

Role of Temperature in Microbial Methane Oxidation in Landfill Cover Soil

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ABSTRACT

Municipal solid waste (MSW) landfills are the third largest anthropogenic source of methane (CH₄) emissions in the United States. Part of the CH₄ generated in landfills is converted to carbon dioxide (CO₂) by CH₄-oxidizing bacteria (MOB) present in the landfill cover soil, whose activity is controlled by various environmental factors including temperature. As landfill temperature fluctuates substantially due to seasonal variations and series of reactions during waste decomposition, which may affect the microbial activity and thus, the rates of CH₄ oxidation. This study aims at analyzing the effect of temperature on CH₄ oxidation potential and microbial community structure of methanotrophs in laboratory-based microcosm studies on landfill cover soil. Landfill cover soil samples were incubated at selectively two temperatures 23°C and 50°C, and rates of CH₄ oxidation were measured, and microbial community structure was analyzed using shotgun metagenome sequencing. CH₄ oxidation occurred at both temperatures in soil microcosm tests with highest activity at 23°C. A corresponding shift in the soil microbiota was observed, with a transition from mesophilic to thermophilic methanotrophs with increased incubation temperature. The study shows that temperature is a critical factor affecting rates of CH₄ oxidation in landfill cover soil, and the changing rates of CH₄ oxidation are in part driven by shift in the methylotroph community.

INTRODUCTION

MSW landfills are one of the major contributors of anthropogenic CH₄ emissions and stand as the third largest source in the USA (USEPA, 2019). Various gases are generated in the MSW landfills due to the waste decomposition process. CH₄ and CO₂, both of which are highly potent greenhouse gases (GHG), constitute the major part of the landfill gas (LFG). In the recent past, many researchers explored the options for mitigating landfill CH₄ emissions and in this regard, they evaluated the CH₄ oxidizing potential of MOB present in the landfill cover soil (Kightley et al. 1995; Cao and Staszewska 2011). MOB, commonly known as methanotrophs, are a part of larger microbial community called methylotrophs. Methanotrophs use CH₄ as their sole source of carbon and energy, whereas the methylotrophs use majority of C1-compounds (Hanson and Hanson, 1996). The enzyme methane monooxygenase (MMO) plays an important role in catalyzing

methanotrophic CH₄ oxidation reactions. The MMO enzymes are classified as soluble methane monooxygenase (sMMO), which forms a subset of methanotrophs and particulate methane monooxygenase (pMMO) (Semrau et al. 2010; Reddy et al. 2019).

Various environmental factors such as moisture content, pH, temperature, soil particle size, and nutrients govern the microbial CH₄ oxidation process (Abushammala et al. 2014; Sadasivam and Reddy, 2014). With respect to all enzymatic processes, temperature is found to be a critical factor governing oxidative activity of MOB (Visvanathan et al. 1999; Scheutz and Kjeldsen, 2004; Reddy et al. 2019). Most of the methanotrophs are mesophilic in nature, growing at moderate temperatures (25 - 35°C) (Sadasivam and Reddy, 2014). Many studies based on the laboratory incubation in landfill cover soil have reported maximum CH₄ oxidation at a temperature range of 20 - 31°C (Visvanathan et al. 1999; Scheutz and Kjeldsen, 2004; Park et al. 2005; Chanton et al. 2008), peat soil (Dunfield et al. 1993), wetland and forest soil (Nesbit and Breitenbeck 1992). Apart from affecting microbial CH₄ oxidation, environmental factors also affect the methanotrophic community structure and diversity. Soil texture, pH, gas concentration and moisture content play crucial role in controlling community structure (Su et al. 2014; Urmann et al. 2009). Apart from these factors, some studies showed the effect of temperature on the microbial diversity using molecular techniques in various ecosystems (Mohanty et al. 2007; Borjesson et al. 2004; He et al. 2012; Horz et al. 2005). However, only few studies have explored the effect of temperature on CH₄ oxidation and microbial diversity together in landfill cover soil. Hence, the aim of this study is to examine the effect of selected temperatures on the CH₄ oxidation potential and microbial community together in landfill cover soil. In this regard, laboratory incubation experiments were performed with soil microcosms from a landfill cover soil at two temperatures (23 and 50°C).

MATERIALS AND METHODS

Soil microcosm batch tests. Soil was collected from the Zion landfill site, located in Greater Chicago area, Illinois, USA. Soil samples were collected from an interim cover at a depth of ~1 to 2 feet and were shipped to the Geotechnical and Geoenvironmental Engineering Laboratory at the University of Illinois at Chicago (UIC), where they were stored at room temperature (23 ± 2°C). Soil samples were air dried, pulverized and screened prior to conducting experiments following similar procedure as explained in Reddy et al. (2019).

Batch tests were performed following similar procedures as explained in Reddy et al. (2019). 10 g of sieved soil was placed in 125 mL-serum vials and deionized water was added to obtain a moisture content of 20% (w/w). The optimal moisture content for CH₄ oxidation is found to be 10 - 25% (Abushammala et al. 2014; Sadasivam and Reddy, 2014) based on which a 20% moisture content was chosen in this study. The vials were sealed hermetically using butyl rubber septa and secured by a crimp cap. 20 mL of air from the headspace of each vial was replaced with equal volume of synthetic LFG comprising of 50% (v/v) CH₄ and 50% (v/v) CO₂ to achieve a headspace concentration of ~5 - 6% (v/v) CH₄ and ~5 - 6% (v/v) CO₂ balanced in air (~88 - 90%). The soil microcosms were then incubated at two different temperatures 23°C (room temperature) and 50°C using Heratherm incubators (Thermo Fisher Scientific). The gas samples were collected from the headspace and analyzed at regular intervals using gas chromatograph (GC) until the headspace concentration dropped to less than 1%. All experiments were conducted in replicate, together with the controls (LFG gas without any substrate). CH₄ oxidation rates were determined from the linear slope of CH₄ concentration versus time following zero-order kinetics. At the end

of each temperature experiment, soil samples were frozen immediately at -20°C prior to nucleic acid extraction and molecular analysis.

Microbial community structure analysis. Genomic DNA (gDNA) was extracted from soil samples using a DNeasy PowerSoil Kit (Qiagen) based on manufacturer's instructions with a slight modification. Samples were heated at 65°C for 10 min before homogenizing with FastPrep-24 5G bead-beating device (MP Biomedicals) at 6 m/s for 40 sec. After homogenization, extraction protocols were automated on a QIAcube instrument (Qiagen), according to the manufacturer's instructions. Genomic DNA was processed for microbial community analysis using shotgun metagenome sequencing (SMS). The samples were processed for shotgun metagenome sequencing using a Nextera XT library preparation kit (Illumina, San Diego, CA) according to the manufacturer's instructions. Libraries were pooled and sequenced on a NextSeq500 instrument, employing a 300 cycle (2x150 paired end reads) mid-output kit. Sequence data were analyzed using the online annotation server from OneCodex, as described previously (Minot et al. 2015). DNA extraction, library preparation and sequencing were performed at the University of Illinois at Chicago Sequencing Core (UICSQC).

Gas analysis. The gas samples were analyzed for CH₄, CO₂ and O₂ concentrations using an SRI 9300 GC equipped with a thermal conductivity detector (TCD) and CTR-1 column that separates N₂ and O₂ for simultaneous analysis of CO₂, CH₄, O₂ and N₂. Gas samples were withdrawn using 1 mL syringe where 0.5 mL of the sample was discarded and remaining 0.5 mL was injected into the GC to reduce any pressure effects due to sampling. A calibration curve for a minimum of three points was established using high purity standard gas mixtures ranging from 1% to 50% CH₄ and 5% to 50% CO₂ (Reddy et al. 2019).

RESULTS AND DISCUSSION

Temperature effect on CH₄ oxidation in soil. Fig. 1 shows CH₄ consumption with time in soil samples incubated at temperatures 23 and 50°C. An initial lag phase of 24 hours was observed in soil samples at 23°C followed by a decline in the CH₄ concentration with time. Similarly, at 50°C after an initial lag phase of 48 - 72 hours (Fig. 2) CH₄ was consumed rapidly with time. The CH₄ oxidation rates were calculated from the linear slopes and resulted to be 85.1 and 60.7 µg CH₄ g⁻¹ d⁻¹ at 23°C and 50°C, respectively. Statistical t-test was performed on these two temperatures to check if the oxidation rates were significantly different from each other. The t-test showed significant difference in the oxidation rates between the two temperatures at 95% confidence level ($p = 0.025$). This difference in the oxidation rates could be attributed to substrate diffusion limitation incubated at higher temperature and/or shift in the methanotrophic community at 50°C. Many studies have reported wide range of oxidation rates at a temperature range of 20-23°C in landfill cover soil ranging from as low as 14.4 µg CH₄ g⁻¹ d⁻¹ (Visvanathan et al. 1990), 73 µg CH₄ g⁻¹ d⁻¹ (Whalen et al. 1990) to as high as 2496 µg CH₄ g⁻¹ d⁻¹ (Scheutz and Kjeldsen, 2004). This difference in oxidation rates were mainly due to differences in their experimental conditions and other environmental factors influencing CH₄ oxidation. No studies on landfill cover soil have shown CH₄ oxidation at a temperature > 50°C (Scheutz and Kjeldsen, 2004; Spokas and Bogner, 2011) except the study by Kallistova et al. (2013) who showed CH₄ oxidation rate of 40.1 µg CH₄ g⁻¹ d⁻¹ at 50°C almost 2 folds higher when compared to oxidation rates at 20°C. Studies have also reported moisture content to be one of the important factors affecting CH₄ oxidation along with

temperature (Christophersen et al. 2000; Einola et al. 2007). For an effective CH₄ oxidation, an optimum moisture content of 10 - 20% (w/w) is recommended (Sadasivam and Reddy, 2014) consistent with this study as moisture content lower than 5% causes microbial water stress affecting CH₄ oxidation and greater than 35% causes gas diffusion limitation (Scheutz and Kjeldsen, 2004).

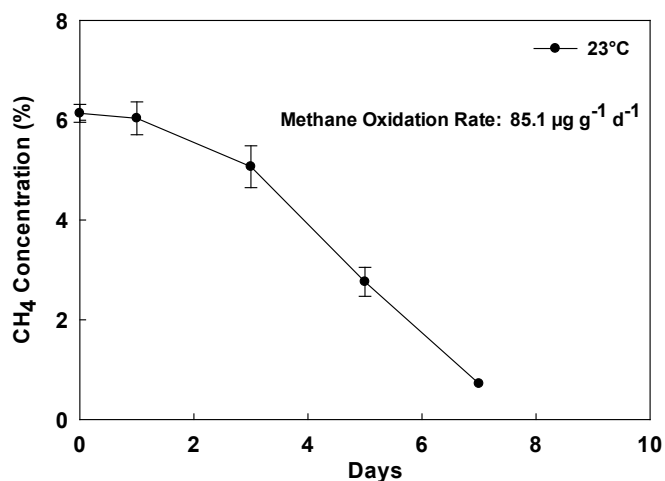


Figure 1. Methane consumption with time in soil microcosms at 20% moisture content (w/w) incubated at temperature 23°C (n=2)

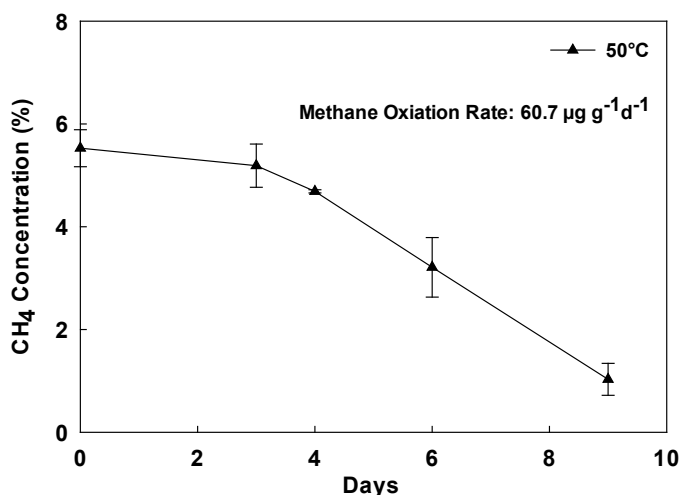


Figure 2. Methane consumption with time in soil microcosms at 20% moisture content (w/w) incubated at temperature 50°C (n=2)

Methylobacter microbial community composition in soil. Soil microcosms were incubated in batch serum vials at temperatures 23°C and 50°C. Microbial genomic DNA was profiled using shotgun metagenome sequencing (Fig. 3). A shift in microbial community structure were observed with the change in temperature. At 23°C, microbial communities methylobacter of the genus *Methylobacter* (Fig. 3a), and of the species *Methylobacter luteus* (Fig. 3b) were found in abundance. Sequences derived from *Methylobacter* accounted for 81% and 0.3% of all methylobacter shotgun sequences identified at temperatures 23°C and 50°C, respectively.

Methylobacter was shown to be dominating at temperature 23°C with the species *Methylobacter luteus* and was negligible at higher incubation temperature of 50°C. *Methylobacter*, a Type-I methanotroph, is one of the major abundant methanotrophs reported in studies of landfill cover soil (Yargicoglu and Reddy 2017; Chi et al. 2015; Chen et al. 2007). The majority of these species are mesophilic (Bowman et al. 1993), though some strains within the genus are psychrophilic, with the capability to grow between 1°C and 20°C (Trotsenko and Khmelenina, 2005). *Methylobacter luteus*, highly abundant in soil microcosms at 23°C, utilize CH₄ and methanol as sole source of carbon and energy and grow at temperatures between 20°C and 37°C (Bowman et al. 1993). Other studies have also reported growth of this species at temperatures 20-30°C in landfill cover soil (Wise et al. 1999; Kallistova et al. 2013) showing consistency with this study. However, at 50°C, the most dominant methanotroph identified belonged to the genus *Methylocaldum* and species *Methylocaldum szegediense*. These are thermophilic bacteria that alter their cell morphology at elevated temperatures and were first isolated from the effluent of an underground hot spring, with an optimal temperature of 55°C (Bodrossy et al. 1997). It was observed that *Methylocaldum szegediense* constituted approximately 97% of the total methanotrophic community at 50°C and negligible at 23°C (< 1%) showing their preference to grow at higher temperatures in comparison to lower temperatures.

In this study it was observed that the microbial community was typically dominated by Type-I methanotrophs. Prior studies on landfills have shown that Type-I methanotrophs outcompetes Type-II methanotrophs under nutrient-rich conditions or at elevated oxygen concentrations and low CH₄ concentrations (Wise et al. 1999; Henckel et al. 2000; Cebon et al. 2007; Li et al. 2013; Chi et al. 2015; Yargicoglu and Reddy, 2017). In addition to the dominant taxa, some of the other methylotrophs (<1%) detected in the soil microcosms belong to the genus *Methylocystis*, *Methylomonas*, *Methylosarcina*, *Methylobacillus* and *Methylomicrobium*.

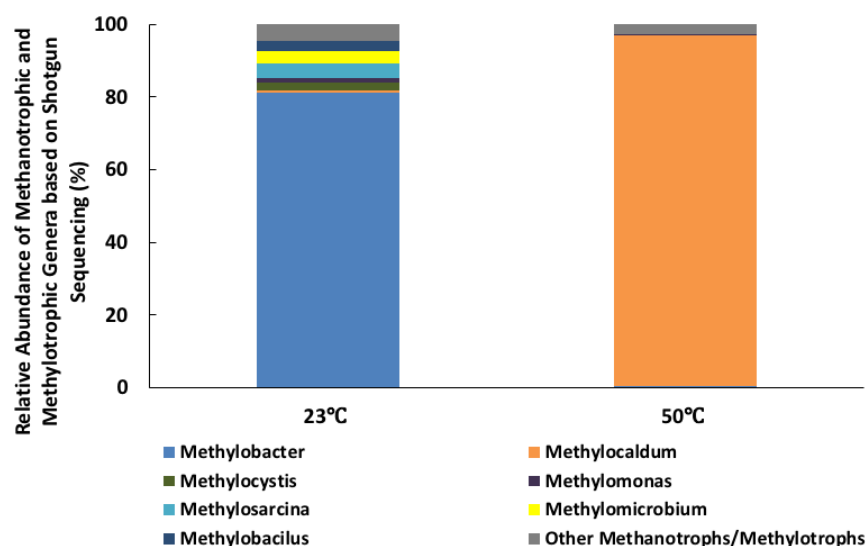


Figure 3a. Relative abundance (%) of sequences related to Methanotrophic and Methylotrophic genera as determined by shotgun gene amplicon sequencing: Soil microcosms at temperatures 23°C and 50°C

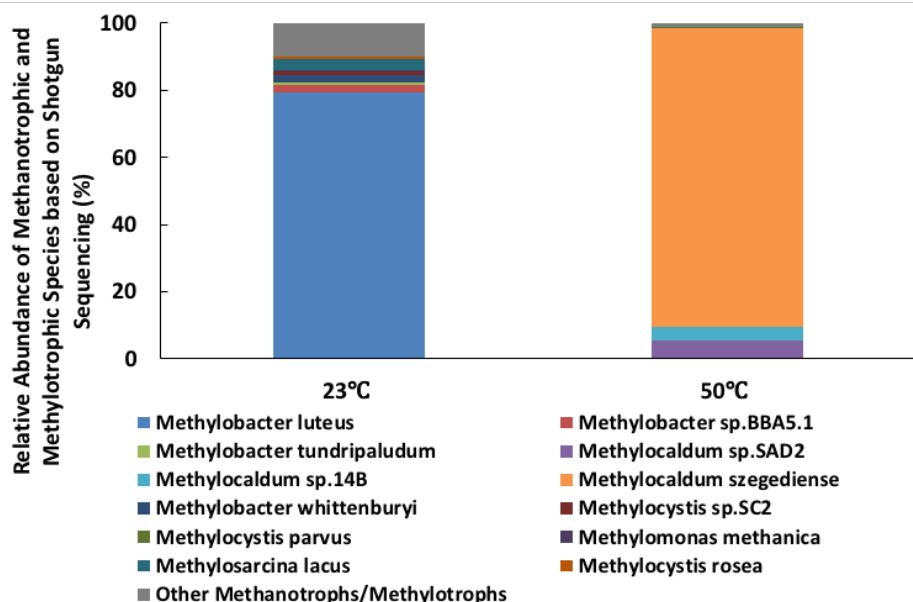


Figure 3b. Relative abundance (%) of sequences related to Methanotrophic and Methylophilic species as determined by shotgun gene amplicon sequencing: Soil microcosms at temperatures 23°C and 50°C

CONCLUSION

The study evaluated the effect of temperature on CH₄ oxidation potential and community structure in soil microcosms. The CH₄ oxidation rate decreased with increase in temperature from 23°C to 50°C. Furthermore, a shift in the methanotrophic community from mesophilic to thermophilic methanotrophic bacteria was pronounced showing direct effect of temperature on the community composition. Overall, temperature is one of the factors influencing CH₄ oxidation and community structure in the landfill cover soil.

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