Mitigating CH\textsubscript{4} Emissions in Semi-Aerobic Landfills: Impacts of Operating Conditions on Abundance and Community Structure of Methanotrophs in Cover Soils

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Introduction

Methane (CH\textsubscript{4}) is the second largest contributor after carbon dioxide (CO\textsubscript{2}) to global warming, with a global warming potential 25 times higher than that of CO\textsubscript{2} [36]. Meanwhile, landfills are considered as one of the major sources of methane emission, accounting for 1.5%–15% of the global methane sources [40]. Consequently, aerobic methane oxidation by methanotrophic bacteria, which are Gram-negative, that use methane as the sole carbon and energy source, becomes one of the most important sink of CH\textsubscript{4}. In addition, anaerobic methane oxidation by methanotrophic Archaea driven by sulfate or nitrite has been discovered [37], but the efficiency of methanotrophic Archaea would be at least 1 order of magnitude lower than the rates of aerobic methane oxidation [16]. Therefore, methanotrophic Archaea was proven to be less important in CH\textsubscript{4} uptake in landfill covers where a large quantity of aerobic methanotrophic bacteria exist. Thus, the methanotrophs studied in the cover layer in this paper are aerobic methanotrophic bacteria.

A number of researchers estimated that 10% to 100% of the CH\textsubscript{4} generated in landfills is oxidized by methanotrophs [9, 13]. Moreover, several studies reported that landfills act as sinks of CH\textsubscript{4} rather than as sources [7, 8, 33]. Therefore,
stimulating the activities of such bacteria in landfill cover soils could possibly reduce the emission of \( \text{CH}_4 \) from landfills, especially in landfills where active gas collection is not required.

Further studies found that there are many factors affecting the activity of methanotrophs, including pH [21], concentration of \( \text{CH}_4 \) and \( \text{O}_2 \) [8, 38], moisture [32], temperature [10, 39], \( \text{NH}_4^+ \) [27, 32], and \( \text{Cu}^{2+} \) [15]. Among these factors, the availability of \( \text{CH}_4 \) and \( \text{O}_2 \) are the most important ones that determine the growth and the type of methanotrophs because they are the substrates for methanotrophs. Traditionally, methanotrophs are classified into two general groups (Type I and II) based on several characteristics, such as cell morphology, membrane arrangement, carbon assimilation pathway, and predominant phospholipid fatty acids. Type I methanotrophs, which belong to Proteobacteria, are composed of Methylococcales, Methylothermataceae, Methylococcaceae, Methylosarcinaceae, Methylosphaeraceae, Methylocorpus, Methylosomentaceae, Methylophalobaceae, Methylocaldaceae, and Methylobacteraceae [21, 31]. Type I methanotrophs can be further divided into two different groups, Type Ia and Ib; the latter is composed of Methyloccaceae, Methylocaldaceae, and Methylothermataceae, whereas the others are Type Ia methanotrophs [6]. Genera that are members of Type II methanotrophs belong to \( \alpha \)-Proteobacteria, including Methylosinus, Methyloccella, Methylocapsa, and Methylocystis [21].

The semi-aerobic landfill (SAL) is a technology of Japanese origin. With properly engineered designs, ambient air naturally flows into the waste body through leachate collection pipes and consequently enhances the waste stabilization processes and leachate quality. Previous estimates suggest that more than 50% of the area in an SAL is aerated as a result of continuous ambient air flow. SAL would therefore be an ideal environment for the growth of methanotrophs because of the interflow of \( \text{CH}_4 \) and \( \text{O}_2 \) in the same region (especially around the venting pipes). Researchers hypothesize that the community structure of methanotrophs in the cover layer of SALs is different from that in sanitary landfills and other habitats because of the special design and therefore \( \text{CH}_4 \) emissions would be mitigated comparing to traditional landfills, but few studies investigated methanotrophic communities in SALs especially in China.

In the present study, the abundance and community structure of methanotrophs from cover soils of two SALs in China were investigated using real-time polymerase chain reaction (real-time-PCR) and denaturing gradient gel electrophoresis (DGGE), respectively. The purpose of this study was to understand the changes in methanotroph communities and abundance in SAL cover soils that may help in designing better management practices for methane emission mitigation in SALs.

**Materials and Methods**

**Landfills and Landfill Gas Measurement**

Soil samples were collected from two SAL cover layers. One is Weifang SAL (23°23’N, 103°23’E) located in Shandong Province, Bohai Sea Region, central Shandong Peninsula. Another is Loudi SAL (27°42’N, 111°59’E) located in central Hunan Province, at the central section of the Yangtze River.

Weifang landfill was put into service in September 2002, having a refuse treatment capacity of 550 t/day and a total volume of \( 8 \times 10^6 \) m\(^3\). Loudi landfill was operated in November 2007, having a refuse treatment capacity of 300 t/day and a total volume of \( 7.7 \times 10^6 \) m\(^3\).

Before soil sampling, the component and concentration of landfill gases, including \( \text{CH}_4 \), \( \text{CO}_2 \), and \( \text{O}_2 \), around the perforated vertical venting pipe of the two landfills were measured using an infrared gas analyzer (X-am7000; Drager, German).

**Sampling**

Samples were collected from the cover layers (10–30 cm in depth) of the two landfills. The cover layer soil of Weifang landfill is brown clay, whereas that of Loudi landfill is red clay. Sampling was conducted around the perforated vertical venting pipes, which are connected to the leachate drainage pipe; ambient air flows through these venting pipes into the waste body. At the same time, the perforated venting pipes act as outlets of landfill gas produced from the waste body; thus, it is an ideal area for invigorating methanotrophs because of the abundant interflow of \( \text{CH}_4 \) and \( \text{O}_2 \). The locations where sampling was done are listed in Table 1, and the basic characteristics of soil samples, including pH, moisture content, organic matter, \( \text{NH}_4^+ \), and \( \text{Cu}^{2+} \), were measured in accordance with a standard method (GB7830-7892-87). Each sample was a mix of three parallel samples (\( n = 3 \)) with the same distance around the perforated vertical venting pipe.

**Table 1.** Locations of samplings in Weifang and Loudi landfills.

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Weifang landfill</th>
<th>Loudi landfill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W1</td>
<td>W2</td>
</tr>
<tr>
<td>Distance from perforated pipe (m)</td>
<td>0</td>
<td>3</td>
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