



Methane Oxidizing Bacteria and Its Potential Application of Methane Emission Control in Landfills

Wenjing Sun^{1,3}, Xiaoyang Liu¹, and Xueping Chen^{2,3}(✉)

¹ Civil Engineering Department, Shanghai University, Shanghai 200444, China

² School of Environmental and Chemical Engineering,
Shanghai University, Shanghai 200444, China
xpchen@shu.edu.cn

³ Institute for the Conservation of Cultural Heritage, Shanghai 200444, China

Abstract. A large amount of methane gas released during the service of a landfill will not only cause the greenhouse effect, but it may also cause an explosion when the methane reaches a certain concentration in the air or soil. In the background of promoting energy conservation and environmental protection, it is of great significance to effectively reduce the methane release content in landfills, ensure the normal service of landfill and the safety of people's life and property. The mechanism of methane oxidation by methane oxidizing bacteria is reviewed and the factors affecting the efficiency of methane oxidizing bacteria to oxidize methane are analyzed in order to reduce the emission reduction of hazardous gases in the landfill and provide a reference for the design of other new landfills.

Keywords: Methane oxidizing bacteria · Landfill · Greenhouse effect
Emission reduction · Biodegradation

1 Research Background

After the second industrial revolution, the world economic level has been developed rapidly. The growing population has led to the continuous growth of solid waste. There are three basic technologies for solid waste treatment, namely landfill, composting and incineration, but landfill is still the first choice in many parts of the world.

Landfill gas may be released for a long time after the landfill is closed, which is produced by anaerobic degradation of organic matter in municipal solid waste and mainly composed of carbon dioxide, methane and some volatile organic compounds. Although the concentration of methane gas is lower than that of carbon dioxide, research shows that methane in the atmosphere is an important greenhouse gas second only to CO₂, with a contribution to the greenhouse effect of about 26% (Zheng 2013). It is estimated that the annual methane emission to atmosphere is between 0.5 billion and 0.6 billion tons. The amount of methane released annually by the landfill accounts for about 6%–12% of the total methane emission worldwide.

Methane is not only a greenhouse gas but also flammable and explosive. When the methane released in the landfill reaches a certain concentration in the air, it is very easy to cause a series of major accidents, such as the explosion and collapse of the landfill, even the casualties and property loss. For example, there have been 20 explosions and fires caused by methane in the United States, resulting in the tragedy of 5 deaths (Han et al. 2000). Therefore, in order to ensure the safe service of landfills and safety of the people's life and property, it is very necessary and urgent to apply methane oxidizing bacteria to the overlying layer of landfill to reduce the greenhouse effect caused by methane emission.

Because the establishment of methane collection system is expensive, especially for the old landfill, it is not economical to install the new gas gathering system. Some old landfill sites are not set up gas collection system. Even if a gas collection system is set up, the methane released by the landfill cannot be completely collected. In the methods of methane emission reduction, Methane oxidizing bacteria reduces methane content by biodegradation, which is considered to be an important global methane sink and plays a vital role in maintaining the relative stability of the global methane content. Studies show that 90% of methane can be oxidized by methane oxidizing bacteria before entering the atmosphere (Gupta et al. 2013). The oxidation efficiency of methane oxidizing bacteria is closely related to temperature, pH value, water content, density and depth of overburden and so on.

In conclusion, applying methane oxidizing bacteria to the landfill overlay can effectively reduce the greenhouse effect caused by methane emissions. According to the survival conditions of methane oxidizing bacteria, it is very important to control the indexes of the overlying soil and improve the efficiency of methane oxidation bacteria to degrade methane gas, which is of great importance to solve the environmental problems.

2 The Mechanism of Methane Oxidation and the Classification of Methane Oxidation Bacteria

Methane oxidizing bacteria are a class of bacteria with methane as the only carbon source and energy. According to the use of the oxygen in the environment as the electron acceptor, methane oxidizing bacteria can be divided into aerobic methane oxidizing bacteria and anaerobic methane oxidizing bacteria.

The mechanism of oxidation of methane by aerobic methane oxidizing bacteria is relatively complicated. It is believed that this approach can be divided into two stages: the first phase is activated by methane monooxygenase (MMO) to generate methanol by CH_4 , and then methanol to formaldehyde; the second stage is mainly through the serine pathway or the monophosphate pathway to assimilate methanol to the microbial biomass or to oxidize it to formic acid, and finally the formic acid is oxidized to CO_2 (Han 2008). Oxidation process is as follows



Figure 1 clearly describes the process of the two stages. In methane oxidizing bacteria cells, MMO is the first key enzyme to use methane. MMO oxidize methane to methanol under the action of molecular oxygen; methanol is oxidized to formaldehyde under the action of methanol dehydrogenase (MDH); then cells are synthesized through the serine cycle or pentose phosphoric acid pathway, and at the same time, the formaldehyde dehydrogenase (FADH) and the methylene dehydrogenase (FADH) are used. Under the action of formic dehydrogenase (FDH), formaldehyde is further oxidized to formic acid, CO_2 and H_2O , and to provide a reducible coenzyme NADH for cell metabolism (Han 2008). CO_2 is the final product of CH_4 deep oxidation.

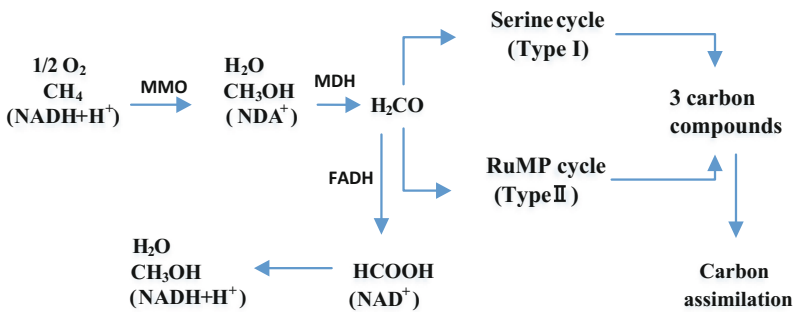
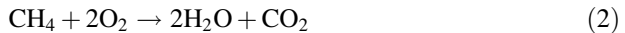
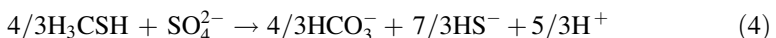


Fig. 1. Mechanism of methane oxidation by aerobic methane oxidizing bacteria (Han 2008)



In quantitative terms, the methane of 1 mol will be oxidized by 2 mol oxygen to produce 1 mol carbon dioxide and 1 mol water.

As early as 1960s, some methane oxidizing bacteria had been found to oxidize methane under anaerobic conditions (Dunfield et al. 2007), but the mechanism of methane oxidation by anaerobic methane oxidizing bacteria is also complex. At present, there are three ways in the environment: anaerobic methane oxidation coupled with sulfate reduction, anaerobic methane oxidation coupled with nitrate reduction and anaerobic methane oxidation coupled metal ion reduction. Morn et al. (2008) also proposed a new methanogenic pathway: methylation pathway. The reaction process is expressed as follows:



That is, methane oxidizing bacteria uses HS^- and methane to form CH_3SH , and CH_3SH is used to produce HCO_3^- and HS^- by desulphurization bacteria (Moran et al. 2008).

In addition to methane oxidation, methane oxidizing bacteria can significantly reduce the concentration of organic compounds in landfill leachate, such as degradation of toxic halogenated hydrocarbons and polycyclic aromatic hydrocarbons in sewage. Therefore, from the point of view of industrial utilization and ecological protection, methane oxidizing bacteria is of great research value.

Bowman et al. (1993) divided methane oxidizing bacteria into Type I, Type II and Type X. Their difference lies in the different methods and ways of assimilating formaldehyde in bacteria. Type I assimilates formaldehyde by monophosphate pathway to assimilate formaldehyde as cell component; Type II uses serine pathway to assimilate formaldehyde as cell component; Type X is similar to Type I, and C assimilation by RuMP pathway (Bowman 1993). The methane oxidizing bacteria in the garbage samples can be classified as *Methylocaldum*, *Methylobacterium* and *Methylocystis*, and the dominant strains were *Methylocaldum* belonging to Type I (Zhang 2012). The following are several examples of methane oxidizing bacteria found in landfills (see Table 1).

Table 1. Methane oxidizing bacteria found in landfills

| Type | Genus | Source | Literature |
|---------|----------------------|---|---------------------|
| Type I | <i>Methylocaldum</i> | A municipal solid waste landfill site in Hebei province | Zhang et al. (2012) |
| Type II | <i>Methylocystis</i> | A municipal solid waste landfill in Hangzhou | Guo et al. (2008) |
| Type I | <i>Methylobacter</i> | A municipal solid waste landfill in Hangzhou | Yu et al. (2008) |
| Type II | <i>Methylocystis</i> | Landfill in Northern Germany | Gebert (2008) |

3 Factors Affecting the Efficiency of Methane Oxidation by Methane Oxidizing Bacteria

The research shows that the physical properties of the landfills, such as temperature, moisture content and pH value, are very closely related to the efficiency of methane oxidation by methane oxidizing bacteria. Therefore, it is of great theoretical and practical significance to study the effects of different physical properties of the overburden on the methane oxidation capacity of methane oxidizing bacteria.

3.1 Water Content

The water content in the soil has a great influence on the activity of methane oxidizing bacteria. When the soil water content is low to a certain value, the metabolism of methane oxidizing bacteria will be reduced, and even the methane oxidizing bacteria will be dormant. Ding and Cai (2003b) found that the influence of water content on the

methane oxidation efficiency of methane oxidizing bacteria depends on the soil quality. Boeckx et al. (1996) described that the water content of the soil could change the permeability of the soil, thus affecting the efficiency of methane oxidation by methane oxidizing bacteria.

3.2 Thickness of Biological Cover Layer

Yang et al. (2010) studied the effect of the thickness of different biological cover layers on methane oxidation in the landfill and found that the efficiency of methane oxidation by methane oxidizing bacteria was different with the depth of the cover layer. From the economic ecological coupling benefit, the oxidation efficiency of methane was the best when the thickness of the landfill cover was 30 cm. The methane oxidation rate of 100% can be reached for a long time.

3.3 CH₄ Concentration and O₂ Concentration

During the service process of landfills, the concentration of methane released from landfills will be changed with the change of service life. Cai (2014) studied the efficiency of methane oxidation by methane oxidizing bacteria under different methane concentration in laboratory conditions. It is found that methane can be completely consumed by methane oxidizing bacteria in 12 h when CH₄ concentration is less than 5%. Methane oxidizing bacteria can still achieve better effects when methane concentration is high. It only takes a long time, for example, for methane with a concentration of about 20% and methane oxidizing bacteria can be eliminated more than 90% in 48 h. As for oxygen, Czepiel et al. (1996) found that the oxygen concentration had a significant effect on the rate of methane oxidation. When the oxygen concentration was below 3%, the rate of methane oxidation was basically zero, so the ideal oxidation rate of methane can only be achieved if a certain amount of oxygen is guaranteed.

3.4 Temperature and PH Value

The effect of temperature and pH value on the oxidation efficiency of methane is mainly manifested by affecting the activity of enzymes in methane oxidizing bacteria. As the monooxygenase of methane oxidizing bacteria is easily inactivated during the purification process, it is very difficult to study the response mechanism of methane oxidizing bacteria to temperature change. There are few reports on the mechanism of methane oxidizing bacteria (Ding and Cai 2003a). Although the studies are fewer, it is not difficult to find that the efficiency of methane oxidation by methane oxidizing bacteria increases in a certain temperature range, but when the temperature is too high, the activity of the monooxygenase will be reduced and even inactivated, thus the effect of methane oxidation will be affected. The pH value over high and low will reduce the activity of monooxygenase and even deactivate the enzyme, which will affect the oxidation efficiency of methane.

3.5 Nitrogen Source

Nitrogen source is essential for the growth and reproduction of methane oxidizing bacteria. Wang et al. (2003) described that the effect of inorganic nitrogen input on the oxidation of soil CH₄ depended on the type of methane oxidizing bacteria, the species and quantity of nitrogen, and the soil condition (Wang et al. 2003). The study of Fei and Wang (2008) and Wei (2015) found that the oxidation of methane to methane by methane oxidizing bacteria was inhibited when the concentration of nitrogen source was higher than a certain value.

It can be seen that there are many factors affecting the efficiency of methane oxidation. When methane oxidizing bacteria is used in landfill, the effects of various factors on the activity of methane oxidizing bacteria should be taken into consideration. Only in this way can the methane oxidizing bacteria be used to ensure the efficiency of methane oxidation.

4 Conclusion and Prospect

At present, the study of methane oxidizers is limited to the screening of bacteria in the laboratory and the study of the efficiency of methane degradation. However, considering the effects of vegetation coverage and the density of overburden, there are few examples of using methane oxidizing bacteria in landfills to achieve emission reduction.

In natural environment, landfill will be affected by natural conditions such as wind, sun and rain, and the physical properties of the overlying layer will be changed under the condition of dry and wet circulation, which will affect the activity of methane oxidizing bacteria. The research and engineering application of methane oxidizing bacteria will be promising on the basis of previous research if we control the density, pH value, water content and oxygen supply of the overlying soil and combine with the study of ecology.

If the real natural conditions are simulated in the laboratory and the cracking of the overlying soil is studied, to some degree, it can provide a theoretical analysis for the large scope of the methane oxidizing bacteria in the landfill. In addition, sensors are added to the landfill to monitor the methane concentration in the landfill in real time and take necessary measures to control it in time which may provide a new idea for the formulation of energy conservation and atmospheric protection schemes.

Acknowledgement. The authors are grateful to the support of Shanghai Key Innovative Team of Cultural Heritage Conservation, and the National Sciences Foundation of China (Grant No. 41572284).

References

- Zheng SW, Tang W, Gu Y et al (2013) Estimation and control of methane emissions in landfills. *Environ Sci Manag* 38(7):45–49
- Han HF, Jin MT, Chi CJ et al (2000) On applicable urban domestic refuse treatment technologies in China. *Environ Pollut Prev* 21(6):40–41
- Han B (2008) Methanogenic bacteria, molecular ecology study on methanotrophs and fundamental research on the applications of methanotrophs. Tsinghua University
- Dunfield PF, Yuryev A, Senin P et al (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum verrucomicrobia. *Nature* 450:879–882
- Moran JJ, Beal EJ, Vrentas JM et al (2008) Methyl sulfides as intermediates in the anaerobic oxidation of methane. *Environ Microbiol* 10(1):162–173
- Bowman JP (1993) Revised taxonomy of the methanotrophs: description of *Methylobacter* gen. nov. emendation of *Methylococcus*, validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family *Methylococcaceae* includes only the group I methanotrophs. *Int J Syst Bacteriol* 43(4):735–753
- Zhang W, Yue B, Huang QF et al (2012) Community analysis of methane oxidizing bacteria in municipal solid waste semi-aerobic landfill. *Chin J Ecol Environ* 8:1462–1467
- Guo M, He PJ, Lv F et al (2008) Type II methanotrophs community structure in the cover soils of landfill. *Chin Environ Sci* 28(6):536–541
- Yu T, He PJ, Lv F et al (2008) Effect of operational modes on community structure of type I methanotroph in the cover soil of municipal solid waste landfill. *Environ Sci* 29(10):2987–2992
- Gebert J, Stralis-Pavese N, Alawi M, Bodrossy L (2008) Analysis of methanotrophic communities in landfill biofilters using. *Environ Microbiol* 10(5):1175–1188
- Ding WX, Cai ZC (2003a) Mechanism of methane oxidation by methanotrophs and effect of soil moisture content on their activity. *Chin J Eco-Agric* 11(1):94–97
- Boeckx P, Cleemput OV, Villaralvo I (1996) Methane emission from a landfill and the methane oxidizing capacity of its covering soil. *Soil Biol Biochem* 28(10–11):1397–1405
- Yang WJ, Dong SK, Zhang XF et al (2010) Effect of bio-cover thicknesses on methane oxidation in landfill. *Environ Pollut Prev* 32(7):20–24
- Cai CH (2014) Experimental study on performance and its influencing factors of methane degradation by methanotrophs. Henan Polytechnic University
- Czepiel PM, Mosher B, Crill PM, Harriss RC (1996) Quantifying the effect of oxidation on landfill methane emissions. *J Geophys Res-Atmos* 101(D11):16721–16729
- Ding WX, Cai ZC (2003b) Effect of temperature on atmospheric CH₄ oxidation in soils. *J Ecol* 22(3):54–58
- Wang ZP, Hu CS, Yang JR (2003) Effect of inorganic nitrogen on CH₄ oxidation in soils. *J Appl Ecol* 14(2):305–309
- Fei PA, Wang Q (2008) Analysis on the mechanism and influence factors of methane oxidation in landfill soil covers. *Renew Energ* 26(1):97–101
- Wei WP, Deng H, Li GX et al (2015) Screening and culture condition of a type II methanotroph. *Appl Environ Biol* 21(3):455–463