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Methane oxidation coupled to denitrification under microaerobic and hypoxic conditions in leach bed bioreactors

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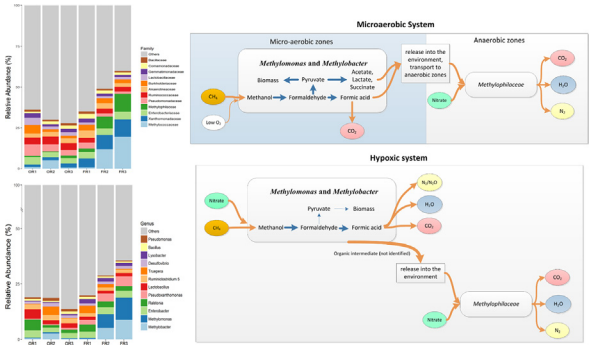
HIGHLIGHTS

- MAME-D and HYME-D have the potential to reduce CH_4 and NO_3^- emissions in landfill.
- C and N mass balances verify these two processes in cultured landfill samples.
- Denitrification rates of MAME-D was higher than that of HYME-D.
- *Methylobacter* and *Methylomonas* oxidized CH_4 using NO_3^- in HYME-D and O_2 in MAME-D.
- *Methylophilaceae* involved in denitrification in the couple system.

GRAPHICAL ABSTRACT



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ABSTRACT

Managing nitrogen and carbon cycles in landfills is an environmental challenge. In this study, our purpose was to test two types of methane oxidation processes coupled to denitrification inside landfills: microaerobic and hypoxic methane oxidation coupled to denitrification (MAME-D and HYME-D). Leach bed bioreactors were designed and operated for >100 d with NO_3^--N concentration ranging from 100 to 400 mg N/L. During six runs of the leach bed bioreactor experiment, leach bed bioreactor 2 (MAME-D) reached 100% denitrification efficiency and the highest average specific denitrification rate of 20.36 mg N/(L·d) in run 5, while leach bed bioreactor 3 (HYME-D) achieved 75% denitrification efficiency and the highest average specific denitrification rate of 8.09 mg N/(L·d) in run 6. Subsequently, waste from leach bed bioreactors 1, 2, and 3 was inoculated into anaerobic bottles to run a batch experiment for 13 d. The total consumed methane, oxygen, and nitrate amounts in the microaerobic system with no methane and oxygen supplement were 2.33, 2.38, and 2.04 mmol, respectively, which almost matched the theoretical equation of aerobic methane oxidation coupled to denitrification. In the hypoxic system, the total consumed methane and nitrate amounts were 0.23 and 0.41 mmol, respectively, the ratio of which closely matched the HYME-D. In addition, via the diverse functional community analysis, methane oxidation in the microaerobic system was confirmed to be conducted by methanotrophs (i.e., *Methylobacter* and *Methylomonas*) using oxygen as an electron acceptor. Subsequently, the generated organic compounds could support denitrifiers (i.e., *Methylophilaceae*) to complete denitrification. In the hypoxic system, *Methylomonas* and *Methylobacter* utilized nitrate as a direct electron acceptor to oxidize methane. The two landfill processes characterized here will expand our understanding of the environmental role of methanotrophs and methylotrophs in both carbon and nitrogen cycles.

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1. Introduction[1]

Municipal solid waste landfill emissions in terms of landfill leachate and gas, are the most serious problems that impede the development of landfill technology. Landfill leachate is high-strength wastewater characterized by extreme pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD5), ammonia, volatile fatty acids, and heavy metals (Bilgili et al., 2012). Treating municipal solid waste by recirculating leachate under aerobic conditions is an efficient method for processing ammonia and BOD5 in leachate and accelerating landfill stabilization (Chung et al., 2015). Nonetheless, nitrate accumulates via ammonia oxidation. In contrast, BOD5 decreases rapidly due to aerobic and anaerobic degradation, thereby causing an imbalance in the C/N ratio. Meanwhile, a large amount of methane is produced by methanogenesis in the anaerobic zones, which contributes to 1.5–15% of global methane emissions (Li et al., 2013). Therefore, combined systems that treat leachate and landfill gas synchronously would be beneficial for solving landfill problems.

The uneven distribution of components (on a macroscale) or the formation of microbial flocs (on a microscale) causes oxygen gradients inside landfills. The utilization of multiple terminal electron acceptors is a strategy of many microorganisms in oxycline habitats (Kits et al., 2015). In this work, the possibility of methane oxidation coupled to denitrification in the microaerobic and hypoxic zones of landfills using a leachate recycling system was tested. As a result, we demonstrate an alternative pathway to decrease methane and nitrate. First, in microaerobic zones, aerobic methanotrophs choose oxygen as the primary electron acceptor to oxidize CH_4 into organic compounds, such as methanol (Werner and Kayser, 1991), citrate (Rhee and Fuhs, 1978), and proteins (Eisentraeger et al., 2001), which are then used by inhabiting denitrifiers as electron donors for denitrification. The process is called microaerobic methane oxidation coupled to denitrification (MAME-D). Second, in hypoxic zones, where oxygen is very limited, methanotrophs can utilize nitrate as the direct electron acceptor to oxidize methane. This process is called hypoxic methane oxidation coupled with denitrification (HYME-D).

While MAME-D has rarely been studied, most studies have focused on aerobic methane oxidation coupled to denitrification (AME-D). The fundamental mechanisms of AME-D and MAME-D are similar, but the difference in oxygen concentration results in activity differences (between methanotrophic bacteria and denitrifying bacteria) and in the reaction efficiency. The methane oxidation coupled to nitrate reduction was first reported by Rhee and Fuhs (1978) at the nitrate removal rate of 79.92 mg N/(L·d). They indicated that the citrate released from methane oxidation could be used by denitrifiers to complete denitrification. Nitrate removal achieved by other methods, such as microbial cell, reached the rate of 1.21 kg NO_3^-N/(m^3(TGV)·d) (Zhang and Angelidaki, 2013; Zhang and Angelidaki, 2014). Microaerobic oxidation of methane at 1% and 10% oxygen was demonstrated by Liu et al. (2014) to release formaldehyde, citrate, and acetate as organic intermediates, and MAME-D–conducting microbial populations were identified to be *Methylococcaceae* and *Methylophilaceae*. The AME-D mechanism and reaction efficiency in the landfill were only reported by Werner and Kayser (1991) at the nitrate removal rate of 60 mg N/(L·d) in an activated sludge reactor. Nevertheless, the methane oxidation and denitrification efficiency, as well as the microbial community of landfill samples cultured in methane and nitrate under microaerobic conditions, have remained unknown. The phenomenon of HYME-D was lately observed by Beck et al. (2013). Meanwhile, data are available suggesting that the oxidative potential of nitrate approximates that of oxygen (Chen and Strous, 2013). Further, it suggests that at least some of the aerobic methanotroph and methylotroph species may be able to thrive in extreme oxygen-limited environments and can potentially utilize alternative electron acceptors, such as nitrate, for methanotrophic and methylotrophic metabolism (Beck et al., 2013; Costa et al., 2000; Modin et al., 2008). The production of N_2O in a HYME-D process was also observed (Kits et al., 2015); however, none of the inoculants were samples from landfills. Thus, whether HYME-D occurs in landfills remains unknown.

The objective of this study was to test whether MAME-D and HYME-D were present in landfills with leachate collection-pretreatment (aeration)-recirculation systems, to determine the nitrate removal rate, and to characterize the microbial community involved. To achieve these goals, we used two experimental setups: (1) a leach bed bioreactor to culture the functional microorganisms and to study the effects of various nitrate concentrations and gas supplements and (2) a batch reactor to study the methane oxidation efficiency, denitrification efficiency, and the correlation between methane oxidation and denitrification. Combined with community analysis, the functional microorganisms and the mechanisms involved in these two processes were revealed.

2. Materials and methods

2.1. Waste samples and leachate preparation and analysis

Waste used for a lab-scale test was taken from the first and second sections of the Emenshan landfill in China. This landfill is a conventional sanitary landfill equipped with a leachate collection-pretreatment (aeration)-recirculation system. The waste samples were collected at three sites from waste ~1, 2, and 10 years old, and at depths of 50–70 and 100–120 cm for each year's deposits. The waste samples were immediately sealed in plastic zip-lock bags and stored at 4 °C. After transport back to the laboratory, the physical and chemical characteristics of the waste samples were analyzed and are given in Table 1.

2.2. Leach bed bioreactor (LBB) experimental system

The experiment was carried out using the nitrate mineral salts (NMS) medium with the following composition (mg/L): KH_2PO_4 200; K_2HPO_4 400; $CaCl_2·2H_2O$ 500; $MgSO_4·7H_2O$ 1000; $FeSO_4·7H_2O$ 9.1; and $NaNO_3$. NO_3^--N concentration ranging from 100 to 400 mg N/L (Modin et al., 2008). The medium also contained 1 mL/L trace element solution. The trace element solution consisted of the following (mg/L): $FeSO_4·7H_2O$ 2486; $MnCl_2·4H_2O$ 500, H_3BO_3 50, $CoCl_2·6H_2O$ 50, $CuSO_4·5H_2O$ 310, $NiSO_4·6H_2O$ 101, and $Na_2MoO_4·2H_2O$ 26 (Modin et al., 2008). The final pH of the NMS medium was adjusted to 7.0.

Three equal-size LBBs were set up for the experiment. The control reactor was labeled as LBB1. Reactor 2 labeled LBB2 was intended for the operation of the MAME-D process, and reactor 3 labeled LBB3 was designed for the operation of the HYME-D process. Fig. 1 schematically illustrates the LBB design. The 14 L stainless steel container with a column of 13 cm internal diameter was subdivided into four chambers: the upper part of 2 L contained steel wool for the desiccation of gases; the second part of 5 L was intended for the storage of gases; the third part of 5 L was the main reaction zone; and the remaining part of 2 L was intended for the storage of liquid. The main reaction zone consisted of three layers (base to top): (1) 5 cm gravel for water drainage, (2) 25 cm landfill waste, and (3) 5 cm sand. Therefore, the carrier volume was approximately 4 L. The gas was distributed from the base to top via gas supply pumps. The gas effluent was collected in a 10 L gas reservoir, and subsequently pumped back to the bottom of the reactor for gas recirculation. Two liters of the NMS medium (Houbron et al., 1999) was pumped into the main reaction zone via peristaltic pumps (LongerPump BT 300-2 J) and spread over the entire top surface of the sand. Next, the liquid percolated through the main reaction zone and was stored in the liquid container, from where it was pumped back to

[1] COD: chemical oxygen demand
MAME-D: microaerobic methane oxidation coupled to denitrification
HYME-D: hypoxic methane oxidation coupled to denitrification
LBB: leach bed bioreactor
SDR: specific denitrification rate
DE: denitrification efficiency.