Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Anaerobic treatment of municipal wastewater at ambient temperature: Analysis of archaeal community structure and recovery of dissolved methane

Wasala M.K.R.T.W. Bandara [a], Tomonori Kindaichi [b], Hisashi Satoh [a,*], Manabu Sasakawa [c], Yoshihito Nakahara [c], Masahiro Takahashi [a], Satoshi Okabe [a]

[a] Division of Environmental Engineering, Faculty of Engineering, Hokkaido University, North-13, West-8, Kita-ku, Sapporo 060-8628, Japan
[b] Department of Civil and Environmental Engineering, Graduate School of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashihiroshima, Hiroshima 739-8527, Japan
[c] Mitsubishi Rayon Co., Ltd., Aqua Development Center, 4-1-2 Ushikawadori, Toyohashi, Aichi 440-8601, Japan

ARTICLE INFO

Article history:
Received 14 June 2012
Received in revised form
25 July 2012
Accepted 30 July 2012
Available online 7 August 2012

Keywords:
Archaeal community structure
Degassing membrane
Dissolved methane
Municipal wastewater
Psychrophilic condition
Upflow anaerobic sludge blanket process

ABSTRACT

Anaerobic treatment is an attractive option for the biological treatment of municipal wastewater. In this study, municipal wastewater was anaerobically treated with a bench-scale upflow anaerobic sludge blanket (UASB) reactor at temperatures from 6 to 31 °C for 18 months to investigate total chemical oxygen demand (COD) removal efficiency, archaeal community structure, and dissolved methane (D-CH_4) recovery efficiency. The COD removal efficiency was more than 50% in summer and below 40% in winter with no evolution of biogas. Analysis of the archaeal community structures of the granular sludge from the UASB using 16S rRNA gene-cloning indicated that after microorganisms had adapted to low temperatures, the archaeal community had a lower diversity and the relative abundance of acetoclastic methanogens decreased together with an increase in hydrogenotrophic methanogens. D-CH_4, which was detected in the UASB effluent throughout the operation, could be collected with a degassing membrane. The ratio of the collection to recovery rates was 60% in summer and 100% in winter. For anaerobic treatment of municipal wastewater at lower temperatures, hydrogenotrophic methanogens play an important role in COD removal and D-CH_4 can be collected to reduce greenhouse gas emissions and avoid wastage of energy resources.

1. Introduction

Anaerobic treatment is an attractive option for the biological treatment of municipal wastewater (Latif et al., 2011; Kayranli and Ugurlu, 2011; Lew et al., 2011; Elefsiniotis et al., 1996). It has numerous advantages, including the generation of useful energy (i.e., biogas), no energy requirement for aeration, and the reduction of the cost of sludge treatment. In temperate regions, the ambient temperature of municipal wastewaters is considerably lower than the optimum value for anaerobic treatment processes (Latif et al., 2011; Dhaked et al., 2010). To operate anaerobic treatment processes under mesophilic conditions (30 °C–40 °C), a significant input of energy is required to heat the influent wastewaters, resulting in a loss of energy. Thus, the operation of an anaerobic bioreactor for municipal wastewater treatment at ambient temperature in

* *Corresponding author*. Tel./fax: +81 (0)11 706 6277.
E-mail address: qsatoh@eng.hokudai.ac.jp (H. Satoh).
0043-1354/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.watres.2012.07.061

temperate regions offers economic advantages over the operation under mesophilic or thermophilic (45 °C–60 °C) conditions.

Anaerobic treatment of low-strength wastewaters at ambient or low temperatures has recently been successfully demonstrated (Latif et al., 2011; Kayranli and Ugurlu, 2011; Lew et al., 2011; Urban et al., 2007; Alvarez et al., 2008). However, further studies are required to anaerobically treat low-strength municipal wastewaters at low temperatures. Specifically, chemical oxygen demand (COD) removal rate and methane (CH_4) production rate are low owing to lower microbial activity under psychrophilic (<20 °C) conditions (Latif et al., 2011; Dhaked et al., 2010). The economic feasibility of the long-term low-temperature anaerobic treatment relies on sufficient microbial activity to ensure reliable wastewater treatment. However, little is known about psychrophilic methanogenesis. In some systems mesophilic methanogenic communities that adapt to low temperatures contributed to methanogenesis, in contrast, specific psychrophilic methanogenic communities carried out methanogenesis in others. (Dhaked et al., 2010). Greater insights into the relationship between process performance and microbial characteristics in the anaerobic treatment of municipal wastewaters at low temperature are required to achieve stable COD removal and biogas production.

It is also important to consider dissolved methane gas (D-CH_4) in the anaerobic treatment of municipal wastewaters. D-CH_4 in anaerobic treatment effluent is not usually recovered, which results in greenhouse gas emission from the anaerobic treatment process and loss of a potential energy resource (Urban et al., 2007). The loss of D-CH_4 is enhanced at lower temperatures because of the increase in CH_4 solubility at reduced temperatures (Bandara et al., 2011). Thus, it is the case with the anaerobic treatment of municipal wastewaters at low temperatures. In these processes, as much D-CH_4 as possible from the anaerobic treatment effluent should be collected. Several studies have investigated the collection of D-CH_4 from the anaerobic treatment of wastewaters by physical gasification based on gas–liquid equilibrium and mixing with gas or a paddle (Hatamoto et al., 2010; Matsuura et al., 2010; Hartley and Lant, 2006; Pauss et al., 1990). However, the collection efficiency of D-CH_4 was low and/or the recovered CH_4 gas concentration was low using these technologies. Our previous research demonstrated that degasification with a degassing membrane (DM) could effectively collect D-CH_4 without reducing its concentration in the anaerobic treatment of a low-strength synthetic wastewater at low temperature (Bandara et al., 2011). The DM only allows gas molecules to pass through the non-porous layer of the DM (Bandara et al., 2011; Matsunaga et al., 2012). Thus, the DM effectively separates dissolved gases from the liquid. In the present research study, we operated a bench-scale upflow anaerobic sludge blanket (UASB) reactor, of which the liquid outlet was connected to the DM reactor, to treat raw municipal wastewater at ambient temperature (from 6 °C to 31 °C) over 18 months. The performance of the UASB reactor was monitored. The archaeal community structures of the UASB reactor were analysed using 16S rRNA gene-cloning techniques. Additionally, the D-CH_4 collection efficiency by degasification was investigated.

2. Materials and methods

2.1. Experimental setup and operating conditions

A UASB reactor (height, 80 cm; diameter, 5 cm; working volume, 1.6 L) was operated from January 2010 to June 2011. The reactor was inoculated with 0.7 L of anaerobic granular sludge obtained from the bench-scale UASB reactor operated in our laboratory (Bandara et al., 2011). It had total and volatile solids concentrations of 24 g/L and 18 g/L, respectively. The UASB reactor was fed with domestic wastewater from the Souseigawa municipal wastewater treatment plant in Sapporo, Japan (Okabe et al., 1999). The concentrations (average ± standard deviation) of total COD (T-COD) and the dissolved fraction of COD (D-COD), and pH in the wastewater were 173 ± 38 mg/L, 78 ± 17 mg/L, and 7.2 ± 0.2, respectively. The hydraulic retention time (HRT) was changed in response to changes in the COD removal efficiency (see Fig. 1b). The temperature, which was not controlled, varied from 6 °C to 31 °C. A 20-cm-high filter media (polyester nonwoven fabric sheets; Japan Vilene Co., Ltd., Tokyo, Japan) was installed in the upper part of the UASB reactor on June 22, 2010, to avoid biomass washout. A DM reactor was connected to the effluent of the UASB reactor to collect the residual D-CH_4 in the effluent according to a study described previously. The characteristics of the DM have been described in detail elsewhere (Bandara et al., 2011). D-CH_4 was collected into the lumen of the hollow fibres of the DM using a vacuum pump and the transmembrane pressure was set to 80 kPa using a vacuum gauge. A transmembrane pressure of 97 kPa was also tested after April 25, 2011, to investigate the effect of transmembrane pressure on D-CH_4 collection efficiency.

2.2. DNA extraction and PCR amplification of 16S rRNA genes

Total DNA was extracted from the granular sludge inoculum, termed INO, and a mature granular sludge, termed PSY, in the UASB reactor on day 416 (February 15, 2011) using a Fast DNA spin kit (MP Biomedicals, Irvine, CA) according to the manufacturer's instructions. To construct archaeal clone libraries, the 16S rRNA gene fragments from the isolated total DNA were amplified using a ONE Shot LA PCR MIX kit (TaKaRa Bio Inc., Ohtsu, Japan) and a primer set of arc109f (Lueders and Friedrich, 2000) and univ1390r (Zheng et al., 1996). The PCR condition for the archaea was as follows: 5 min of initial denaturation at 94 °C, followed by 25 cycles of 30 s at 94 °C, 30 s at 50 °C, and 1 min at 72 °C. The final extension was conducted for 5 min at 72 °C. All PCRs were performed using a total volume of 50 μL containing 1 μg of DNA as the template. The PCR products were electrophoresed in a 1% (wt/vol) agarose gel.

2.3. Clone library construction and phylogenetic analysis

The archaeal clone libraries were constructed using previously described methods (Kindaichi et al., 2011). DNA sequencing was performed by Dragon Genomics Center, TaKaRa Bio Inc. (Yokkaichi, Japan). The 16S rRNA gene