Bioresource Technology 102 (2011) 10299–10304

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Dissolved methane oxidation and competition for oxygen in down-flow hanging sponge reactor for post-treatment of anaerobic wastewater treatment

Masashi Hatamoto [a,b,*], Tomo Miyauchi [c], Tomonori Kindaichi [a], Noriatsu Ozaki [a], Akiyoshi Ohashi [a]

[a] Department of Civil and Environmental Engineering, Graduate School of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashihiroshima, Hiroshima 739-8527, Japan
[b] Department of Environmental Systems Engineering, Nagaoka University of Technology, 1603-1, Kamitomiokamachi, Nagaoka, Niigata 940-2188, Japan
[c] Civil & Environmental Engineering Program, Faculty of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashihiroshima, Hiroshima 739-8527, Japan

ARTICLE INFO

Article history:
Received 22 May 2011
Received in revised form 22 August 2011
Accepted 22 August 2011
Available online 27 August 2011

Keywords:
Greenhouse gas
Methane oxidation
Anaerobic treatment
Post-treatment
Down-flow hanging sponge (DHS)

ABSTRACT

Post-treatment of anaerobic wastewater was undertaken to biologically oxidize dissolved methane, with the aim of preventing methane emission. The performance of dissolved methane oxidation and competition for oxygen among methane, ammonium, organic matter, and sulfide oxidizing bacteria were investigated using a lab-scale closed-type down-flow hanging sponge (DHS) reactor. Under the oxygen abundant condition of a hydraulic retention time of 2 h and volumetric air supply rate of 12.95 m^3-air m^{-3} day^{-1}, greater than 90% oxidation of dissolved methane, ammonium, sulfide, and organic matter was achieved. With reduction in the air supply rate, ammonium oxidation first ceased, after which methane oxidation deteriorated. Sulfide oxidation was disrupted in the final step, indicating that COD and sulfide oxidation occurred prior to methane oxidation. A microbial community analysis revealed that peculiar methanotrophic communities dominating the *Methylocaldum* species were formed in the DHS reactor operation.

1. Introduction

Anaerobic waste/wastewater treatment technology has several advantages over the conventional aerobic treatment system, such as a lower energy requirement and reduced production of excess sludge. In recent years, anaerobic treatment process has been focused not only on its cost-saving advantages but also its eco-friendly nature in terms of the energy recovery as methane gas and reduction in CO_2 emissions. And thus its applications are expanding in various types of wastewaters, including low-strength wastewaters (Kassab et al., 2010; Takahashi et al., 2011). However, the anaerobic process discharges unrecovered methane as dissolved methane in the effluent. Methane is a greenhouse gas with a 25-fold greater effect on global warming than CO_2 (Forster et al., 2007), thus reduction of dissolved methane emission is significantly important for further popularization of anaerobic treatment technologies.

To date, few studies have been conducted on the removal and/or recovery of dissolved methane from anaerobic wastewater treatment. Hartley and Lant (2006) applied microaeration to reduce the dissolved methane effusion. To recover and remove the dissolved methane, Matsuura et al. (2010) employed a two-stage closed down-flow hanging sponge (DHS) reactor; the first DHS reactor recovered the dissolved methane as burnable gas, whereas the second reactor oxidized residual dissolved methane. We have been developing a biological dissolved methane oxidation system using the single closed DHS reactor (Hatamoto et al., 2010), which revealed that methane oxidation occurred preferentially over ammonium oxidation in the reactor; however, the interactions of methane oxidation with sulfide and organic carbon oxidation remain to be investigated. The DHS reactor has been developing as a low cost post-treatment for the anaerobic treatment process and applied to several types of wastewaters (Machdar and Faisal, 2011; Takahashi et al., 2011; Tandukar et al., 2007); thus, it is a promising method for removing dissolved methane as well as polish up the anaerobically treated effluent.

In this study, in order to clarify the effects of hydraulic retention time (HRT) and air supply rate on methane, ammonium, sulfide, and COD removal, a lab-scale closed DHS reactor was operated using artificial anaerobic wastewater, and the reactor performance was evaluated. Further, the microbial community structure based on the 16S rRNA gene sequence and the succession of methane-oxidizing bacterial communities based on the particulate methane monooxygenase (*pmoA*) gene were also investigated.

2. Methods

2.1. Experimental set-up and operational conditions

In this study, a closed DHS reactor of a 4 L column (112 cm in height and 6.8 cm in diameter) was used. In the closed DHS reactor,

* Corresponding author at: Department of Civil and Environmental Engineering, Graduate School of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashihiroshima, Hiroshima 739-8527, Japan. Tel.: +81 258 47 9642; fax: +81 258 47 9637.
E-mail address: hatamoto@vos.nagaokaut.ac.jp (M. Hatamoto).

0960-8524/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2011.08.099

a string of 31 polyurethane sponge-cubes ($2 \times 2 \times 2$ cm in size) connected diagonally in series with each other was hanged. The hanging string of sponge-cubes was 1 m in vertical length with a working volume of 0.25 L, based on sponge volume (Fig. 1). To inoculate the sludge, the string of sponge-cubes was soaked in diluted activated sludge from a wastewater treatment plant treating municipal sewage. The artificial wastewater with dissolved methane, which was made by purging artificial wastewater with methane gas (100% [v/v]), was fed into the system from the top of the reactor. The artificial wastewater contained in 1 L: 11 mg KH_2PO_4; 5 mg $CaCl_2{\cdot}2H_2O$; 33 mg $MgCl_2{\cdot}6H_2O$; 16 mg KCl; 150 mg NH_4Cl; 40.5 mg Na_2S; 25 mg COD propionate; and 25 mg COD acetate. The trace elements solution was added at the previously described concentration (Hatamoto et al., 2010). The pH in the feed was maintained at 8.1 by addition of 500 mg L^{-1} $NaHCO_3$. The reactor was maintained at 20 °C and was operated as shown in Table 1.

2.2. Analytical methods

The concentrations of NH_4^+, NO_2^-, NO_3^-, and SO_4^{2-} were routinely measured by ion chromatography (Hatamoto et al., 2010). COD was analyzed using an HACH water quality analyzer (HACH DR4000). For measurement of effluent COD, a small amount of hydrochloric acid was added and then purged with nitrogen gas to remove sulfide. Gas compositions in the influent, inside of the column from the gas sampling port, and the off gas were determined by a gas chromatograph equipped with thermal conductivity detector (Shimadzu GC-14BPT). Acetate and propionate concentrations of influent and effluent were measured using a gas chromatograph equipped with flame ionization detector (Shimadzu GC-14BPF). At the end of each operational phase, sludge samples were collected from the upper, middle, and lower parts of the reactor sponge and used for further analysis. Dissolved CH_4 in the influent and effluent was measured by the headspace technique described previously (Hatamoto et al., 2010). Dissolved methane concentration was calculated based on Henry's Law using quantities of equilibrated headspace methane and Bunsen's coefficient. Concentrations of suspended solids and volatile suspended solids (VSS) in the retained sludge were determined according to the standard method (APHA-AWWA-WEF, 1995). Elemental sulfur was extracted from sludges with acetone as the solvent in an ultrasonic water bath for 1 h. The concentration of elemental sulfur was determined using high performance liquid chromatography (HPLC LC-10A; Shimadzu, Kyoto, Japan) with a COSMOSIL type $5C_{18}$-AR-II column (Nacalai Tesque, Kyoto, Japan) and UV detector (SPD-10AVvp; Shimadzu Co., Kyoto, Japan). The mobile phase was methanol at a flow rate of 1 mL min^{-1} and sulfur was detected at 230 nm.

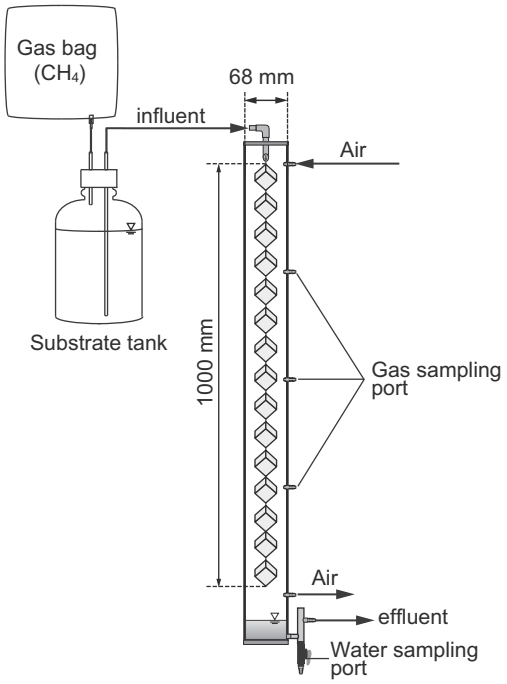

Fig. 1. Schematic diagram of the closed DHS reactor. The artificial wastewater is purged with pure methane gas to ensure a 100% methane atmosphere.

The calculations for flow and removal rate were based on sponge volume. Oxygen consumption rates of CH_4, NH_4^+, and sulfide oxidations were calculated based on the theoretical oxygen demands of 4 g-O_2 g-CH_4^{-1}, 4.57 g-O_2 g-N^{-1}, and 1.5 g-O_2 g-S^{-1} from the CH_4 and NH_4^+ removal rate and SO_4^{2-} production rate, respectively.

2.3. Cloning and sequencing of the 16S rRNA gene and phylogenetic analysis

Sludge samples were squeezed and collected from the upper, middle, and lower parts of the reactor sponge and washed with phosphate buffer. DNA was extracted from the washed sludges using the Fast DNA spin kit for soil (MP Biomedicals, Irvine, CA), as described in the manufacturer's instructions. Extracted DNA was used for amplification of bacterial 16S rRNA gene fragments with primer pairs of EUB338F/1492R (Hatamoto et al., 2007). Three clone libraries were constructed using previously described methods (Hatamoto et al., 2010). The cloned 16S rRNA gene fragments were sequenced at the Dragon Genomics Center (TAKARA BIO, Yokkaichi, Japan). Chimeric sequences were identified and removed using the Bellerophon program (Huber et al., 2004). 16S rRNA gene sequences with $\geqslant$97% identity were grouped into the same phylogenetic clone type by using the FastGroupII program (Yu et al., 2006). The ribosomal database project classifier tool was employed to assign the 16S rRNA genes to appropriate taxonomic groups (Wang et al., 2007). Phylogenetic analysis using the ARB program was also performed for poorly classified sequences.

2.4. T-RFLP analysis of pmoA genes

Sludge collection and DNA extraction was performed as described above. PCR amplification of the *pmoA* gene was done with primer pairs of A189f labeled with Beckman-dye D4 and mb661 (Costello and Lidstrom, 1999). PCR reaction was initiated with 5 min of initial denaturation at 94 °C and 25 cycles of 30 s at 94 °C, 30 s at 56 °C, and 1 min at 72 °C. The final extension step was at 72 °C for 4 min. The PCR products were digested by MspI restriction endonuclease. Digested samples were analyzed on the CEG-2000XL (Beckman Coulter, Fullerton, CA) as described previously (Hatamoto et al., 2008).

2.5. Statistical analysis of the T-RFLP patterns

The terminal restriction fragment (T-RF) data of the *pmoA* gene was used for multidimensional scaling (MDS) statistical analyses according to a previous report (Yamada et al., 2008). Briefly, the relative abundances of each T-RF fragment were normalized by calculating the proportion of a given peak height to the total peak height. The small T-RF peaks, e.g., peak height below 100 relative fluorescence units or peak area below 1% of the total area, were removed as background noise. The bp numbers of T-RFs having the decimal numbers rounded to whole numbers. MDS analyses were performed using the Bray–Curtis coefficient based on the relative