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Long-term performance and bacterial community dynamics in biocovers for mitigating methane and malodorous gases

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ABSTRACT

The long-term performance of lab-scale biocovers for the simulation of engineered landfill cover soils was evaluated. Methane (CH_4), trimethylamine (TMA), and dimethyl sulfide (DMS) were introduced into the biocovers as landfill gases for 134 days and the removal performance was evaluated. The biocover systems were capable of simultaneously removing methane, TMA, and DMS. Methane was mostly eliminated in the top layer of the systems, while TMA and DMS were removed in the bottom layer. Overall, the methane removal capacity and efficiency were 224.8 ± 55.6 g-CH_4 m^{-2} d^{-1} and 66.6 ± 12.8%, respectively, whereas 100% removal efficiencies of both TMA and DMS were achieved. Using quantitative PCR and pyrosequencing assay, the bacterial and methanotrophic communities in the top and bottom layers were analyzed along with the removal performance of landfill gases in the biocovers. The top and bottom soil layers possessed distinct communities from the original inoculum, but their structure dynamics were different from each other. While the structures of the bacterial and methanotrophic communities showed little change in the top layer, both communities in the bottom layer were considerably shifted by adding TMA and DMA. These findings provide information that can extend the understanding of full-scale biocover performance in landfills.

1. Introduction

Humans are considered to be the dominant detectable influence on climate change over the past 60 years (Houghton et al., 2001; Karl and Trenberth, 2003). As a result of human activities, anthropogenic emissions of non-CO_2 greenhouse gases significantly contribute to the Earth's climate change (Montzka et al., 2011). Landfills are considered as one of the significant sources of non-CO_2 greenhouse gases, including methane, nitrous oxide, and malodorous gases (Bogner et al., 2007; Karl and Trenberth, 2003; Montzka et al., 2011). An estimation of methane emissions from landfills accounted for 18% of the global anthropogenic methane emissions, which was approximately 30 Tg in 2005 (Bogner et al., 2007; Bogner et al., 2008). To mitigate non-CO_2 greenhouse gas emissions from landfills, a variety of approaches, such as biofilters and biocovers, have been proposed (Kim et al., 2013a; Moon et al., 2014). A biocover system is a typical technique that was developed to simulate landfill topsoil, which usually consist of a coarse gas distribution layer and an appropriate substrate layer (Huber-Humer et al., 2009). In case of field application, biocovers usually used as an interim or final cover that can be used during landfill operation, aftercare or remediation (Abushammala et al., 2014; Huber-Humer et al., 2008). Organic and inorganic "engineered" materials (e.g., compost, clay, peat, wood chips, sand, sewage sludge, soil, earthworm cast, activated carbon, tobermolite, saprolite, etc.) can be used as a substrate layer for biological methane removal. The biocover system serves to minimize methane emission by optimizing the environmental conditions for methanotrophs and enhancing biotic methane oxidation (Huber-Humer et al., 2009; Stern et al., 2007). This approach is based on biological removal processes of landfill gases (e.g., methane and malodorous gases) that are generated from landfills via anaerobic digestion of wastes (Themelis and Ulloa, 2007). To date, efforts have been made to investigate the biological potential for methane removal in landfills and these studies have been conducted using biocover systems to simulate landfill cover soils (topsoils) (Kim et al., 2011; Lee et al., 2014; Moon et al., 2014; Park et al., 2008). Biological methane removal is the most important biological sink in which methane is aerobically oxidized by methane-oxidizing bacteria (Svend Jørgen Binnerup, 2005). Methane-oxidizing bacteria, also known as methanotrophs or methanotrophic bacteria, utilize methane as a sole carbon and energy source which can metabolize methane to carbon dioxide. Thus, active maintenance of a well-established methanotrophic community is crucial for successful

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performance of methane-removing biocovers. Although the performance and removal efficiency of biocovers for biological methane removal has been reported, most systems were operated for relatively short periods of time. Furthermore, the biological removal process depends on the microbial activity and communities. Thus, quantitative and qualitative analyses of the microbial community are necessary to elucidate their relationships.

In this study, we investigated the long-term performance of biocovers for methane removal in the operating systems for 134 days. The capacity and efficiency of methane removal was evaluated by profiling gases as a function of the depth of the biocovers. Along with the methane removal performance of the biocovers, the bacterial and methanotrophic communities were also analyzed in depth to determine their relationships with biological methane removal characteristics. Concurrently, the effects of malodorous gases as landfill trace gases on methane removal were examined. Trimethylamine (TMA) and dimethyl sulfide (DMS) were used as model landfill malodorous gases and they were additionally supplied into the biocover systems. Quantitative PCR (q-PCR) and pyrosequencing assays were performed to evaluate the bacterial and methanotrophic populations and communities.

2. Materials and methods

2.1. Operation of lab-scale biocover systems

Two identical lab-scale biocover systems were operated to simulate landfill cover soils. Acryl columns were used to construct the biocover systems, which consisted of two sections with packing and ventilating parts. The inner diameter, height, and volume of the packing section were 8 cm, 50 cm, and 2.5 L, respectively. The composition and configuration of the biocover systems have been described previously in detail (Moon et al., 2014). Briefly, a gas inlet port was positioned at the bottom of the system, where a synthetic gas was fed. Gas sampling ports were located in the packing section at 10 cm intervals and the gases were periodically monitored. The biocovers were operated in three experimental stages for 134 days at room temperature ($30 \pm 5\,^{\circ}C$). The operating stage of the biocover changed depending on the inlet gas composition. Methane (CH_4) was only supplied into the inlet port for 39 days (days 0–39 of the operating period, stage 1 (S1)). Subsequently, TMA was additionally introduced with methane for 65 days (days 40–104 of the operating period, stage 2 (S2)) and then, DMS was simultaneously fed with methane and TMA for 30 days (days 105–134 of the operating period, stage 3 (S3)). Methane (mixed with CO_2, 2:3 v/v ratio, Seoul Special Gas Inc., Seoul, South Korea) was continuously supplied into the biocover systems at a flow rate of 5 mL min^{-1} (which is equivalent to a space velocity of 0.12 h^{-1}). The other gases (e.g., TMA and DMS) were supplied by passing methane gas through a liquid storage container. A five hundred milliliter glass serum bottle was used as a liquid storage container. The bottle contained 150 mL each of TMA (Sigma-Aldrich Co., St. Louis, USA) and DMS (Sigma-Aldrich Co., St. Louis, USA). Vegetable oil was subsequently added into the serum bottle to prevent rapid evaporation of TMA and DMS, which forms a 2 cm thick separation layer in the upper TMA and DMS liquid. By connecting the methane gas line into the liquid storage container, gaseous TMA and DMS were generated via spontaneous volatilization. The inlet concentrations of methane, TMA, and DMS were 350,000–400,000, 700–2000, and 700–900 ppm (v/v), respectively. Two biocover systems were identically operated.

2.2. Packing material preparation

A mixture of tobermolite, landfill cover soil, and earthworm cast (2:1:1, w/w) was used as the packing material to simulate the landfill topsoil environment. Tobermolite was purchased from JawooBio, Daejeon, South Korea and had a diameter of 3–8 mm. The cover soil was sampled at a depth of 10–30 cm from the surface of a landfill site at Gapyeong-gun, Gyeonggi-do, South Korea. The earthworm cast was obtained as a courtesy of the Nangi Municipal Sewage Treatment Plant, Goyang, Gyeonggi-do, South Korea. The cover soil and earthworm cast was sieved with a 2 mm mesh to remove larger particles, and the tobermolite was washed 4 or 5 times with tap water to eliminate impurities prior to use. Note that the earthworm cast was used as an inoculum source of methanotrophs. The mixture of the packing material was homogeneously blended and subsequently added into the biocover systems.

2.3. Gas analysis

The methane, TMA, and DMS concentrations were periodically monitored at the gas sampling ports using gas chromatography (GC, 6850 N, 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, PA, USA), as previously described by Lee et al. (Lee et al., 2010). The O_2, N_2, and CO_2 gas concentrations were measured using GC (7890A, Agilent, Santa Clara, USA) equipped with a thermal conductivity detector (TCD) and its analysis conditions were previously described by Lee et al. (Lee et al., 2014). The gas analyses were performed in triplicate. The removal efficiencies were calculated from the inlet and outlet gas fluxes of the biocover systems, which were estimated based on the flow rates and gas concentrations.

2.4. Bacterial community analysis based on the pyrosequencing assay

The packing mixture was collected from the top (0–10 cm) and bottom (40–50 cm) layers of the biocover systems at the respective operating stages (38, 104, and 128 days of operation) for the bacterial and methanotrophic community analyses. The collected mixture was immediately stored at −70 °C and was homogeneously mixed prior to analysis. Genomic DNA was extracted in duplicate from 0.5 g (dry weight) of each packing mixture using a NucleoSpin soil kit (Macherey–Nagel GmbH & Co. KG, Düren, Germany) according to the manufacturer's instructions. The DNA was eluted in 100 μL of elution buffer and its concentration was measured using an ASP-2680 spectrophotometer (ACTGene Inc., Piscataway, USA).

The pyrosequencing assay was carried out in duplicate using the genomic DNA extracted from the top and bottom layers of each biocover. A primer set of 340F and 805R was used for PCR amplification of the region of the 16S rRNA gene (Kim et al., 2013b). Six different composite primer sets were designed based on the 340F–805R set for multiplex pyrosequencing. The PCR mixtures and conditions were described previously (Kim et al., 2013b; Moon et al., 2014). The purified DNA concentrations were measured using an ASP-2680 spectrophotometer, and equal amounts of purified DNA were subsequently pooled into vials for the pyrosequencing analysis. Pyrosequencing was performed using a Genome Sequencer 454 FLX Titanium system (Roche Diagnostics Inc., Mannheim, Germany).

The primer, low quality (length <400 nt, average quality score <25, and with an ambiguity), and chimera sequences were trimmed and excluded using the ribosomal database project (RDP) pyrosequencing pipeline (Cole et al., 2009) and Black Box Chimera Check software with the default settings (Gontcharova et al., 2010) to obtain high-quality sequences. The RDP pyrosequencing pipeline was employed in this study to analyze the pyrosequencing data as previously described by Kim et al. (Kim et al., 2013b). The sequence of each operational taxonomic unit (OTU) at 3 and 1% distance levels of the bacterial and methanotrophic communities were BLAST