Microbial community and methanol conversion during long-term continuous operation of a methanol-fed UASB reactor

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Abstract
A mesophilic (35˚C) UASB reactor was operated for treating synthetic wastewater containing methanol as sole carbon source at various organic loading rate. The reactor was successfully operated at OLR 30kg COD/m³/d for approximately 300 days with over 95% soluble COD₃ removal efficiency. Granules of 0.1 to 2 mm in diameter mainly consist of aggregated coccoid cells. Specific methanogenic activity tests indicate that the methanol-methane pathway and the methanol-H₂/CO₂-methane pathway are predominant in the methane fermentation of the methanol. The results of cloning and fluorescence in situ hybridization analysis suggests that Methanomethylovorans hollandica were predominant in the reactor, and formed a large cluster as a granule, which is different from the Methanosarcina-dominant community previously considered in the researches on anaerobic methanol treatment.

Keywords
Up-flow anaerobic sludge blanket reactor; Methanol; Granulation; Microbial community; Metabolic pathway

INTRODUCTION
Anaerobic treatment of methanol-containing wastewater using up-flow anaerobic sludge blanket reactor is an economically attractive process. However, its dispersed granule with poor settling ability can limit high-rate treatment. It is not clear whether granulation on methanol as sole carbon source proceeds satisfactorily in the long term (Weijma and Stams, 2001) although granulation on methanol with volatile fatty acids (VFA) such as acetate and propionate as co-substrate proceeds well (Fukuzaki and Nishio, 1997; Nishio et al., 1993). Although anaerobic treatment of methanolic wastewater has been investigated (Lettinga et al., 1979; Bhatti et al., 1995; Fukuzaki and Nishio, 1997; Zandvoort et al., 2004), microbial community depending on methanol conversion pathway is still unclear. To sustain granules satisfactorily in a long-term operation of methanol-fed UASB process, it is important to understand the mechanism governing microbial community structure in the reactor.

The objective of this study is to investigate the relationship between microbial community and methanol conversion pathway in the long-term methanol-fed UASB reactor.

MATERIAL AND METHODS
Experimental setup
The seed granule was taken from a full-scale UASB reactor treating food-processing wastewater. A UASB reactor with a working volume of 5 L was used in this study. The temperature of the reactor was controlled at 35˚C. The composition of 1 L of the synthetic wastewater used in this study was as follows: methanol, 10000 mg as COD₃; K₂HPO₄, 250 mg; KH₂PO₄, 100 mg; NH₄Cl, 850 mg; MgSO₄ 7H₂O, 100 mg; KCl, 750 mg; MgCl₂ 6H₂O, 125 mg; FeCl₂ 4H₂O, 42 mg; CoCl₂ 6H₂O, 4.2 mg; NiCl₂ 6H₂O, 4.2 mg; CaCl₂, 15 mg; NaHCO₃, 5000 mg.
Specific methanogenic activity test
Specific methanogenic activity (SMA) test was studied by batch test using 120 ml serum vials filled with 50 ml of media and 2 g granules taken from the reactor during phase 2. In this study, we used 4 different media, in which methanol, acetate, formate and $\text{H}_2/\text{CO}_2$ as sole carbon source, respectively. Except $\text{H}_2/\text{CO}_2$, 2 g/L as COD$_{Cr}$ of each carbon sources was added.

Microbial community analysis
The granule sludge used in this experiment was taken from the sampling port of the reactor (250 mm height from the bottom) during phase 2.

RESULTS AND DISCUSSION
Operation performance
The time courses of the (a) COD$_{Cr}$ loading rate, (b) Biogas production rate, (c) pH, (d) Effluent COD$_{Cr}$ in the reactor are shown in Fig. 1. Fig.2 shows the average methane production rate (a), the average COD$_{Cr}$ removal efficiency (b) and the SS profiles at day 227 and 372 in phase 2 (c) at different OLR. Between OLR 2.5 and 60 kg COD$_{Cr}$/m$^3$/d, the average methane production rate proportionally increased with OLR. The average total and soluble COD$_{Cr}$ removal efficiencies were within 90-97% and 98-99% between OLR 20 and 60 kg COD$_{Cr}$/m$^3$/d, respectively.
Specific methanogenic activity
Table 1 shows SMA profiles of the granules at different height during phase 2. SMAs for acetate and formate were too low to be detected while those for methanol and H₂/CO₂ were clearly detected. Both upper and downer located granules showed larger SMAs for methanol than those for H₂/CO₂. These results suggest the methanol-methane pathway and the methanol-H₂/CO₂-methane pathway are predominant in the methane fermentation of the methanol.

Typical structure of the granule
Scanning electron microscope (SEM) images of a typical granule taken from the reactor is illustrated in Fig.3. Granules were irregular-shaped and from 0.1 to 2 mm in diameter. Whole of a granule mostly consists of identical shaped coccoid cells. Each cells aggregated and formed a cluster. Predominance of coccoid cells in methanol-fed UASB reactors was reported by some researchers (Bhatti et al., 1995; Fermoso et al., 2008).

Microbial community
Table 2 summarizes the archaeal 16S rRNA gene clone library constructed using DNA extracted from the granules. All of the analyzed clones were classified into 5 OTUs, and 40 clones (83% of the total clones) were affiliated with *Methanomethylovorans hollandica* with 99% sequence identity. *M.hollandica* can directly convert methanol into methane, and moreover, it is characterized by formation of a cluster consisting aggregated coccoid cells. Three clones were 97% sequence identity to *Methanobacterium aarhusense*, and 1 clone was 100% sequence identity to *Methanobacterium subterraneum*. One clones was close related to *Methanolinia tarda*. All of *M.aarhusense*, *M.subterraneum* and *M.tarda* are formate and H₂ utilizing methanogen. Two clones were affiliated with *Methanoseta concilli*, which was acetoclastic methanogen.

Fig.4 illustrates a 4',6-diamidino -2-phenylindole (DAPI) staining image (a), hybridization image with probe ARC915 (b) and hybridization with probe Mmv667 (c) in an identical microscopic field in the FISH experiment using granule samples. As shown in this figure, most archaeal cells present in the granule were stained by the probe targeting at the genus *Methanomethylovorans*. The *Methanomethylovorans*-targeted cells formed clusters as observed in SEM experiment. Considering the results of SMA test, clone analysis and FISH, *M.hollandica* were predominant in the reactor, and responsible for the direct conversion of methanol into methane. It is noted that our finding is different from the *Methanosarcina*-dominant community previously considered in the researches (Nishio et al., 1993; Bhatti et al., 1995; Fermoso et al., 2008) on mesophilic anaerobic methanol treatment.

![Fig.3 SEM images of a typical granule taken from the reactor](image-url)
Table 2 Archaeal 16S rRNA gene clone library in the reactor

<table>
<thead>
<tr>
<th>OTU</th>
<th>Relative abundance</th>
<th>Closest isolates</th>
<th>Identity</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me1</td>
<td>40/48</td>
<td>Methanomethylovorans hollandica (AY260433)</td>
<td>623/627 (99%)</td>
<td>Methylamine</td>
</tr>
<tr>
<td>Me2</td>
<td>3/48</td>
<td>Methanobacterium aarhusense (AY386124)</td>
<td>613/630 (97%)</td>
<td>H₂, Formate</td>
</tr>
<tr>
<td>Me3</td>
<td>2/48</td>
<td>Methanosaeta concilii (M59146)</td>
<td>622/634 (98%)</td>
<td>Acetate</td>
</tr>
<tr>
<td>Me4</td>
<td>1/48</td>
<td>Methanolinea tarda (AB162774)</td>
<td>597/629 (94%)</td>
<td>H₂, Formate</td>
</tr>
<tr>
<td>Me5</td>
<td>1/48</td>
<td>Methanobacterium subterraneum (DQ649330)</td>
<td>628/628 (100%)</td>
<td>H₂, Formate</td>
</tr>
</tbody>
</table>

Fig.4 Fluorescence in situ hybridization of homogenized sludge taken from the reactor. A DAPI staining image (a), hybridization image with probe ARC915 (fluorescein labeled) (b) and hybridization with probe (Cy3 labeled) (c) in an identical microscopic field.

CONCLUSION
Conclusions of this study are summarized as follows:
1) Stable and successful operation with over 95% soluble COD removal efficiency was carried out at OLR 30 kg COD/m³/d for approximately 300 days.
2) Granulation successfully proceeded during OLR 30 kg COD/m³/d.
3) SMA tests indicate that the methanol-methane pathway and the methanol-H₂/CO₂-methane pathway are predominant in the methane fermentation of the methanol.
4) The results of cloning and fluorescence in situ hybridization analysis suggests that Methanomethylovorans hollandaica were predominant in the reactor, and formed a large cluster as a granule.

REFERENCES