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Effects of trichloroethylene on community structure and activity of methanotrophs in landfill cover soils

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ABSTRACT

Landfill cover soil (LCS) plays an important role in mitigating the emission of CH_4 and volatile organic gases from landfills to the atmosphere. In this study, effect of trichloroethylene (TCE) on community structure and activity of methanotrophs, as well as TCE degradation efficiency was investigated in waste biocover soil (WBS), which was collected from a landfill bioreactor treating organic waste, in comparison with LCS. The CH_4 oxidation activity and TCE degradation rate were higher in WBS compared to those in LCS. The TCE degradation rates in both soils were enhanced with the increase of TCE concentration within 50 ppmv. Compared to LCS, the TCE inhibitory concentration that caused inhibition of CH_4 oxidation activity was greater for WBS. The abundance of *mmoX* was similar in both soils during the whole experiment, while the average abundance of *pmoA* in WBS was about two orders of magnitudes higher than in LCS. Type I methanotrophs (*Methylocaldum*, *Methylomonas*, *Methylosarcina* and *Methylobacter*) and type II methanotrophs (*Methylocystis*) were abundant in both soils. Among them, type I methanotrophs *Methylocaldum* and *Methylobacter* dominated in WBS, while type II methanotrophs *Methylocystis* predominated in LCS. The relative abundance of *Methylobacter* increased with an increase of TCE concentration and exposure time in both soils, especially in WBS, indicating that *Methylobacter* seemed tolerant to TCE and/or may play an important role in the TCE degradation.

1. Introduction

Landfill gas (LFG) mainly originates from the biodegradation of organic matter in landfills, which consists of methane (CH_4) (55–60%, v/v), carbon dioxide (CO_2) (40–45%, v/v) and trace gases such as halogenated, aromatic hydrocarbons and sulfur-containing compounds (Scheutz and Kjeldsen, 2004). Both CH_4 and CO_2 are important greenhouse gases. The global warming potential of CH_4 is about 25 times higher than that of CO_2 on a 100-year time frame (IPCC, 2007). Landfills, as one of the major anthropogenic sources of CH_4 emissions, account for 6–12% of the total global CH_4 emissions. It is estimated that 35–69 Tg CH_4 is released annually into the atmosphere from landfills (IPCC, 2007). Although the concentration of trace gases is less than 2% (v/v) in LFG, many trace gases such as toluene and trichloroethylene (TCE) are on the United State Environmental Protection Agency priority pollutant list and pose a potential serious risk to public health (Assmuth and Kalevi, 1992).

Landfill cover soil (LCS) is the environmental interface between deposited waste and the atmosphere, which can serve as a biofilter to reduce the emission of LFG pollutants. It has been reported that 6–100% of CH_4 that escapes from landfills is consumed by LCS (Barlaz et al., 2004; Börjesson et al., 2004; Einola et al., 2007; Scheutz et al., 2009). Aerobic methanotrophs are the primary mediators of CH_4 consumption in oxic layers of LCS before CH_4 releases into the atmosphere (Henneberger et al., 2012). Novel thermoacidophilic aerobic methanotrophs within the *Verrucomicrobia* were recently discovered in geothermal areas (Dunfield et al., 2007; Pol et al., 2007; Islam et al., 2008). Aerobic methanotrophs mainly belong to the *Proteobacteria* and can be divided into two taxonomic groups: type I (belonging to the Methylococcaceae family of γ-*Proteobacteria*) and type II methanotrophs (including the genera *Methylocystis*, *Methylosinus*, *Methylocella* and *Methylocapsa* in α-*Proteobacteria*), based on their cell morphology, ultra-structure, phylogeny and metabolic pathways (Hanson and Hanson, 1996; Semrau et al., 2010). CH_4 is oxidized to methanol by methane monooxygenase (MMO), which exists in two forms: the membrane bound particulate MMO (pMMO) and the cytoplasmic soluble MMO (sMMO) (Hanson and Hanson, 1996; Semrau et al., 2010). pMMO is known to be present in all known methanotrophs except

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for the genera *Methylocella* (Dedysh et al., 2000) and *Methyloferula* (Vorobev et al., 2011), whereas sMMO appears to occur only in certain methanotroph strains. MMO, especially sMMO, has a broad substrate specificity and can oxidize a range of recalcitrant hydrocarbons, including TCE, ethanes and ethenes (Semrau et al., 2010). It has been reported that *Methylocystis* sp. strain M, *Methylocystis* strain SB2, *Methylomonas* sp. strain MM2, *Methylomonas methanica* 68-1, *Methylomicrobium album* BG8, *Methylosinus trichosporium* OB3b and methanotrophic culture can simultaneously cometabolize halogenated and aromatic hydrocarbons in the presence of CH_4 (Oldenhuis et al., 1989; Koh et al., 1993; Vlieg et al., 1996; Han et al., 1999; Shukla et al., 2009, 2010; Im and Semrau, 2011). Under low-copper growth conditions, *Methylosinus trichosporium* OB3b can degrade TCE at the biotransformation capacity of ~0.25 mg mg^{-1} dry weight of cell mass (Taylor et al., 1993).

CH_4 oxidation rates vary with physical and chemical characteristics of landfill covers, such as soil texture, particle composition, water content, organic carbon content, temperature and nutrient (Hanson and Hanson, 1996; Scheutz and Kjeldsen, 2004; Scheutz et al., 2009; Wang et al., 2011). In addition to the capacity of CH_4 oxidation, LCS has a significant potential for degradation and cometabolic degradation of volatile organics such as vinyl chloride (VC) and TCE (Scheutz et al., 2004). However, MMO-mediated degradation of chlorinated hydrocarbons can generate toxic intermediates that may inactivate cells. For examples, oxidation of TCE by MMO will produce TCE-epoxide (Little et al., 1988). Some of hydrolysis products of TCE-epoxide can covalently bind to MMO, which may result in the death of cells (Fox et al., 1990; Nakajima et al., 1992). In addition, there is competition between CH_4 and the chlorinated co-substrate for the (s)MMO active site (Smith and Dalton, 2004). Therefore, understanding the effect of trace gases in LFG such as TCE on CH_4 biological process would be helpful to control the emission of LFG pollutants into the atmosphere.

Biocover soils, such as compost, waste biocover soil (WBS) and mineralized waste, have been demonstrated to have a high CH_4 oxidation due to their well-distributed particle size, high organic matter content and active microorganism activity (Scheutz and Kjeldsen, 2004; Bogner et al., 2010; He et al., 2012a). However, little is known about the effect of halogenated compounds on CH_4 oxidation and methanotrophic community in biocover soils. In this study, TCE was chosen as the target compound to evaluate the CH_4 oxidation potential and TCE degradation efficiency under varied TCE concentrations in WBS compared with LCS. The CH_4 oxidation activity and biodegradation rate of TCE were tracked in microcosms at a series of TCE concentrations (0, 0.5, 5, 20 and 50 ppmv) representing reported TCE concentrations at landfills that range from 0.001 to 20 ppmv (Chiemchaisri et al., 2001; Shafi et al., 2006). Genes *pmoA* and *mmoX*, respectively, encoding a key subunit of pMMO and sMMO, were used to analyze the identity and diversity of methanotrophs in both soils using quantitative PCR (Q-PCR), terminal restriction fragment length polymorphism (T-RFLP) and cloning. It was hypothesized that methanotrophs in both soils would have different CH_4 oxidation activity and present different response to the TCE addition. Methanotrophs dominating in the soil samples exposed to high concentrations of TCE might be tolerant to TCE and play an important role in the TCE degradation.

2. Material and methods

2.1. Experimental soils

Two types of soils, namely WBS and LCS, were used in this study. The WBS was collected from a landfill bioreactor (2 m^3) treating organic waste with leachate recycle located in a village in Xindeng town, Zhejiang Province. The raw solid waste composition in the bioreactor was described by Wang et al. (2011). The landfill bioreactor had been operated for ~2 years before taking the stabilized waste (WBS). LCS was collected at the 10–20 cm depth of cover soil from Dawuao landfill, which is located in Dawuao Mountain in Pingshui Town, Zhejiang Province. The sampling site located in an area where LCS had been in place for ~12 yr. After removing plants and large particles, the soils were air-dried and sieved through a 4 mm mesh. The physical and chemical properties of WBS were described previously (Wang et al., 2011; He et al., 2012a). The particle composition of the WBS was 61% of 2–4 mm, 33% of 0.02–2 mm and 6% of <0.02 mm. The pH was 7.7. The organic matter and total nitrogen contents of the WBS were 31 g kg^{-1} and 1.3 g kg^{-1}, respectively. The particle composition of the LCS was 42.3% of 2–4 mm, 46.9% of 0.5–2 mm and 10.8% of ≤0.5 mm. The pH was 6.8. The organic matter and total nitrogen contents of the LCS were 17 g kg^{-1} and 0.4 g kg^{-1}, respectively.

2.2. Landfill cover microcosms

Approximately 50 g of the air-dried experimental soil was placed into a 400 ml serum bottle. The WBS sample was adjusted to the water content of 45% (w/w), at which the WBS was reported to have the highest CH_4 oxidation activity (Wang et al., 2011). Because of its low water holding capacity (i.e. the saturated water content of LCS is about 30% (w/w)), it is impossible to adjust the LCS to the same water content as the WBS. Thus, the LCS sample was adjusted to the water content of 20% (w/w), at which the LCS sample was detected to have the highest CH_4 oxidation capacity. The serum bottles were covered with cling film and allowed to equilibrate the soil water contents overnight (~12 h) at 30 °C, and then sealed with butyl rubber stoppers. A certain volume of air was withdrawn from the serum bottle prior to injecting simulated LFG. Simulated LFG (CH_4:CO_2 = 1:1) was injected into the serum bottles to obtain the CH_4 and CO_2 concentrations of 10% (v/v). High purity O_2 was injected into the serum bottles to keep the O_2 concentration at ~21% (v/v). Gas samples were withdrawn periodically from the headspace of the serum bottles for measuring the concentrations of CH_4, CO_2 and O_2. The serum bottles were flushed with air and the initial concentrations of CH_4, CO_2 and O_2 were resupplied to re-establish the initial gases concentrations each day.

After one month of pre-incubation with simulated LFG, the WBS and LCS were denoted as the original material (0 d) for the experiment. The organic matter and total nitrogen contents were 17.7 and 0.6 g kg^{-1} for the original WBS, and 8.2 and 0.3 g kg^{-1} for the original LCS, respectively. Then TCE was injected into the serum bottles to achieve the concentrations of 0, 0.5, 5, 20 and 50 ppmv, respectively. The treatment with sterilized soils and NaN_3 (0.13 mg g^{-1} (dry weight)) were used as non-microbial controls to account for such things as adsorption. All treatments were performed in triplicate, and incubated at 30 °C. Gas samples were withdrawn periodically from the headspace of the serum bottles for measuring CH_4, CO_2, O_2 and TCE concentrations. The serum bottles were flushed with air, and then the initial concentrations of CH_4, CO_2, O_2 and TCE were re-established in the fume hood as described above each day. The whole experiment lasted 54 days.

2.3. Sampling and analysis

Gas samples (100 μl) were periodically taken from the headspace of the serum bottles for measuring CH_4 concentrations as described previously (Wang et al., 2011). The CH_4 oxidation activity was calculated by applying zero-order kinetics as described by Kightley et al. (1995), and expressed by the mass of the CH_4 oxidized per dried soil per hour (μg g^{-1} h^{-1}). Gas samples (500 μl) were withdrawn from the headspace of the serum bottles for the