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/m^3/d for approximately 300 days with over 95% soluble COD_{Cr} 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-H_2/CO_2-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 COD_{Cr}; K_2HPO_4, 250 mg; KH_2PO_4, 100 mg; NH_4Cl, 850 mg; $MgSO_4$ $7H_2O$, 100 mg; KCl, 750 mg; $MgCl_2$ $6H_2O$, 125 mg; $FeCl_2$ $4H_2O$, 42 mg; $CoCl_2$ $6H_2O$, 4.2 mg; $NiCl_2$ $6H_2O$, 4.2 mg; $CaCl_2$, 15 mg; $NaHCO_3$, 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 H_2/CO_2 as sole carbon source, respectively. Except H_2/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.

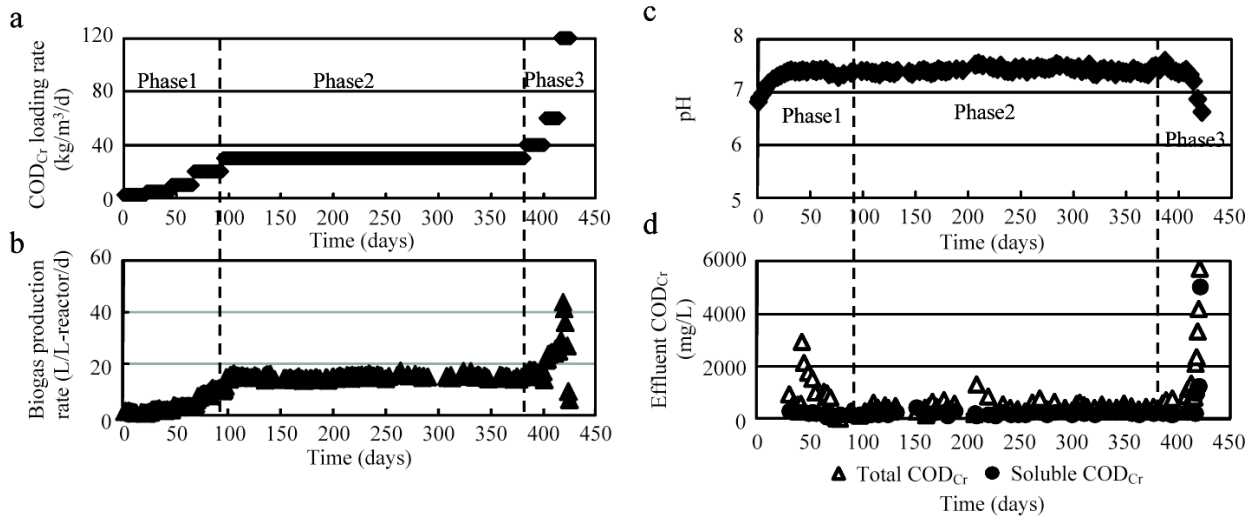

Fig.1 Evolution of reactor performance during experimental period. (a) COD_{Cr} loading rate, (b) Biogas production rate, (c) pH, (d) Effluent COD_{Cr}.

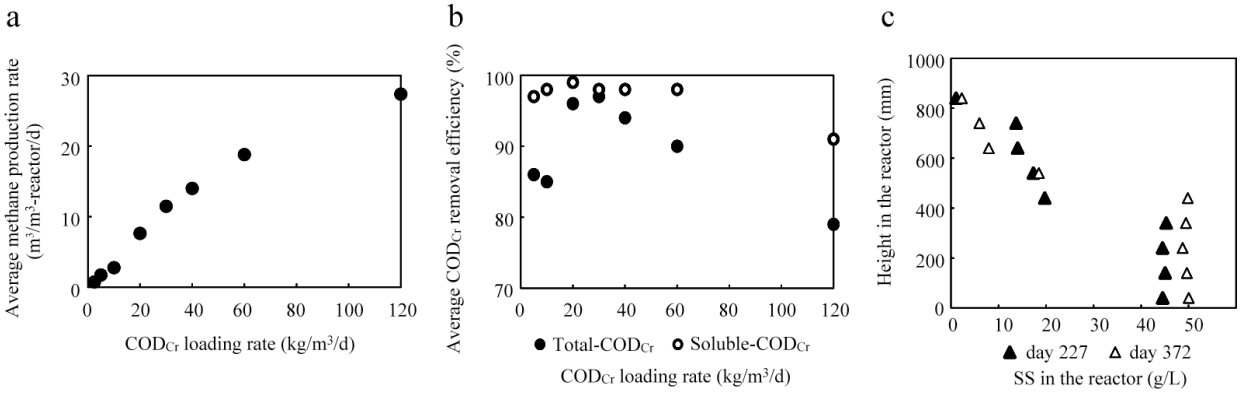

Fig.2 Average methane production rate (a). Average CODCr removal efficiency (b). SS profiles in the reactor (c).

Table 1 Specific methanogenic activity of granules during phase 2

Substrate	Methanogenic activity (ml CH_4/g VSS/d)	
	Hight (40 mm)	Hight (440 mm)
Acetate	Not detected	Not detected
Formate	Not detected	Not detected
H_2/CO_2	366	196
Methanol	516	384

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 H_2/CO_2 were clearly detected. Both upper and downer located granules showed larger SMAs for methanol than those for H_2/CO_2. These results suggest the methanol-methane pathway and the methanol-H_2/CO_2-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 H_2 utilizing methanogen. Two clones were affiliated with *Methanosaeta 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.

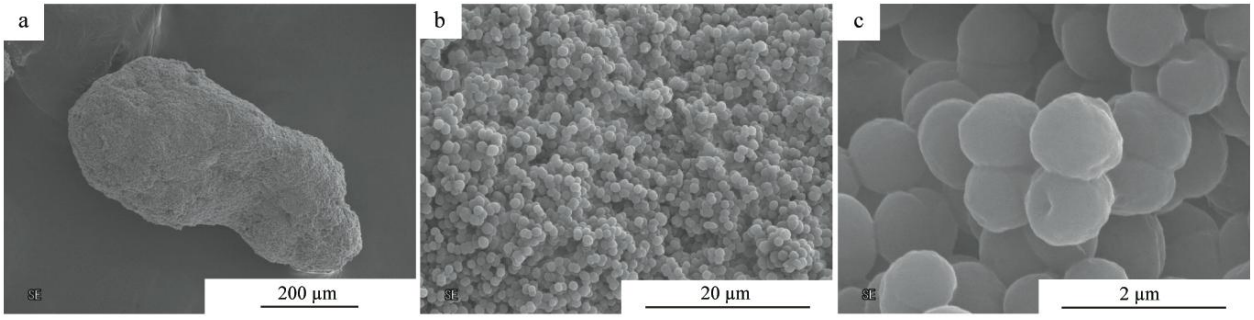

Fig.3 SEM images of a typical granule taken from the reactor

Table 2 Archaeal 16S rRNA gene clone library in the reactor

OTU	Relative abundance	Closest isolates	Identity	Substrates
Me1	40/48	*Methanomethylovorans hollandica* (AY260433)	623/627 (99%)	Methylamine
Me2	3/48	*Methanobacterium aarhusense* (AY386124)	613/630 (97%)	H_2, Formate
Me3	2/48	*Methanosaeta concilii* (M59146)	622/634 (98%)	Acetate
Me4	1/48	*Methanolinea tarda* (AB162774)	597/629 (94%)	H_2, Formate
Me5	1/48	*Methanobacterium subterraneum* (DQ649330)	628/628 (100%)	H_2, Formate

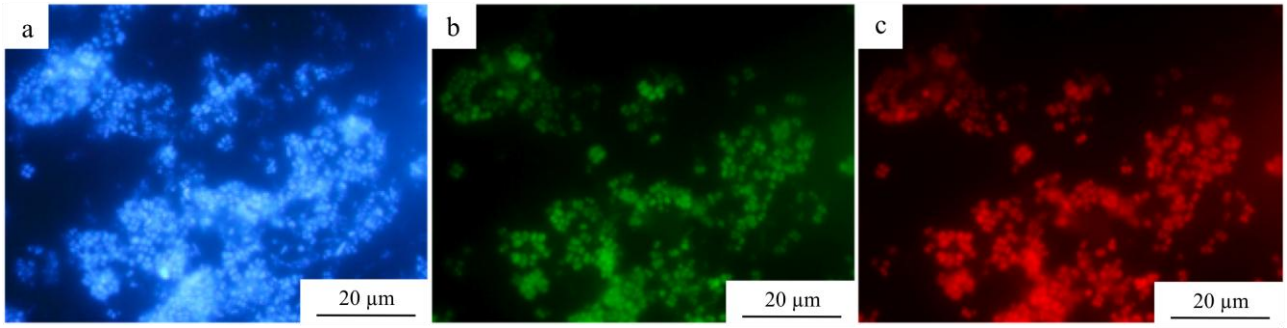

Fig.4 Fluorescence in situ hybridization of homogenized sludge taken from the reactor. A DAPI staining image (a), hybridization image with probe ARC915 (fluorescein labeled) (b) and hybridization with probe (Cy3 labeled) (c) in an identical microscopic field.

CONCLUSION

Conclusions of this study are summarized as follows:

1) Stable and successful operation with over 95% soluble COD_{Cr} removal efficiency was carried out at OLR 30kg COD/m^3/d for approximately 300 days.

2) Granulation successfully proceeded during OLR 30kg COD/m^3/d.

3) SMA tests indicate that the methanol-methane pathway and the methanol-H_2/CO_2-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 hollandica* were predominant in the reactor, and formed a large cluster as a granule.

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