Journal of Biotechnology 184 (2014) 56–62

Contents lists available at ScienceDirect

Journal of Biotechnology

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

Depth profiles of methane oxidation potentials and methanotrophic community in a lab-scale biocover

Eun-Hee Lee [1], Kyung-Eun Moon [1], Tae Gwan Kim, Kyung-Suk Cho *

Global Top5 Research Program, Department of Environmental Science and Engineering, Ewha Womans University, 52, Ewhayeodae-gil, Seodaemun-gu, Seoul, 120-750, Republic of Korea

ARTICLE INFO

Article history:
Received 16 December 2013
Received in revised form 8 May 2014
Accepted 13 May 2014
Available online 23 May 2014

Keywords:
Methane
Biocover
Depth profile
Methanotrophs
Bioresource

ABSTRACT

The depth profiles of the CH_4 oxidation potentials and the methanotrophic community were characterized in a lab-scale soil mixture biocover. The soil mixture samples were collected from the top (0–10 cm), middle (10–40 cm), and bottom (40–50 cm) layers of the biocover where most of methane was oxidized at the top layer due to consumption of O_2. Batch tests using serum bottles showed that the middle and bottom samples displayed CH_4 oxidation activity under aerobic conditions, and their CH_4 oxidation rates were 85 and 71% of the rate of top sample (8.40 μmol g dry sample^{-1} h^{-1}), respectively. The numbers of methanotrophs in the middle and bottom were not significantly different from those in the top sample. There was no statistical difference in the community stability indices (diversity and evenness) among the methanotrophic communities of the three layer samples, even though the community structures were distinguished from each other. Based on microarray analysis, type I and type II methanotrophs were equally present in the top sample, while type I was more dominant than type II in the middle and bottom samples. We suggested that the qualitative difference in the community structures was probably caused by the difference in the depth profiles of the CH_4 and O_2 concentrations. The results for the CH_4 oxidation potential, methanotrophic biomass, and community stability indices in the middle and bottom layer samples indicated that the deeper layer in the methanotrophic biocover serves as a bioresource reservoir for sustainable CH_4 mitigation.

1. Introduction

Landfills are a significant source of anthropogenic methane, accounting for approximately 500–800 Mt CO_2 eq year^{-1} of global methane emissions (Bogner and Matthews, 2003). Large engineered landfills can readily emit several hundred m^3 of landfill gas h^{-1}, and they have been studied for the recovery of landfill methane as a renewable energy source (Spokas et al., 2006). The recovery of landfill methane is conducted at more than 1150 plants worldwide, with methane mitigation producing more than 105 Mt CO_2 eq year^{-1} (Bogner et al., 2008). However, many landfill sites flare or incinerate landfill gases without energy recovery. Even energy recovery sites should be used to mitigate residual emissions after the closing of the landfill sites. Landfill biocovers, a methane mitigation technique for the secondary control of landfill methane emissions, use microbial methane oxidation by indigenous methanotrophs in cover soils. Methanotrophs can co-oxidize non-CH_4 organic compounds such as aromatic and chlorinated compounds as well as methane, resulting in the mitigation of gas emissions from landfills to the atmosphere (Schuetz et al., 2003). Therefore, methanotrophic activity is a major factor used to determine the performance quality of biocovers.

Biological methane oxidation is limited by the transport of CH_4 flux from the anaerobic zones of landfills and O_2 flux from the atmosphere since methanotrophs are strictly aerobic microorganisms (Bogner et al., 2008). Therefore, many studies have focused on methane oxidation in the upper layer of the landfill cover soil, where the O_2 level is high (De Visscher et al., 1999; Kightley et al., 1995). Although methanotrophs are strict aerobes, they can oxidize methane at low oxygen levels of 1–3% (Czepiel et al., 1996). This indicates that methane can possibly be removed at the deeper landfill soil layers as well as at the surface layer. However, the methane oxidation potentials and their methanotrophic communities in deeper soil layers are still controversial despite the possibility of methane mitigation in landfills.

In order to investigate the possibility of methane mitigation in deeper layers of the landfill cover soil, a lab-scale biocover was set

* Corresponding author. Tel.: +82 2 3277 2393; fax: +82 2 3277 3275.
E-mail address: kscho@ewha.ac.kr (K.-S. Cho).
[1] These authors contributed equally to this work (co-first authors).

http://dx.doi.org/10.1016/j.jbiotec.2014.05.006
0168-1656/

up and was operated for the removal of stimulated landfill biogas including CH_4 and non-CH_4 volatile organic compounds such as dimethyl sulfide (DMS), benzene (B), and toluene (T) (Kim et al., 2013). In the present study, we characterized the depth profiles of the methane oxidation potentials and evaluated their methanotrophic communities in different soil layers in a lab-scale biocover. In addition, we discussed the effects of environmental variables such as biocover depth and CH_4 and O_2 levels on the methane oxidation potentials and the methanotrophic communities.

2. Materials and methods

2.1. Gas profiles of a lab-scale landfill gas-removing biocover and related soil sampling

A lab-scale biocover was constructed with landfill soil, granular-activated carbon (GAC), earthworm cast, and saprolite (4:2:1:1, w/w, approximately 4 kg) (Kim et al., 2013). The moisture content was adjusted to 20% with distilled water. A synthetic gas consisting of CH_4/CO_2/DMS/B/T (400,000/600,000/115–3,000/100–800/20–150 ppm_v) was continuously fed into the bottom of the biocover at a flow rate of 5 ml min^{-1} for 26 days. The biocover consisted of the packing and ventilating sections with an inner diameter of 8 cm (Kim et al., 2013). The working height and total volume in the packing section were 50 cm and 2.5 l, respectively. The ventilating section was assembled with air inlet and gas outlet ports with a height of 15 cm. The specific operating and maintenance conditions of the biocover were previously described (Kim et al., 2013). Compressed air was introduced into the top of the ventilation section at a flow rate of 100 ml min^{-1}. The air was diffused and vented at the top of the biocover system. The CH_4, CO_2, N_2, O_2, DMS, B, and T concentrations were monitored at depths of 0, 10, 20, 30, 40, and 50 cm from the top of the biocover on day 14. The methane removal efficiency and elimination capacity were calculated with a consideration of a dilution factor of 20. On the same day, soil mixtures were collected in duplicate from the top (0–10 cm), middle (10–40 cm), and bottom (40–50 cm) layers for the characterization of the methane oxidation potentials and their methanotrophic community structures. The collected soil mixtures were immediately stored at −70 °C prior to analysis of the community structures.

2.2. Methane oxidation potential and sMMO activity in the top, middle, and bottom layers of the biocover

In order to evaluate the methane oxidation potentials of the samples obtained from the top, middle, and bottom layers of the biocover, approximately 2 g of each sample was added to 600-ml serum bottles containing 20 ml of nitrate mineral salt (NMS) medium. The bottles were sealed with butyl rubbers and parafilm, and then methane was injected into each bottle at 5% (v/v) of the final concentration from a methane gas cylinder (99%, Seoul Special Gases, Seoul, Korea). The NMS medium consists of $MgSO_4{\cdot}7H_2O$ 1 g, $CaCl_2{\cdot}2H_2O$ 0.295 g, KNO_3 1 g, KH_2PO_4 0.26 g, $Na_2HPO_4{\cdot}2H_2O$ 0.41 g, and $CuSO_4{\cdot}5H_2O$ 0.0025 g per 1-liter; plus trace elements of $FeSO_4{\cdot}7H_2O$ 500 μg, $ZnSO_4{\cdot}7H_2O$ 400 μg, $MnCl_2{\cdot}4H_2O$ 20 μg, H_3BO_4 15 μg, $CoC1_2{\cdot}6H_2O$ 50 μg, $NiCl_2{\cdot}6H_2O$ 10 μg, and EDTA 250 μg.

In order to analyze the soluble methane monooxygenase (sMMO) activity of each layer sample, the bottles were prepared as described above with addition of the allylthiourea (Sigma, Saint Louis, USA). Allylthiourea is a well-known inhibitor of the synthesis of particulate methane monooxygenase (pMMO) (Yu et al., 2009). Allylthiourea was supplemented into the bottles by adding it into the NMS medium at 50 mM. The serum bottles were incubated at 30 °C with 150 rpm of agitation. The concentration of the methane in the headspace was monitored every 4 or 5 h using a gas-tight syringe. All of the experiments were performed in five replicates.

2.3. Gas analysis

The methane, DMS, B, and T concentrations were measured using gas chromatography (GC, 6850N, Agilent Technologies Inc., Santa Clara, USA) equipped with a flame ionization detector (FID) and a wax column (30 m × 0.32 mm × 0.25 μm, Supelco Inc., Bellefonte, USA), as previously described by Lee et al. (2010). The O_2 and N_2 concentrations were monitored using a GC (7890A, Agilent, Santa Clara, USA) equipped with a thermal conductivity detector (TCD) and a capillary column (30 m × 0.53 mm × 28 μm, HP Molesieve 5A, Agilent, Santa Clara, USA). The oven, injector, and detector temperatures were set at 50, 200, and 250 °C, respectively. The CO_2 concentration was analyzed using a GC (6890N, Agilent, Santa Clara, USA) equipped with a TCD and an HP-PLOT/Q column (30 m × 0.53 mm × 40 μm, Agilent, Santa Clara, USA). The oven, injector, and detector temperatures were set at 60, 100, and 250 °C, respectively. The standard curves for the calculation of the gas concentrations were established using CH_4, DMS, B, T, N_2, O_2, and CO_2 standard gases as described by Kim et al. (2013).

2.4. Nucleic acid extraction and q-PCR

Genomic DNA was extracted in duplicate from 0.5 g of each sample. The DNA was extracted using a NucleoSpin Soil Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) with modification. The soil mixture samples were disrupted with a BeadBeater-8 system (BioSpec Inc., Bartlesville, USA) at 5000 rpm for 30 s. The DNA was eluted with 100 μl of the elution buffer and stored at −20 °C prior to use. The extracted DNA was quantified using an ASP-2680 spectrophotometer (ACTGene Inc., Piscataway, USA).

Quantitative real-time PCR (q-PCR) was used to quantify the methanotrophs using the primer set of A189f and mb661r, which targets the α-subunit of the particulate methane mono-oxygenase gene (*pmoA*) (Kolb et al., 2003). The *pmoA* gene of *Methylobacter luteus* (NCIMB11914) was used in order to establish a standard curve for the quantitative detection. The PCR mixture and operating conditions were described in detail by Kim et al. (Kim et al., 2013; Lee et al., 2010). The *pmoA* copy number was calculated by measuring the DNA concentration. The DNA standard ranged from 3.26×10^3 to 3.26×10^7 copies. The q-PCR was performed in duplicate.

2.5. Microarray

For the microarray analysis, in vitro RNA transcription was performed as described by Bodrossy et al. (2003), with modifications. The PCR reactions were performed in 50 μl volumes with the primer pair A189f and T7-mb661r targeting the *pmoA* gene (Bodrossy et al., 2003). The PCR conditions were described in detail by Kim et al. (2011). The PCR amplicons were purified using the RBC HiYield Gel/PCR DNA mini kit (Real Biotech Co., Banqiao, Taiwan). In vitro transcription was carried out using the MEGAscript® T7 Kit (Ambion Inc., Austin, U.S.), with Cy 3 or Cy 5-UTP (PerkinElmer Inc., Waltham, U.S.).

Microarrays, with 59 oligonucleotide-probes (Bodrossy et al., 2003), were custom-made by Digital Genomics (Seoul, Korea). Each probe was spotted in three replicates per block, with two blocks per glass slide, resulting in six replicates per probe. The microarray procedures and data analysis were identical to those described by Kim et al. (2012).