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### Table 2. COD mass balance under different pH conditions.

<table>
<thead>
<tr>
<th>Stage</th>
<th>COD_{inlet}</th>
<th>COD_{removal}</th>
<th>Acetate_{removal}</th>
<th>Propionate_{removal}</th>
<th>COD balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.5</td>
<td>3,017 ± 50.5</td>
<td>102.2 ± 16.9</td>
<td>96.7 ± 1.5</td>
<td>42.7 ± 2.5</td>
<td>96.7 ± 1.5</td>
</tr>
<tr>
<td>pH 7.1</td>
<td>3,056 ± 75.6</td>
<td>222.5 ± 30.4</td>
<td>92.7 ± 2.3</td>
<td>148.2 ± 3.7</td>
<td>92.7 ± 2.3</td>
</tr>
<tr>
<td>pH 6.8</td>
<td>3,010 ± 45.2</td>
<td>217.9 ± 41.5</td>
<td>91.6 ± 1.6</td>
<td>190.7 ± 10.5</td>
<td>91.6 ± 1.6</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>3,005 ± 50.3</td>
<td>445.3 ± 32.6</td>
<td>85.2 ± 1.3</td>
<td>344.1 ± 13.6</td>
<td>85.2 ± 1.3</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>3,020 ± 80.7</td>
<td>480.9 ± 43.7</td>
<td>84.1 ± 2.5</td>
<td>418.5 ± 50.3</td>
<td>84.1 ± 2.5</td>
</tr>
<tr>
<td>pH 5.5</td>
<td>3,153 ± 66.9</td>
<td>581.5 ± 57.2</td>
<td>81.4 ± 0.9</td>
<td>536.3 ± 25.6</td>
<td>81.4 ± 0.9</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>2,995 ± 77.5</td>
<td>737.5 ± 35.1</td>
<td>75.4 ± 2.7</td>
<td>690.9 ± 35.7</td>
<td>75.4 ± 2.7</td>
</tr>
<tr>
<td>pH 4.5</td>
<td>2,936 ± 65.3</td>
<td>903.7 ± 45.3</td>
<td>69.2 ± 1.8</td>
<td>834.5 ± 13.6</td>
<td>69.2 ± 1.8</td>
</tr>
<tr>
<td>pH 4.0</td>
<td>3,075 ± 46.9</td>
<td>1,107 ± 95.1</td>
<td>68.3 ± 3.9</td>
<td>1,038 ± 101.7</td>
<td>68.3 ± 3.9</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Stage</th>
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</tr>
</tbody>
</table>

*1 mg VSS = 142 mgCOD

VSS_{removed} = [(VSS_{t-1} − VSS_{t})/(V/L) × 1000] × 142 mgCOD/[Time (day)] × (Total influent/day)]

(V, Working volume of reactor)

(“−”, VSS_{removed} (mg COD/l) could not be calculated because the VSS was reduced.)
stage, PCR-DGGE of the partial 16S rRNA gene sequence was performed (Fig. 4A). DGGE-based molecular analysis has been extensively applied to monitor the changes in the microbial diversity in organics in anaerobic treatment systems [24, 46, 50]. Understanding changes in community distributions provides insight into the metabolic function of microorganisms in biological treatment systems and allows the optimization of operational conditions [46]. As shown in Fig. 4A, seven bands were obtained from the DGGE gel and sequenced. The phylogenetic placement based on the partial 16S rRNA gene sequences determined from these bands and reference 16S rRNA gene sequences obtained from the NCBI database was constructed by MEGA 3.1 software (Fig. 4B).

As shown in Fig. 4B, all of the obtained sequences were grouped into three groups in the phylogenetic tree. 16S rRNA gene sequences of three bands were related to previously identified propionate-oxidizing bacteria. Two DGGE bands belonged to the genus Pelotomaculum in the phylum Firmicutes. The DGGE band B3 showed 96% sequence similarity with P. schinkii, and band B4 was related to P. propionicicum. These two propionate-oxidizing bacteria from Pelotomaculum are obligately syntrophic bacteria, which grow only in co-culture with methanogens [17, 27]. The band B6 was related to the genus Smithella within phylum Proteobacteria with 99% sequence similarity to S. propionica. P. propionicicum and S. propionica can grow in a pH range of 6.5–7.5 and 6.3–7.8, respectively. However, the pH range of P. schinkii for growth is unknown [33]. Pelotomaculum spp. degrade propionate by the methylmalonyl-coenzyme A pathway. S. propionica oxidizes propionate to acetate and butyrate via an integration of two molecules of

**Fig. 4.** The DGGE map (A) and phylogenetic relationship (B) of bacteria in different pH conditions. All reference 16S rRNA gene sequences are from the NCBI database. The tree was constructed using the neighbor-joining method. The bar represents five substitutions per 100 nucleotide positions. Bootstrap probabilities are indicated at branch nodes.
propionate, followed by syntrophic β-oxidation of butyrate to acetate [33]. Butyrate degradation is thermodynamically easier than propionate so that product inhibition is easily eliminated, leading to the promotion of propionate oxidation by *S. propionica* [38]. This unique metabolic pathway of *S. propionica* may be favorable for propionate degradation under acidic condition.

The present study showed that the genera *Smithella* and *Pelotomaculum* may be the main propionate-oxidizing bacteria in this UASB system. Similarly, *Smithella* dominated in a mesophilic anaerobic reactor fed with different concentrations of propionate under neutral conditions [5]. In chemostat experiments containing propionate as the sole carbon source, high dilution rates were found to promote *Pelotomaculum* and low dilution rates promoted *Syntrophobacter* spp. at pH 7.0 [45]. In contrast, Worm *et al.* [51] found that *Syntrophobacter* was stable in UASB reactors treating propionate synthetic wastewater at pH 7.0. However, *Smithella* and *Pelotomaculum* competed with *Syntrophobacter* for propionate consumption when the UASB reactor was not supplied with molybdenum, tungsten, and selenium for a long term [51].

As shown in Fig. 4, propionate-oxidizing bacteria relevant band B3 was enhanced with the pH decrease from 7.1 to 6.0, indicating that this propionate degrader (band B3) is slightly acid tolerant. In contrast, the signals of band B4 and band B6 decreased as pH decreased. Overall, the pH decrease reduced propionate-oxidizing bacteria, resulting in a decrease in propionate removal by the sludge blanket (Fig. 3). These propionate-oxidizing bacteria (bands B3, B4, and B6) were also present in the suspended layer at pH 5.5–4.5, likely because the pH in the suspended layer was higher than that of the sludge blanket (Figs. 2 and 4). Other bands have not shown obvious succession with decrease in pH from 7.5 to 6.0 and then to be reduced.

Although propionate was used as the sole carbon source, some non-propionate-oxidizing bacteria were also present in this system. These non-propionate-oxidizing bacteria might utilize the products of microbial disintegration or intermediates of propionate degradation as substrates for growth. As shown in Fig. 4B, bands B1 and B7 showed 99% sequence similarity with *Petrimonas sulfuriphila* BN3 from the phylum Bacteroidetes. *P. sulfuriphila* BN3 is a mesophilic fermentative bacterium isolated from a biodegraded oil reservoir in Canada, whose favorable pH for growth is 7.2 [22]. Band B5 was closely related to the Bacteroidetes QEEB2AH08 (CU918164), a bacterium whose function has not been characterized. Interestingly, *Syntrophomonas* spp. were also observed in this UASB reactor. Band B2 was similar to *S. zehnderi* OL-4, which is a mesophilic syntrophic fatty-acid-oxidizing bacterium. It can degrade C_4–C_8 compounds when in co-culture with methanogens [47]. The existence of this bacterium might be related to the presence of *Smithella propionica* in the reactor. Intermediate butyrate can be produced during the *S. propionica* oxidation of propionate, and butyrate can be used as a substrate for *S. zehnderi* [33].

**Archaea.** Propionate anaerobic oxidation depends on the syntrophy of propionate-oxidizing bacteria and methanogens in methanogenic environments [9, 37]. Methanogens are important for eliminating the products from propionate oxidation and then driving the propionate degradation process. In this study, methanogens allowed the acetate concentration to always be less than 140 mg/l in the effluent. Moreover, the hydrogen content was lower than the detection limit of the gas chromatograph during the whole operational period (Fig. 2). Therefore, it is significant to know the change in the population of the methanogens as pH decreases in the UASB system (Figs. 5 and 6).

The 13 excised DGGE bands were successfully sequenced...
and a similar search using the BLAST program in the NCBI database was performed. As shown in Fig. 6, all of the obtained sequences grouped into three orders in the phylogenetic tree. The DGGE bands A1–A4 and A9 were affiliated with genus *Methanobacterium* within order *Methanobacterales*, with 97%–100% sequence similarity to *Methanobacterium* sp. MO-MB1, *M. beijingense* 4-1, and *M. petrolearium*. They can use H$_2$/CO$_2$ and formate as substrate for growth at pH 6.5–8.6 [28, 36, 41]. Four bands (A5–A7 and A10) were grouped into the order *Methanosarcinales*, showing 99–100% similarity with genus *Methanoseta*, in which species are specialists in utilizing acetate [34]. *Methanoseta* species were previously identified as the dominant acetotrophic methanogens in a variety of anaerobic reactors with low acetate concentrations [54]. The presence of *Methanoseta* species usually leads to an improved granulation process and results in a more stable bioreactor performance [29, 49]. Bands A8 and A12 with other 16S rRNA gene sequences formed a unique branch in the phylogenetic tree, corresponding to the order *Methanomicrobiales*. The band A8 was matched with *Methanospirillum hungatei*, a hydrogenotrophic methanogen, whose optimal pH range for growth was 6.6–7.4 [20]. Band A12 was related to *Methanolfis limitans*, which was isolated from a fluidized bed reactor and can use H$_2$/CO$_2$ and formate as substrates [53]. Two bands (A11 and A13) showed 99% sequence similarity with *Methanosarcina barkeri*, which can use H$_2$/CO$_2$ and formate as substrates.

**Fig. 6.** Phylogenetic relationships of methanogens in different pH conditions. All references 16S rRNA gene were sequenced from the NCBI database. The tree was constructed using the neighbor-joining method. The bar represents two substitutions per 100 nucleotide positions. Bootstrap probabilities are indicated at branch nodes.
and acetate with low affinity [21].

It was obvious from Figs. 5 and 6 that the methanogenic composition was remarkably changed by pH decrease, especially the hydrogenotrophic methanogens. In the sludge blanket, the bands A1, A2, and A9 (Methanobacterium) were detected at pH 5.0–4.0, pH 6.0–4.0 and pH 6.8–5.0, respectively. The signal of band A8 (Methanospirillum) was enhanced as pH decreased from 7.5 to 6.0 and then stabilized. Bands A3 and A4 (Methanobacterium) maintained similar signal intensity during the whole operational period. The Methanobrevibacter species group (band A12) was unique at pH 4.5 in the suspended layer. Although the pure cultures about hydrogenotrophic methanogens can grow at pH 6.5–8.6, this study indicated that these methanogens can grow at pH as low as 4.5 [28, 36, 41]. These results showed that most detectable hydrogenotrophic methanogens can grow at low pH conditions. Similarly, Kim et al. [30] reported that the hydrogen-utilizing methanogens were more tolerant to acidic conditions than the other methanogens. Some hydrogenotrophic methanogens could be observed at the acidogenic phase (pH 6.0–6.2) in an anaerobic baffled reactor with four compartments treating sugar refinery [10]. In addition, hydrogenotrophic methanogenesis existed in an acidic (pH 4.4) peat and attempts to obtain acetotrophic methanogenic enrichment was unsuccessful [25]. Presumably, it is because the conversion of H2/CO2 to CH4 is energetically more favorable than that of acetate [34].

For acetotrophic methanogens, all members of the Methanoseta species group (bands A5, A6, A7, and A10) were reduced with pH decrease. In the sludge blanket, the bands A5, A6, and A7 disappeared at pH 5.5, 6.5, or 5.0, separately. However, bands A5 and A7 were observed at pH 5.5–4.5 in the suspended layer, consistent with a higher pH (Fig. 2). The shift of band A11 (Methanosarcina) was similar to band A10, whereas band A13 was only detected in the suspend layer. Although acetotrophic methanogens were reduced with pH decrease, the acetate concentration in the effluent remained low. Acetate might be syntrophically oxidized to CO2 by the concerted activity of acetate-oxidizing anaerobes and hydrogenotrophic methanogens in this system [25].

A better understanding of the microbial community succession with pH change in the UASB reactor will allow improvement of propionate removal and stability during anaerobic digestion. The stepwise pH decrease from 7.5 to 4.0 significantly inhibited propionate removal (98.6% to 70.1%). PCR-DGGE analysis confirmed that propionate-oxidizing bacteria (Pelotomaculum and Smithella) were remarkably reduced at low pH (pH ≤5.5) conditions. Most detectable hydrogenotrophic methanogens can endure a low pH (pH 5.5–4.0), but acetotrophic methanogens deceased significantly at pH ≤5.5.

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References


