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Original Research

Methane-Oxidizing Microorganism Properties in Landfills

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Abstract

Bio-oxidation of methane in a landfill environment is important for mitigating global methane emissions into the atmosphere. In the present study, the characteristics of methane bio-oxidation and methane-oxidizing microorganisms were studied by enrichment cultivation using fresh and aged leachate (collected from Qizishan Landfill, Suzhou, China). Both aerobic and anaerobic methane oxidation were detected, methane oxidation capacities of the culture liquid were 5.24–7.26 μmol/mL/d under aerobic conditions and 4.41-3.70 μmol/mL/d under anaerobic conditions. The stoichiometry of anaerobic oxidation of methane (AOM) showed the complexity of AOM mechanisms in the leachate culture, with the types of sulfate-dependent, denitrification-dependent and iron-dependent AOM. The 16S rRNA gene sequence analysis and SEM analysis results showed that the genus *Methylocystis* was the dominant bacteria in aerobic cultures (relative abundance 35.96–78.37%). Genus *Moheibacter* (41.38%) and *Cupriavidus* (43.08%) were the most dominant taxa in anaerobic cultures, with aerobic methanotrophs *Methylocaldum* and *Methylocystis* in low abundance, and no anaerobic methane-oxidizing archaea (ANME) was found. Further research is needed to confirm whether aerobic methanotrophs can oxidize methane under anaerobic conditions.

Keywords: landfill, methane oxidation, leachate, methane-oxidizing microorganisms, methanotrophs, anaerobic oxidation of methane

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Introduction

Methane is a well-known greenhouse gas. Atmospheric methane concentration has more than doubled since the preindustrial era, from approximately 0.7 ppm to about 1.8 ppm today. Approximately 60% of its global emissions are derived from human activity [1, 2]. According to estimates from the International Panel on Climate Change, the global release of methane was approximately 3×10^{11} kg in 2000 and has continued to increase. Refuse landfill sites are significant sources of methane release and account for 6-12% of global methane emissions [3]. Hence, on-site control of methane emissions in refuse landfill sites has been highlighted.

The methane oxidation capability of landfill covers has been studied, with the final cover demonstrating effective reduction of methane release [4, 5]. The feasibility of various materials to enhance methane oxidation capacity of landfill covers has been investigated [6-9]. Aged refuse from waste landfills closed for 8 years was found to contain methanotrophs capable of methane bio-oxidation [8,10]. Both Type I and II methanotrophs were found in the aged refuse, including the genus Methylobacter, Methylocaldum, and Methylocystis [10]. These findings suggest that methanotrophs may be present inside the landfill.

Many studies have shown that some methane that was previously thought to be consumed by aerobic oxidation was actually consumed by anaerobic oxidation of methane (AOM) [11-13]. Subsequent studies found that AOM is widespread and has been detected in various environments, including landfills and the surrounding environment [14-18]. AOM was discovered in an alluvial aquifer contaminated with leachate from an unlined municipal landfill [19, 20]. The rate of AOM at this site was 1-3 orders of magnitude lower than that in ocean sediments with rich sulfate, and 3 orders of magnitude lower than that of aerobic oxidation of methane in the landfill cover [19]. Han found that sulfate-reducing bacteria (SRB) existed in almost all layers of landfill bodies, and aged refuse at the bottom contained most [21]. Both aerobic and anaerobic methane oxidation effects were detected in garbage samples, with anaerobic oxidation accounting for more than 20%.

AOM has been considered important for controlling global methane emissions. There has been much research on AOM in deep sea and sediments, and ANME are thought to couple methane oxidation to sulfate reduction in partnership with SRB in the deep sea [22-24]. The findings of AOM in various environments, such as lake sediments, a contaminated aquifer, and a mud volcano, have deepened our understanding of its mechanisms [25-28]. The anaerobic oxidation of methane may influence methane emissions in a landfill because the anoxic region is prevalent in this environment. But little is known about methane oxidation in a landfill, and its mechanism and the related microorganisms are not clear.

Therefore, the AOM mechanism in landfills and related microbial populations should be investigated. In this study, leachate was used to enrich methane-oxidizing microorganisms to reveal the characteristics of the methane oxidation community in the landfill at different refuse degradation phases, and the AOM mechanism was discussed by stoichiometric analysis. This research provided useful reference for further study of the anaerobic oxidation process and microorganisms in a landfill. It is helpful for understanding the carbon conversion process within the landfill and providing guidance for effective methane emissions control at landfill sites.

Materials and Methods

Enrichment Cultivation of Methane-Oxidizing Microorganisms

Two types of leachate were used for enrichment cultivation: leachate M1, during the hydrolysis acidification stage, was collected from a laboratory simulation landfill lysimeter containing refuse that had been sealed for over 6 months; and leachate M2 was collected from Qizishan Landfill (with a cover time of over 8 years) in Suzhou, China (Fig. 1). The leachate of 6 months is not a typical environment for methane-oxidizing bacteria, but significant methane oxidation was observed in the leachate of this stage in our previous experiments, so leachate of 6 months was used for enrichment experiments here.

Materials packed in the lysimeter were prepared according to the organic composition of refuse in landfill sites [29], and consisted of 70% kitchen waste, 20% paper, and 10% wood and fabric. The main properties of the material were as follows: a moisture content of approximately 70% and a density of approximately 235 kg/m^3. The prepared household waste was broken down before being mixed and placed in the lysimeter, which was then sealed. The experimental rig was made according to Mei [10].

Leachate was used to inoculate nitrate minimal salt (NMS) medium to enrich methane-oxidizing microorganisms [10]. Enrichments were carried out by batch incubation experiments using two methods: under aerobic conditions and under anaerobic conditions. Samples MO1 and MO2 are cultures enriched with leachate M1 and M2 under aerobic conditions; MA1 and MA2 are cultures enriched with leachate M1 and M2 under anaerobic conditions. Leachate (2 mL) and 15 mL NMS medium were placed in 300 mL serum bottles. For aerobic cultivation, the serum bottles were sealed with rubber stoppers and 50 mL methane was injected to replace the air in the bottle. For anaerobic cultivation, leachate and NMS medium were anaerobically transferred into the bottle with 2 mL Na_2SO_4 (0.125 mol/L) added as an electron acceptor, the bottles were swept with nitrogen for 15 min,

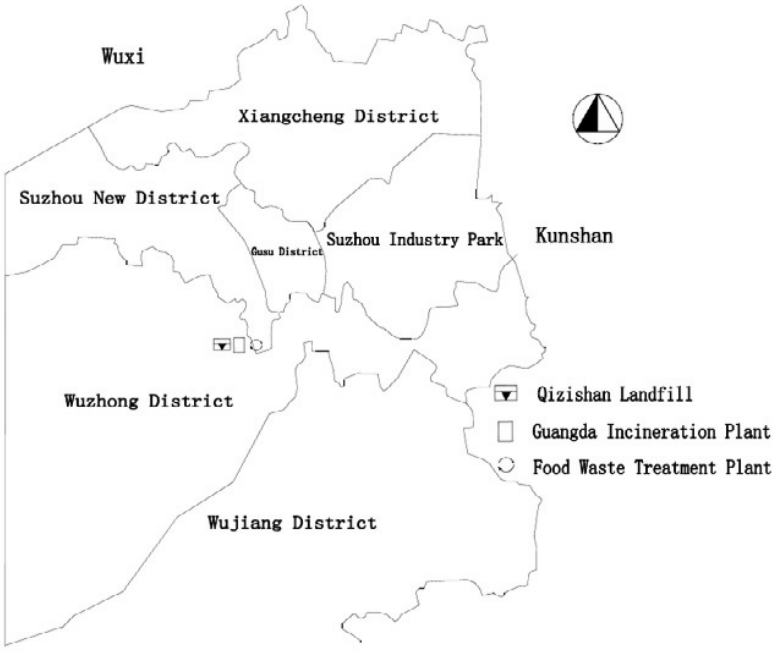

Fig. 1. The location of Suzhou Qizishan Landfill, which disposes municipal solid waste in Suzhou New District, Suzhou Industry Park, Gusu District, Xiangcheng District and Wuzhong District.

sealed with rubber stoppers and injected with 50 mL methane. All bottles were incubated at 36ºC, on a rotary shaker operating at 130 rpm. High methane oxidation rates were found to be between 28 and 37ºC in our previous research [8], therefore methane-oxidizing microorganisms were enriched at 36ºC in this study. Methane concentrations in the bottles were analyzed before and after enrichment to determine methane oxidation rates. Each culture included three parallel samples.

DNA Extraction, PCR, and Pyrosequencing

The cultures with the highest methane oxidation rate were prepared for DNA extraction. The culture liquid was centrifuged (8,000 × g, 3 min) and DNA was extracted from the deposit using the E.Z.N.A. Soil DNA Isolation Kit (Omega Bio-Tek, Inc., USA). The bacterial primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the V3-V4 region of the 16S rRNA gene. Archaea community analysis was conducted using the bacterial primers Arch334F (5'-ACGGGGYGCAGCAGGCGCGA-3') and Arch915R (5'-GTGCTCCCCCGCCAATTCCT-3').

The pmoA gene was PCR amplified from total DNA extracted from the same samples using primer mb661 in conjunction with primer A189gc [30, 31]. Primers A189gc and mb661 can amplify an approximately 470-bp internal section of pmoA and produce strong signals with many methanotrophs. The PCR amplification reactions were carried out using previously published laboratory protocols [31].

Nucleotide Sequence Accession Numbers

High-throughput pyrosequencing was performed on the Illumina MiSeq platform at Majorbio Bio-Pharm Technology Co., Ltd., China. The GenBank accession numbers for the nucleotide sequences determined in this study were MH177848 to MH177850.

Scanning Electron Microscopy (SEM)

The culture liquid was centrifuged (8,000 × g, 3 min) and the deposits were washed using 0.1 M phosphoric acid buffer (PHB) three times, and were then fixed using 2.5% glutaraldehyde solution (pH 6.8) at 4ºC for 12 h. The fixed samples were washed again with PHB three times for 5 min each time, then dehydrated by covering the samples with 30, 50, 70, 90 and 95% ethanol for 10 min each time and finally dehydrated with 100% ethanol three times. Air-dried samples were placed on aluminum specimen mounts and were gold sputtered and viewed with a Hitachi S-3200N scanning electron microscope operating at 20 kV.

Chemometrics Analysis of Anaerobic Methane Oxidation

Besides the added Na_2SO_4, nitrate and sulfate are components of NMS medium and can also serve as electron acceptors for AOM according to reported AOM mechanisms [32-34]. Phosphate is also considered a potential electron acceptor. The concentrations of these electron acceptors in NMS medium were calculated as n Na_2SO_4, n NO_3^-, n SO_4^{2-}, and n PO_4^{3-}, respectively.

Below are the chemometrics of anaerobic methane oxidation related to sulfate reduction, nitrate reduction and phosphate reduction, respectively:

$$CH_4+SO_4^{2-}\rightarrow HCO_3^- + HS^- + H_2O \quad (1)$$

$$5CH_4+8NO_3^-+8H^+\rightarrow 5CO_2+4N_2+14H_2O \quad (2)$$

$$CH_4 +PO_4^{3-} \rightarrow HCO_3^- + H_3P \quad (3)$$

According to the above equations, the equivalent quantity of the electron acceptors of moles of methane are shown below, calculated as N Na_2SO4, N NO_3^-, N SO_4^{2-}, and N PO_4^{3-}, respectively.

$$N\ SO_4^{2-} = n\ SO_4^{2-} \quad (4)$$

$$N\ NO_3^- = 0.625n\ NO_3^- \quad (5)$$

$$N\ PO_4^{3-} = n\ PO_4^{3-} \quad (6)$$

The total number of possible electron acceptors in NMS medium (counted as Ne) was calculated by Eq. (7):

$$Ne = n\ Na_2SO_4 + n\ SO_4^{2-} + 0.625n\ NO_3^- + n\ PO_4^{3-} \quad (7)$$

Analytical Methods

CH_4 was analyzed in a 200 μL sample by gas chromatography (GC-14B, Shimadzu, Japan) with a stainless steel column packed with Carbosive SII (diameter of 3.2 mm and 2.0 m length) and thermal conductivity detector (TCD). The temperature of the injection, column and detector was set at 40, 80 and 90ºC, respectively. The carrier was nitrogen and the flow rate was 30 mL/min.

For the leachate, total nitrogen (TN) was measured according to the distillation-titration method; total phosphate (TP) was determined by the molybdate spectrophotometric method [35]; TOC was determined by TOC-VCPH/CPN (Shimadzu Corporation, Japan), oxidation reduction potential (ORP) was measured using an ORP electrode with a special pH meter for mV readings, negative ion concentrations were analyzed by ICS-1000 (Dionex corporation, USA), and metal ion concentrations were measured by Optima 2100 DV (Perkin Elmer) after microwave digestion by ETHOSE (Mile Stones.r.l, Italy) [36].

Table 1. Physicochemical properties of the leachate.

Parameter	Unit	Leachate M1	Leachate M2
pH		7.90±0.18	7.70±0.17
ORP	mV	-52±17	-38±10
TOC	mg/L	295.4±7.5	27.8±1.1
IC	mg/L	361.6±36.9	568.5±7.4
TN	mg/L	32.0±7.2	1986.5±52.1
TP	mg/L	0.20±0.02	0.26±0.02
Fe	mg/L	0.44±0.06	1.66±0.22
Mn	mg/L	0.49±0.03	0.27±0.02

Results and Discussion

Physicochemical Properties of the Leachate Used for Enrichment Cultivation

Table 1 shows the main physicochemical properties of leachates M1 and M2. M1 sampled from a landfill and simulating a lysimeter sealed for over 6 months represents fresh leachate during the hydrolysis acidification phase of organic decomposition. M2 represents aged leachate of over 8 years.

Methane Oxidation Activity under Different Cultivation Conditions

The method of inoculating leachate into NMS medium to enrich methane oxidation microorganisms was feasible. Both aerobic methane oxidation and anaerobic methane oxidation were observed in the cultivation experiments (Fig. 2). The oxidation rates of the anaerobic culture liquid were 36.28-39.40% in 14 days, lower than those of the aerobic culture liquid (55.84-77.31%). Methane oxidation rates of fresh leachate samples were higher than those of aged leachate samples. For investigating the AOM mechanism, laboratory enrichment cultures of the responsible

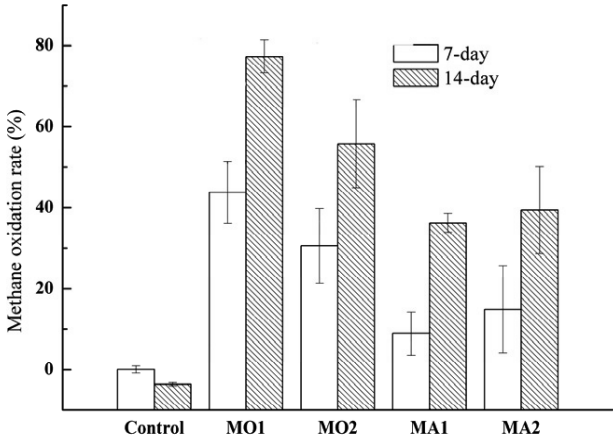

Fig. 2. Methane oxidation rates of different enrichment cultures: MO1 and MO2 are cultures enriched with leachate M1 and M2 under aerobic conditions; MA1 and MA2 are cultures enriched with leachate M1 and M2 under anaerobic conditions.

Table 2. Stoichiometry of oxidized methane and different electron acceptors in the cultivation system.

Number of reactants/ mmol	CH_4	Possible electron acceptors			
		Na_2SO_4	SO_4^{2-}	NO_3^-	PO_4^{3-}
	1.972	0.250	0.035	0.193	0.300
Maximum possible oxidation rate		12.7%			
		14.5%			
		24.3%			
		39.5%			

microorganisms are of crucial importance. High methane oxidation rate of the leachate culture suggested that anaerobic methane oxidation microorganisms in the landfill were enriched successfully here.

According to the ideal gas state equation, the initial methane amount (n_{CH4}) in the serum bottle in the enrichment cultivation test can be calculated. In this experiment temperature, pressure and the volume of methane injected in the bottles were 309K, 101.1 KPa, and 50 mL, respectively. The initial methane amount (n_{CH4}) was approximately 1.972 mmol. Based on n_{CH4}, the average methane oxidation capacities under anaerobic conditions were 3.41-3.70 μmol/mL/d. These values were higher than those observed in other studies [18, 34]. Scheller et al. studied AOM in deep-sea sediments. They performed anaerobic microcosm experiments using methane seep sediment (containing ANME-2a and ANME-2c). After 21 days incubation at 4°C, the initial rates of AOM with different electron acceptors were 0.12-1.62 μmol/mL/d over the first 6 days [34]. Low ambient temperature of 4ºC might cause a low methane oxidation rate.

Mixed culture may be another reason for the high methane oxidation rate in this study. Much research on AOM mechanisms is based on the isolation and purification of related microorganisms – cultivation that often takes a very long time [25-28]. To reveal the mechanisms of denitrification-dependent AOM (DAOM) and iron-dependent AOM, the enrichment cultures were isolated and purified over 16 months continuously [18, 37]. In this study, the enrichment culture was achieved by batch cultivation in a short time (2 weeks), without the isolation and purification of AOM-related microorganisms. Further studies on the changes of methane oxidation rate and microbial population after subculture and purification are needed.

The Stoichiometry of Anaerobic Methane Oxidation

AOM are classified into three types according to electron acceptors, sulfate-dependent AOM (SAOM), DAOM, and iron-dependent AOM [32]. The type of AOM is often proved and determined using stoichiometric analysis [18, 37]. The stoichiometric analysis showed that the amount of methane oxidation exceeded the maximum possible number of various electron acceptors in the cultivation system. Na_2SO_4 was added to NMS medium as an electron acceptor of AOM. The number of moles of added Na_2SO_4 was 12.7% that of methane in the bottles. According to the mechanism of SAOM, the maximum methane oxidation rate should be 12.7% (Table 2). However, the 14-day methane oxidation rates for MA1 and MA2 were 36.28% and 39.40%, respectively (Fig. 2). The amount of oxidized methane was obviously more than the amount of added Na_2SO_4.

Based on the existing anaerobic methane oxidation mechanisms, SO_4^{2-}, NO_3^-, and PO_4^{3-} in the NMS medium also acted as electron receptors [32]. Considering all the possible electron receptors in the NMS medium, the maximum possible oxidation rate was 39.5%. This showed there were various types of AOM in the culture, including SAOM and DAOM. Iron-dependent AOM should also occur because the anaerobic methane oxidation rate was found to be over 50% in some subsequent subcultures. Metal irons, such as Fe^{3+} and Mn^{4+}, could be derived into the medium from the inoculated leachate. The chemical forms construction of Fe and Mn in the landfill and the contribution of Fe^{3+} and Mn^{4+} to methane oxidation should be further studied in the future.

Community Structure of Methane Oxidation Microorganisms

Microbial community analysis of the enrichment cultures was conducted by high-throughput pyrosequencing, which yielded 297,518 reads with an average length of 440.7 bp for each read. 148,759 valid reads were obtained for subsequent analysis. Reference-based chimera detection was performed using Silva's

Table 3. Recovered sequences, OTUs, and richness/diversity estimators of the culture samples (16S rRNA genes).

Samples	Read number	Average length /bp	OTU number	Richness index/ ACE	Diversity index/ Shannon	Coverage /%
MO1	34155	439.52	152	88.9	1.505	99.95%
MO2	37382	429.46	67	76.9	1.049	99.97%
MA1	41581	445.88	91	98.8	1.984	99.97%
MA2	35641	443.93	97	101.7	2.384	99.98%

representative set of 16S rRNA gene sequences, and the valid reads were clustered in operational taxonomy units (OTUs) at 97% similarity. Representative sequences for each OTU (163 OTUs retrieved) were aligned and their taxonomy was assigned using the Silva database. A phylogenetic tree was then constructed using META5.1.

On the whole, the microbial richness and diversity of all enrichment cultures were low (Table 2). The ACE index values of MA were higher than those of MO, indicating that microbial species richness was higher in the anaerobic cultures. According to the Shannon index, species diversity of the aerobic culture was lower. The dominance of main bacteria in aerobic culture samples was higher, and the enrichment effect of oxidizing bacteria was more obvious.

As shown in Fig. 3, Proteobacteria was the dominant group at the phylum level in all samples (relative abundance of 51.26-97.93%), followed by Bacteroidetes (1.35-44.76%), Chloroflexi (8.69% in MA), and Actinobacteria (5.47% in MA), together accounting for 98.95-99.95% of all the classified sequences. For the dominant bacterial phylum, Plantomycetes, there were different subgroups in different samples. Betaproteobacteria was the most abundant clade in MO1 and MA2 (41.4-69.4%), Alphaproteobacteria was the most abundant in MO2, and Gammaproteobacteria was the most abundant in MA1.

The majority of Proteobacteria sequences in the aerobic samples were attributed to aerobic methanotrophs. Genus Methylocystis was the most dominant clade in MO1 and MO2 (35.96% and 78.37%, respectively). Methylocystis has been found in landfill environments, and Methylocystis strain JTA1 was isolated from the Laogang landfill in Shanghai (China), and can utilize methane as well as acetate [38]. In MO1, Methylophilus (42.45%) was more dominant than Methylocystis (35.96%). Moheibacter (41.38%) and Cupriavidus (43.08%) were the most dominant genera of MA1 and MA2, respectively. Methanotrophs were a minor component of anaerobic samples, with 29.68% Methylocaldum in MA1 and 6.36% Methylocystis in MA2. A total of 212 metabolic pathways were detected

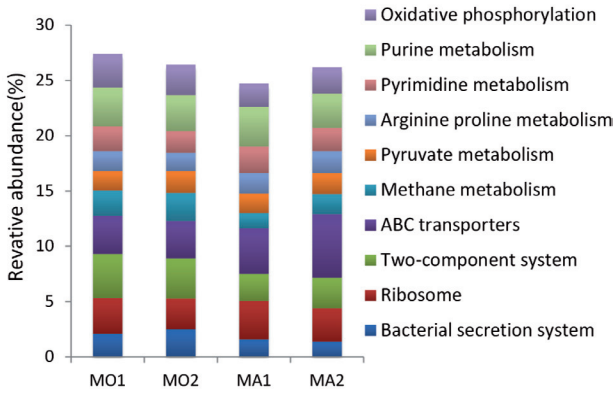

Fig. 4. Top 9 pathways based on 16S rRNA bacterial sequence analysis.

in all samples. Methane metabolism was one of the main pathways in each sample (Fig. 4). This indicated that methane metabolism was the main and important metabolic process of nutrition in the samples. The relative abundance (2.28-2.55%) of methane metabolism in aerobic samples was significantly higher than that in anaerobic samples (1.39-1.81%).

No research has pointed out the relationship between Moheibacter and Cupriavidus with AOM microorganisms. Moheibacter was detected in underground rocks of the Mohe permafrost area, where there is a great potential for gas hydrate accumulation [39]. The discovered strain was aerobic bacteria, which could hydrolyze casein and other proteins, but could not reduce nitrate or sulfate. This should be related to organic matter degradation in the leachate. Cupriavidus is a widespread genus with oxidative metabolism and can grow in the moist and oligotrophic environment. It has been found to be resistant to metals. Some strains can oxidize ferrous in nitrate environment and reduce arsenic, and some resist cadmium [40]. So they can be used to control soil heavy metal pollution. The influence of these two groups on AOM can be further investigated through subcultures.

Microbial community analysis of pmoA gene sequences for MO resulted in only 3 OTUs. OTU2

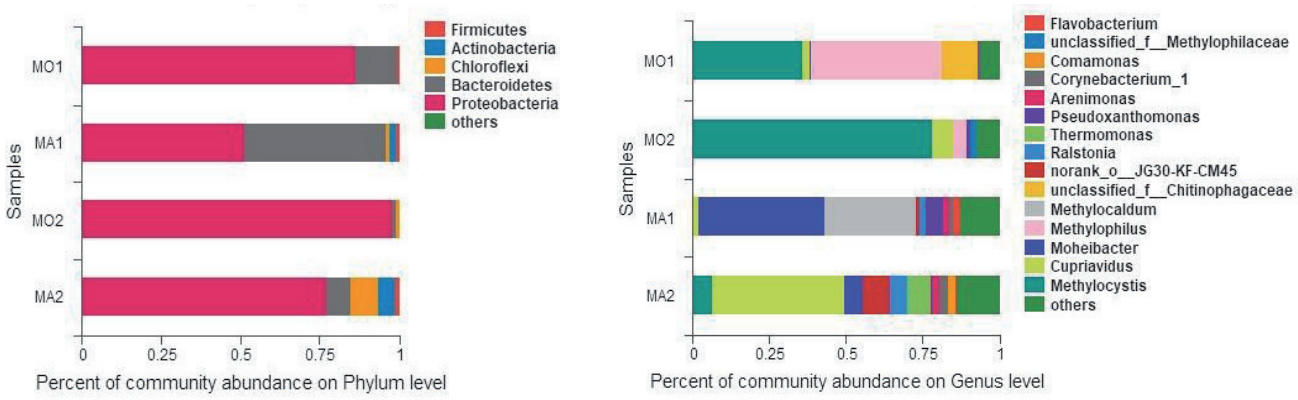

Fig. 3. 16S rRNA bacterial community composition of enriched cultures.

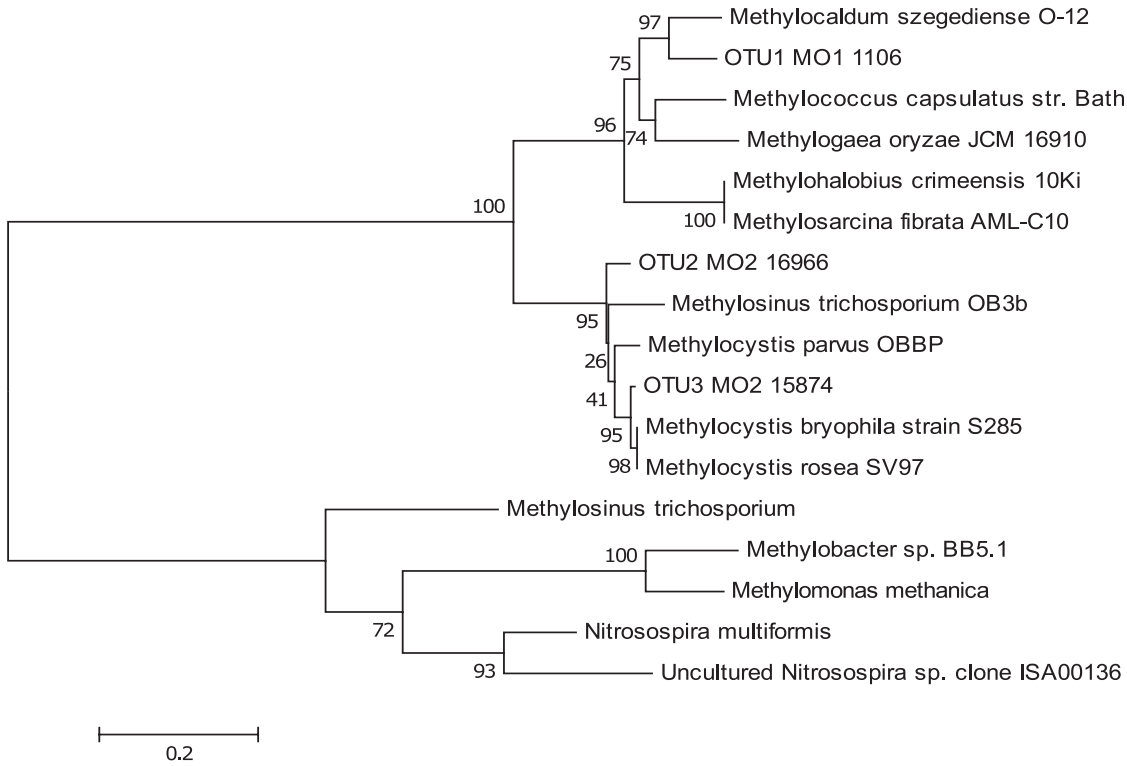

Fig. 5. Phylogenetic analysis of pmoA gene clone sequences retrieved from enrichment culture; bootstrap values are shown near the clades, and the bar indicates the estimated number of base changes per nucleotide sequence position.

(99.37%) and OTU3 (99.28%) were the dominant populations in MO1 and MO2, respectively. Related and similar sequences for the representative sequences of these 3 OTUs obtained from the NBCI database, a phylogenetic tree was constructed (Fig. 5). OTU2 and OTU3 were similar to *Methylocystis* sp. found in other studies, and were also similar to Methylosinus trichosporium OB3b (the sequence similarities were 94% and 95%, respectively). This difficulty in classifying methane-oxidizing organisms has been found in other studies. Carlos also found this while comparing Methylocystis parvus OBBP and *Methylocystis* sp. strain Rockwell and suggested that both species should be taxonomically classified in different genera [41]. OTU1 was Methylocaldum and its relative abundance (0.17%) in MO2 was very low.

Aerobic methanotrophs were found in anaerobic samples in this study. Some previous research also suggest that AOM is a complex process, with more related microbes than previously thought. Aerobic methanotrophs such as Methylobacter have been proven to be involved in AOM [42-47]. Martinez-Cruz et al. studied the microorganisms involved in AOM in sub-Arctic lake sediments using DNA- and phospholipid-fatty acid (PLFA)-based stable isotope probing. They found that aerobic methanotrophs Methylobacter assimilated carbon from CH_4 as one of the main genera, with the mean AOM rate 1.76±0.20 μg/g dw/d according to the slope of $^{13}CH_4$ abundance [42].

Aerobic methanotrophs have been previously recognized to perform methane oxidation under anoxic conditions in lake sediments, and active Methylobacter-type methanotrophs have been found in anoxic and oxic zones in different lakes [43, 44]. Methylobacter, together with Methylotenera-type methanotrophs, were identified as the dominant genera in incubations with low initial O_2 concentrations [45]. Svenning et al. found that a Methylobacter genome also encodes respiratory $NO_3^-$and NO_2^- reductases; therefore, denitrification might be the key to the presence of Methylobacter in anaerobic environments [46, 47]. But the concentrations of NO_3^- and NO_2^- were too low to account for the observed CH_4 oxidation occurring at O_2 concentrations below detection limit.

In our study, aerobic methanotrophs such as genus Methylocaldum and Methylocystis were found to be involved in AOM according to the 16S rRNA analysis. The stoichiometry of anaerobic methane oxidation also indicated the complexity of AOM mechanisms in this process. These results suggest the need for further studies regarding the role of aerobic methanotrophs in AOM.

Morphological Characteristics of Methane-Oxidizing Microorganisms

In the SEM images (Fig. 6), microorganisms in both the aerobic and anaerobic samples were connected by extracellular organic matter in a polymerization state.

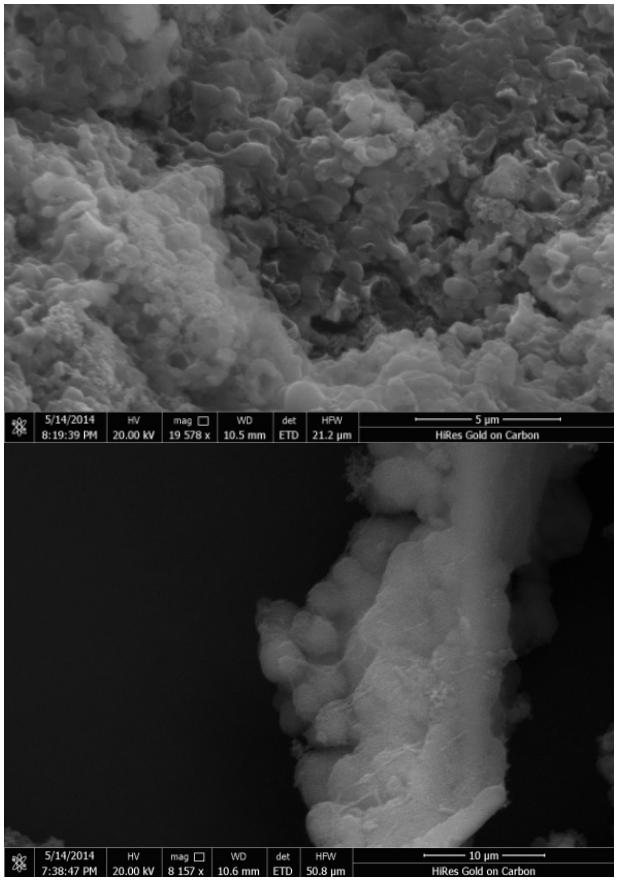

Fig. 6. SEM images of microbial groups in the culture liquid; the left is the MO sample, the right is the MA sample.

The morphological characteristics of the microbes in the aerobic sample could be distinguished by the scattered bacteria on the surface. The cells were rod-shaped, with a round-bowl-shaped concave in the center. Cell diameter ranged from 0.4-0.8 µm. These features were the same as Methylocystis strain JTA1 in the aged refuse [38]. This result is in accordance with the sequencing analysis [48].

Larger particles (diameter 4.5-7 µm) were found in the anaerobic samples. These spherical particles were formed by aggregated microbes with an outer cover [49], and it was difficult to identify the morphological characteristics and types of microorganisms. SEM analysis showed that these aggregates were associated with inorganic minerals, indicating that methane oxidation was accompanied by a mineralization process.

Conclusions

Both aerobic and anaerobic methane oxidation were detected in the landfill environment by the enrichment cultivation method. Methane oxidation rates of aerobic cultures (14-day average oxidation rate 56-77%) were higher than those of anaerobic cultures (36-39%). The methane oxidation capacity under anaerobic conditions reached 5.24 µmol mL^{-1} d^{-1}, which was obviously higher than the oxidation capacity (below 1 µmol/mL/d) measured in enrichment cultivation using leachate without NMS medium. The results showed that it was effective for enriching an anaerobic methane group with NMS as culture medium.

The 16S rRNA gene sequence analysis showed the obvious differences between aerobic and anaerobic cultures. In the samples cultured under anaerobic conditions, methanotrops Methylocystis is the dominant bacteria, with the relative abundance of 35.96-78.37%. In anaerobic cultured samples, Moheibacter (41.38% in MA1) and Cupriavidus (43.08% in MA2) are the most dominant species. Methanotrophs Methylocaldum and Methylocystis were found in the anaerobic cultured samples, with low relative abundance.

SEM analysis showed that the microbes in the aerobic sample were similar to the Methylocystis strain JTA1 in the aged refuse. In the anaerobic samples, larger particles (diameter 4.5-7 µm) formed by aggregated microbes with outer cover were found, and it was hard to identify the types of microorganisms.

The stoichiometry of anaerobic methane oxidation indicated the complexity of AOM mechanisms in this experiment. There were various types of AOM in the culture, including SAOM, DAOM and iron-dependent AOM. Aerobic methanotrophs such as genus Methylocaldum and Methylocystis were found in anaerobic samples according to the 16S rRNA analysis, and further research is needed to confirm whether aerobic methanotrophs can oxidize methane under anaerobic conditions.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

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