Bioresource Technology 151 (2014) 144–150

Contents lists available at ScienceDirect

Bioresource Technology

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

Microbial community composition of a down-flow hanging sponge (DHS) reactor combined with an up-flow anaerobic sludge blanket (UASB) reactor for the treatment of municipal sewage

Kengo Kubota [a,*], Mikio Hayashi [a], Kengo Matsunaga [a,b], Akinori Iguchi [c], Akiyoshi Ohashi [d], Yu-You Li [a], Takashi Yamaguchi [b], Hideki Harada [a]

[a] Department of Civil and Environmental Engineering, Tohoku University, 6-6-06 Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8579, Japan
[b] Department of Civil and Environmental Engineering, Nagaoka University of Technology, 1603-1 Kamitomioka, Nagaoka, Niigata 940-2188, Japan
[c] Faculty of Applied Life Sciences, Niigata University of Pharmacy and Applied Life Sciences, 265-1 Higashijima, Akiha-ku, Niigata, Niigata 956-8603, Japan
[d] Department of Social and Environmental Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashihiroshima, Hiroshima 739-8527, Japan

HIGHLIGHTS

- The microbial community composition of a DHS reactor was investigated.
- Differences were observed at different reactor heights and in different seasons.
- The dominant OTUs in the upper part were likely responsible for organic removal.
- More AOB colonized in the middle and lower parts of the reactor.
- A separation of microbial habitats was found in the DHS reactor.

ARTICLE INFO

Article history:
Received 27 August 2013
Received in revised form 13 October 2013
Accepted 15 October 2013
Available online 23 October 2013

Keywords:
Ammonia oxidation
Down-flow hanging sponge (DHS)
Microbial community composition
Sewage treatment

ABSTRACT

The microbial community composition of a down-flow hanging sponge (DHS) reactor in an up-flow anaerobic sludge blanket (UASB)–DHS system used for the treatment of municipal sewage was investigated. The clone libraries showed marked differences in microbial community composition at different reactor heights and in different seasons. The dominant phylotypes residing in the upper part of the reactor were likely responsible for removing organic matters because a significant reduction in organic matter in the upper part was observed. Quantification of the *amoA* genes revealed that the proportions of ammonia oxidizing bacteria (AOB) varied along the vertical length of the reactor, with more AOB colonizing the middle and lower parts of the reactor than the top of the reactor. The findings indicated that sewage treatment was achieved by a separation of microbial habitats responsible for organic matter removal and nitrification in the DHS reactor.

1. Introduction

The development of sewage treatment technologies appropriate for use in developing countries is a major concern because extensive water pollution, especially in urban areas, is often a serious environmental issue in these regions. The activated sludge process is used extensively in developed countries to treat water. However, the technology uses considerable amounts of electric power and requires highly skilled engineers to maintain the system. Systems based on anaerobic treatment processes, such as the up-flow anaerobic sludge blanket (UASB), have recently been applied to the treatment of sewage in many countries in tropical and subtropical regions (Monroy et al., 2000; Florencio et al., 2001; Wiegant, 2001). However, anaerobic systems alone are often not able to produce effluent with sufficiently high water quality that would allow these treatment facilities to discharge the treated effluent into public water bodies. As a result, post-treatment systems, such as final polishing units, are employed extensively for this purpose. However, despite the additional polishing, the quality of the water discharged from these facilities often does not meet the required water quality standards (Wiegant, 2001; Sato et al., 2006).

The down-flow hanging sponge (DHS) reactor was previously developed as a post-treatment process for anaerobic reactors, and the system has been combined with the UASB process (UASB–DHS system) to treat domestic sewage (Machdar et al., 1997, 2000; Tandukar et al., 2005, 2007; Tawfik et al., 2006). The principle underlying the treatment of wastewater using a DHS reactor is as follows: UASB effluent is introduced to the top of

* Corresponding author. Tel./fax: +81 22 795 5011.
E-mail address: kengok@epl1.civil.tohoku.ac.jp (K. Kubota).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.10.058

the DHS reactor and wastewater trickles downward to the bottom of the reactor. During this down-flow process, the wastewater passes through numerous sponge carriers. Since these sponge carriers are exposed to air and are not submerged in wastewater, oxygen can be supplied to the wastewater from the atmosphere, which obviates the need for external aeration. Effluent water quality from a DHS reactor is comparable to water treated using an activated sludge process (Tandukar et al., 2007). Water quality profiles of chemical oxygen demand (COD_{Cr}), dissolved oxygen (DO), ammonium nitrogen (NH_4^+–N), nitrite nitrogen (NO_2^-–N), and nitrate nitrogen (NO_3^-–N) have shown that organic matter is mineralized in the upper part of the reactor, and that ammonia and nitrite oxidation occur in the middle and lower parts of the reactor (Machdar et al., 2000; Tandukar et al., 2007). UASB effluent contains almost no DO, but the addition of a DHS reactor increases DO levels to 5–6 mg/L, even without aeration (Machdar et al., 1997, 2000; Tandukar et al., 2005, 2007; Tawfik et al., 2006). Microelectrode studies have shown that the outer portion of the sponge is aerobic and that the oxygen concentration decreases toward the center of the sponge before finally falling below detection limits, which would indicate the existence of anoxic or anaerobic conditions (Araki et al., 1999; Machdar et al., 2000). Thus, both aerobic and anaerobic treatments can be performed in a single DHS reactor, which implies that denitrification occurs in a DHS reactor. Furthermore, since the sponges retain large amounts of sludge in their pores, the sludge retention time (SRT) of a DHS reactor is typically very long (>90 days) (Tandukar et al., 2005, 2007; Tawfik et al., 2006), and production of excess sludge by DHS reactors is extremely low (Tandukar et al., 2007).

Although the microenvironments at different points along the vertical length of a DHS reactor and in the individual sponge carriers vary dramatically in terms of organic matter content, nitrogen load, and oxygen concentration, the identity and roles of the microorganisms in DHS reactors are still largely unknown; even though these microorganisms are of considerable interest for engineers and microbiologists. To our knowledge, this is the first report on the microbial community composition of a DHS reactor in a UASB–DHS system that was used for municipal sewage treatment. Specifically, the microbial community of a DHS reactor was investigated by quantifying the 16S rRNA genes and the alpha subunit of the ammonia monooxygenase (*amoA*) genes of *Bacteria* and *Archaea*, and by constructing bacterial 16S rRNA gene clone libraries. The microbial community compositions elucidated in this study will likely provide new insights into understanding microbial wastewater treatment and further potential applications of DHS reactors in process engineering.

2. Methods

2.1. UASB–DHS system

A pilot-scale UASB–DHS system was installed at a municipal sewage treatment plant (Supplementary Fig. S1). Before being treated by the DHS reactor, sewage was processed using a cylindrical UASB reactor with a 1148-L volume (including gas–solid separator) and a height of 4 m. The volume and height of the DHS reactor was 454 L, based on the sponge volume, and 4 m, respectively. The reactor was composed of 10 boxes arranged vertically, which gave a total of 16,240 sponge carriers. Boxes were numbered such that the topmost box was "box 1" and the bottommost box was "box 10" (Fig. S1). The sponge carriers were constructed by inserting a cubic sponge (33 mm × 33 mm × 33 mm) into a polypropylene tube (33 mm diameter × 33 mm long) (Fig. S1). The hydraulic retention times of the UASB and DHS reactors were 8 and 3.2 h, respectively.

2.2. Sludge samples and DNA extraction

Sludge sampling was performed at 283 and 441 days after starting up the DHS reactor. Days 283 and 441 occurred in May and November, respectively, when water temperatures were 20.0 °C and 21.7 °C, respectively. At the time of sampling, three sponge carriers were removed from the sampling port of each box and the sludge was squeezed from the sponges into phosphate-buffered saline (PBS). The sludge samples were then washed with PBS and subjected to DNA extraction using an Ultra Clean Soil DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions.

2.3. Quantitative real-time PCR

Quantitative real-time PCR was performed using the SYBR *Premix ExTaq* II (Perfect Real Time, TaKaRa Bio, Inc., Otsu, Japan) with a LightCycler instrument (Roche Applied Science, Tokyo, Japan). The bacterial and archaeal rRNA genes and *amoA* genes were quantified using the primers Eub8f/Univ518r (bacterial rRNA gene), Arc109f/Arc912r (archaeal rRNA gene), amoA-1F/amoA-2R (bacterial *amoA* gene), and Arch-amoAF/Arch-amoAR (archaeal *amoA* gene) (Table S1). Standards were prepared from plasmids containing each gene by PCR with vector-specific primer set (i.e., M13 forward and M13 reverse). After purifying with the MinElute PCR Purification Kit (Qiagen, Tokyo, Japan), the amount of PCR product was measured with a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and the copy number was calculated. The purity of the amplified products after quantitative real-time PCR was checked by melting analysis and agarose gel electrophoresis, and all experiments were performed in duplicate. Relative abundance of ammonia-oxidizing bacteria (AOB) to *Bacteria* was calculated by dividing the copy number of the bacterial *amoA* gene by the copy number of the bacterial rRNA gene.

2.4. Cloning of the 16S rRNA gene and phylogenetic analysis

The bacterial 16S rRNA gene was amplified with the Eub338f-mix/Univ1500r primer set (Table S1) using the *TaKaRa ExTaq* Hot Start Version (TaKaRa Bio, Inc.). The PCR conditions consisted of an initial denaturation step at 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 2 min, with a final extension step of 72 °C for 10 min. The PCR products were purified with a MinElute PCR Purification Kit, and ligation and transformation were performed using a TOPO TA Cloning Kit for Sequencing and TOP10 competent cells (Invitrogen). Approximately 500 bases of each cloned gene were sequenced starting from position 338 (*Escherichia coli* numbering). The sequences were grouped into OTUs with a threshold of 97% identity using the mothur open-source bioinformatics software package (Schloss et al., 2009). In addition, to identify the closest relatives, the sequences were analyzed using the SILVA database (Pruesse et al., 2007) with ARB software (Ludwig et al., 2004) and BLAST from the NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and similarities among the communities were calculated using Fast UniFrac (Hamady et al., 2010). Almost full-length sequences were determined for selected clones. All sequences were deposited in the DDBJ/NCBI/EMBL database under Accession Numbers AB479546–AB479708, AB618290–AB618481 and AB818471–AB818472.

3. Results and discussion

3.1. System performance

Both the UASB and DHS reactors were operated without temperature control (10–28 °C) for approximately 2 years, and a detailed description of the system's performance is described