Microbiology, Vol. 74, No. 5, 2005, pp. 608–614. Translated from Mikrobiologiya, Vol. 74, No. 5, 2005, pp. 699–706.
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EXPERIMENTAL ARTICLES

Methane Oxidation in Landfill Cover Soil

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Received February 21, 2005

Abstract—Methane oxidation in the cover soil of the Khmet'evo municipal landfill in Moscow oblast was investigated. Methane emission from the experimental site of the landfill was highly heterogeneous. At a depth of 45–60 cm, the pore gas mainly consisted of CH_4 (60–70%) and CO_2 (30–40%). In the upper layers of the cover soil, the concentration of these gases sharply decreased. Methods for estimation of the methane-oxidizing activity in the cover soil of the landfill were tested. The rate of methane oxidation in the soil correlated with the cell number of culturable methanotrophic bacteria and was the factor limiting methane emission from the surface of the landfill. The method of indirect immunofluorescence revealed ten known species of methanotrophic bacteria in enrichment cultures obtained from samples of the cover soil. Our results also indicate the presence of unknown psychrotolerant methanotrophs that are active at the low temperatures characteristic of Moscow oblast.

Key words: municipal landfill, methane emission, methane-oxidizing activity, methanotrophs.

Landfills for solid municipal waste (SMW landfills) are among the main anthropogenic sources of the greenhouse gas methane (6–12% of the total emission) [1]. In Russia, SMW landfills occupy 0.8 million hectares and emit up to 500000–700000 tons of methane per year [2]. A variety of technological approaches exists for collection and utilization of the biogas (a mixture of CH_4 and CO_2) formed at these landfills. However, the low quality of the landfill biogas (high content of CO_2, H_2S, and other gaseous and volatile impurities) and its price, which is higher than that of natural gas, limit its use. In the absence of biogas collection systems, methane oxidation by the dense population of methanotrophic bacteria that develops in the aerobic layer of cover soil is highly important for the reduction of methane emission from SMW landfills. In landfills with a high depth of buried waste (tens of meters), methanotrophs oxidize up to 50% of the methane during the warm season [3], and, at small SMW landfills or dumps, up to 100% [4]. Qualitative estimation of the methane-oxidizing activity at SMW landfills is a rather complex task. No direct estimation method exists, and incubation of samples of cover soil under different conditions allows only the potential methane-oxidizing activity to be determined [5]. Determination of the actual methane-oxidizing activity of cover soil is important for the development of techniques for SMW landfill recultivation and for decreasing methane emission. Publications dealing with the influence of environmental factors on the cell number and taxonomic composition of methanotrophs in the cover soil at SMW landfills are scarce. For the landfills in Moscow oblast, investigation of the density and composition of the methanotrophic microbial population during the warm and the cold season is of special importance, since strong seasonal temperature fluctuations and a prolonged cold winter are characteristic of the climate of this area.

The goal of the present study was to investigate microbial methane oxidation in the cover soil at an SMW landfill, including estimation of methane-oxidizing activity, the cell number and species composition of methanotrophic bacteria and the effect of environmental factors.

MATERIALS AND METHODS

The investigation was performed in spring and summer 2002 on a site of the Khmet'evo SMW landfill that had been out of operation for five years prior to the measurements. The Khmet'evo landfill is located 65 km north of Moscow (Solnechnogorsk region, Moscow oblast). The maximum thickness of the SMW deposits at the site studied was 13 m. The cover soil layer was heterogeneous in structure and contained sand with clay and small stones. The thickness of this layer was 30–60 cm. From early November until late March, the landfill is covered with snow, and the cover soil is completely frozen from early December until

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late March. The area of the site chosen for the field and laboratory investigations was 40 × 40 m in size. The vegetation on the site consisted of mosses and grass. Some zones were devoid of vegetation; the highest methane flux was found there. The determination of methane emission and cover soil sampling were performed in April–June 2002 at 17 evenly distributed points; the distance between sampling points was 10–13 m.

Methane emission was determined using the chamber method [6]. The steel bottom of the chamber (0.16 m^2) was inserted into the soil to a depth of 5–10 cm. The 48-l chamber, made of Plexiglass and equipped with a fan, was mounted on the bottom and hermetically sealed. 10-cm^3 samples of overlaying air were taken from the chamber with syringes three times at 5-min intervals. The syringes were sealed with rubber stoppers and transported to the laboratory for gas chromatographic analyses. The methane flux was calculated from the rate of change in methane concentrations in the chamber using linear regression.

Gas samples for determination of the methane emission were analyzed by gas chromatography using a model 3700 chromatograph (Russia) equipped with a flame ionization detector and a column with Porapak Q as the sorbent. Gas samples for determination of the methane-oxidizing activity were analyzed using Chrom-5 chromatograph (Czech Republic) equipped with a thermal conductivity detector and a column with AG-3 activated charcoal as the sorbent.

Samples of pore gas were collected using a Yastrebov bore (a steel tube with an inner diameter of 1.5–2.0 mm and an outer diameter of 1.5 cm at the top and 0.7 cm at the bottom). Gas samples of 30 cm^3, collected from various depths (0–65 cm), were analyzed using a PGA-7 portable gas analyzer (Russia). The sensitivity was 0.1% for CH_4 and 0.02% for CO_2. The temperature of the cover soil was determined using a TET-2 transistor electric thermometer (Russia).

The cover soil was sampled, using a blast hole, from depths of 0–20, 20–40, and 40–60 cm. The methane-oxidizing activity was determined in soil samples possessing their original humidity and in soil suspensions. In the first case, 7-g soil samples were placed in 120-ml vials and hermetically sealed; methane was added to 10% a concentration in the gas phase. The vials were incubated at 20°C, and the CH_4 concentrations were regularly measured. Soil suspensions were prepared by the addition of distilled water to the soil (1 : 2). Aliquots containing 7 g of soil were incubated similarly to the native soil samples. All the experiments were performed in triplicate. The initial methane oxidation rate was determined from methane consumption during the first three days of incubation. The maximal rate was calculated via the angle of the tangent line at the steepest part of methane consumption curve. The absolutely dry mass (ADM) of the soil samples and soil suspensions was determined by drying at 105°C to a constant weight.

The most probable numbers of culturable methanotrophs in the soil suspensions were determined by serial tenfold dilutions in vials with liquid P mineral medium [7] and 10% CH_4 in the gas phase. The vials were incubated for 2.5 months at 20°C; methane concentrations were measured regularly. The cell numbers of methanotrophs were assessed using the tables in [8].

Enrichment cultures were obtained from cover soil samples collected in April–June 2002. The vials with liquid P mineral medium [7] and 10% CH_4 in the gas phase were inoculated with soil suspensions and incubated at 10 and 20°C.

In the enrichment cultures obtained from the samples of cover soil, methanotrophs were identified using the indirect immunofluorescence method. Immune serums to the following 11 species of methanotrophs were used: *Methylomonas methanica, Methylobacter bovis, Mb. chroococcum, Methylocystis echinoides, Mcs. methanolicus, Mcs. pyriformis, Mcs. parvus, Methylosinus sporium, Ms. trichosporium, Methylocapsa acidiphila*, and *Methylococcus capsulatus.* Drops of the enrichment cultures were placed on microscopic slides, dried in air, dehydrated in ethanol (50, 80, and 90% for 3 min in each), and dried in air again. The immune serum staining was performed according to the standard procedure [9]. The total microbial numbers in the enrichment cultures were determined using 4',6-diamidino-2-phenylindole (DAPI) universal fluorescent DNA-dye. A DAPI solution (0.5 ng μl^{-1}) was applied to the microscopic slides with enrichment cultures; after incubation for 10 min in the dark, the solution was removed, and the slides were dried in air. The number of methanotroph cells and the total number of microbial cells were calculated as averages for 20 fields of a LYuMAM I-1 ultraviolet microscope (Russia) with different sets of filters for the cells stained with DAPI or with the immune serums.

RESULTS AND DISCUSSION

Methane emission. In our previous papers [5, 10, 11], we presented detailed data on methane emission from the surface of the experimental site of the Khmet'evo SMW landfill. The extreme heterogeneity of the methane fluxes reported for this site is characteristic for a number of different SMW landfills [2, 12–15]. The high average value of methane emission from the surface, over 1.0 mol CH_4 m^{-2} day^{-1}, is characteristic of "young" landfills and indicates intense decomposition of organic matter in the anaerobic zone of the landfill body, which results in methane production. The heterogeneity of the methane emission (from 0.002 to 18 mol CH_4 m^{-2} day^{-1}) can be explained by the heterogeneity of waste with respect to its deposition and content of organic matter. High methane concentrations, availability of oxygen, and sufficient humidity and temperature of the soil lead to rapid development of methanotrophic microbial populations in the cover soil of SMW landfills [4, 16]. At some points of the site,

Table 1. Methane emission in April and June 2002 from the surface of the experimental site of the Khmet'evo SMW landfill

Point no.	April 23		June 25	
	CH_4 flux	sd*	CH_4 flux	sd
	mmol CH_4 m^{-2} day^{-1}			
1	1.0	0.4	11.3	5.3
2	–1.0	0.6	–37.0	9.4
3	31.8	4.0	1853.4	591.8
4	18115.0	232.0	16851.2	1714.0
5	–0.2	0.3	–3.1	1.9
7	–4.2	1.2	–1.4	2.0
8	9.8	1.6	0.6	0.5
9	0.9	1.5	–3.5	0.6
11	0.1	0.2	0.4	1.5
12	–1.8	0.2	48.6	4.4
13	225.4	11.0	234.3	40.8
14	7.6	0.8	3.7	1.6
15	2.2	1.2	2.0	1.2
16	–0.5	0.3	8.2	3.7
d9**	468	230	ND	ND

* sd, standard deviation.
** d, a point located in the center of the site.

Table 2. Concentrations of CH_4 and CO_2 in the pore gas along the cover soil profile of Khmet'evo SMW landfill as measured in April 2002

Point	2		4		d9	
Depth, cm	Concentrations, %					
	CH_4	CO_2	CH_4	CO_2	CH_4	CO_2
5	0.0	0.1	26.3	22.2	0.0	25.0
25	0.0	1.9	57.0	43.0	34.0	25.0
35	5.3	10.9	56.0	43.2	35.0	35.6
45	38.5	24.0	50.0	34.8	48.8	40.0

methane consumption from the near-surface air was detected (up to 6.0 ± 3.4 mmol CH_4 m^{-2} day^{-1}), indicating the activity of methane-oxidizing bacteria.

Methane fluxes from the surface of SMW landfills usually decrease in summer [11]. In the investigated site of the Khmet'evo landfill, however, a small increase in emission was observed (Table 1). As will be demonstrated below, this was caused by the decreased methane-oxidation activity of the cover soil during the hot and dry summer period.

In order to demonstrate the important role of methanotrophic bacteria in methane oxidation at SMW landfills, we performed a number of field and laboratory investigations, including measurements of CH_4, CO_2, and O_2 concentrations along the depth of the cover soil layer; determination of methane-oxidizing activity of the soil samples; and determination of the cell numbers and species composition of the culturable methanotrophs.

Analysis of the pore gas in the cover soil layers. The concentrations of CH_4 and CO_2 along the cover soil profile (0–65 cm) were measured at different points of the experimental site of the Khmet'evo landfill. At a depth of 45–60 cm, the pore gas consisted mostly of CH_4 (60–70%) and CO_2 (30–40%). The concentrations of these gases decreased in the upper soil layers. Methane concentration in the pore gas usually correlated with the methane flux at a particular point: the highest CH_4 concentration in the pore gas at a depth of 45–60 cm was found at points with high methane emission; in contrast, at points where the methane concentration rapidly decreased along the soil profile, the emission was low or absent.

The data on CH_4 and CO_2 concentrations along the soil profile for three experimental points with different levels of methane emission—low (point 2), high (point 4), and intermediate (point d9)—are presented in Table 2. Methane emission from the surface at these points (as measured in April 2002) is presented in Table 1. The decrease in the concentrations of CH_4 and CO_2 along the soil profile was caused by gas dissipation into the atmosphere, dilution by external air, and microbial methane oxidation.

Factors affecting the methane-oxidizing ability of the cover soil. Methane oxidation in the cover soil is highly important, since it decreases methane emission from the landfill surface. Environmental factors such as substrate availability, humidity, and temperature are the main influences on the degree of activity of soil microbial populations [2, 12]. In the system investigated, the methane permanently supplied from the anaerobic zone was the substrate. Oxygen, the second substrate, can act as a limiting factor. As a rule, oxygen access into the cover soil of landfills is limited only in the anomalous patches with an extremely high methane flux. Oxygen usually penetrates to a depth of up to 60 cm. Its concentration in the pore gas, however, decreases along the soil profile. For instance, the O_2 concentration for experimental point 4 was 18% at a depth of 5 cm and only 7.6% at a depth of 40 cm. Oxygen penetration can be sometimes hampered for a certain period due to water-logging of the soil caused by prolonged intense rainfall.

The methane-oxidizing activity of the cover soil of the experimental site was determined in soil–water suspensions placed in hermetically sealed vials at 20°C, with 10% CH_4 and 18% O_2 in the gas phase. This gas ratio is in accordance with the average methane concentration in the cover soil of the landfill and provides sufficient oxygen to oxidize this amount of methane. Substrate limitation was therefore practically excluded. At the same time, these conditions were more favorable

for methane oxidizing bacteria than the native conditions of the landfill. Incubation of the soil samples from different points and different depths revealed that, in some cases, methane oxidation commenced immediately and continued at a constant rate until 50–90% of the substrate (methane) was consumed. Such a pattern of methane oxidation, represented by curve *1* in Fig. 1, indicates the presence of an active bacterial population oxidizing methane at a high rate. In the other soil samples, methane oxidation started after a certain delay, and, afterwards, its rate increased sharply (Fig. 1, curve *2*). Such a pattern indicates that methane-oxidizing bacteria were present in these samples in an inactive state. Their rapid growth followed the transition to more favorable conditions. Curve *2* corresponds to a typical pattern of microbial growth. Therefore, we determined both the initial and maximal rates of methane oxidation.

In order to investigate the effect of suspending the soil on its methane-oxidizing activity, samples from the experimental site of the Khmet'evo landfill were taken from depths of 0–20, 20–40, and 40–60 cm. The methane-oxidizing activity was determined by incubation of the samples at native humidity and by incubation of water-suspended samples. Incubation was performed at 20°C. The results are presented in Table 3. Incubation of water-suspended samples from all the depths resulted in 1.2- to 4.7-fold higher rates of methane oxidation compared to the samples of native soil without water added. These results indicate that the methane-oxidizing microbial population present in the original samples of the cover soil is usually inhibited by lack of water. After the addition of water, this population is rapidly activated. The importance of humidification in attaining high rates of this process is evident from comparison of the maximal methane oxidation rates in suspensions and in the native soil. Lack of water certainly inhibits microbial metabolism; however, excessive humidity may lead to compactization of the soil and thus limit oxygen availability.

The composition of cover soil is highly important for its colonization and for development of microorganisms, including methane-oxidizing bacteria. The soil used for SMW landfills is of low quality; moreover, mixtures of sand and clay are usually applied. This material has either a weak or too strong a capacity to retain water [11]. A high content of sand in the cover soil, due to its high permeability for water, results in soil aridity and, therefore, in decreased methane oxidation and increased methane emission from the landfill.

Methane oxidation at different points of the investigated site. The microbial methane-oxidizing activity in the cover soil was determined in samples from the 5–35 cm layer collected at different points of the investigated site of the Khmet'evo landfill. For all the points, both with and without vegetation, low methane emission was characteristic. The dynamics of methane oxidation was studied at 20°C. The initial and maximal rates of methane oxidation at these points are

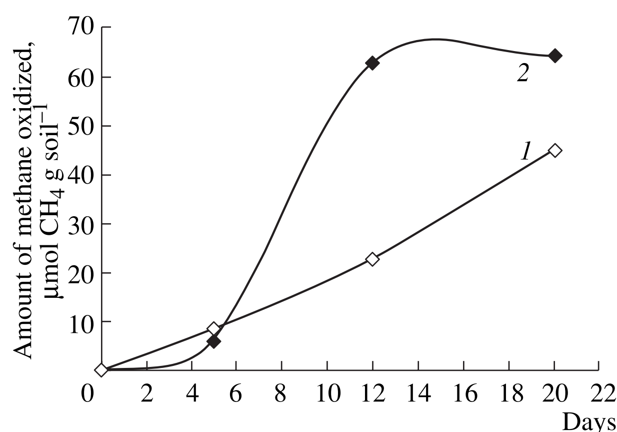

Fig. 1. Methane oxidation at 20°C in the samples of cover soil (30–40 cm) collected from (*1*) point 9 and (*2*) point 8 of the experimental site of the Khmet'evo SMW landfill.

presented in Fig. 2. The initial rate of methane oxidation varied significantly from point to point (Fig. 2a), while variations in the maximal rate were less pronounced (from 3.0 to 6.4 mmol CH_4 kg soil^{-1} day^{-1}) (Fig. 2b). These data indicate the high activity of the methanotrophic microbial population in the investigated site of the SMW landfill. The initial rate of methane oxidation was, however, strongly affected by the heterogeneity of the soil (its composition, humidity, and the presence or absence of biogenic elements) at different points. The initial rate of methane oxidation possibly reflects the real situation at the landfill. The maximal rate reflects, rather, the potential ability of the cover soil to oxidize methane under optimal conditions.

The cell numbers of culturable methanotrophs along the soil profile. The relationship between the cell numbers of culturable methanotrophic bacteria and the depth and temperature of the cover soil is presented in Fig. 3. The cover soil samples were collected at experimental point 2 in the spring (Fig. 3a) and summer (Fig. 3b) seasons. The cell numbers of methanotrophs were found to vary significantly depending on the season. In spring, the cell number of culturable methanotrophs peaked in the 20–40 cm layer of cover soil. In summer, the cell numbers of methanotrophs along the soil profile

Table 3. Rates of methane oxidation by native samples of cover soil and by soil–water suspension (Khmet'evo SMW landfill, 20°C)

Depth, cm	Rate of methane oxidation, µmol g soil^{-1} day^{-1}			
	Suspension		Native soil	
	initial	maximal	initial	maximal
0–20	3.2	9.6	1.5	8.1
20–40	7.6	17.7	2.9	10.3
40–60	8.9	9.0	1.9	1.9

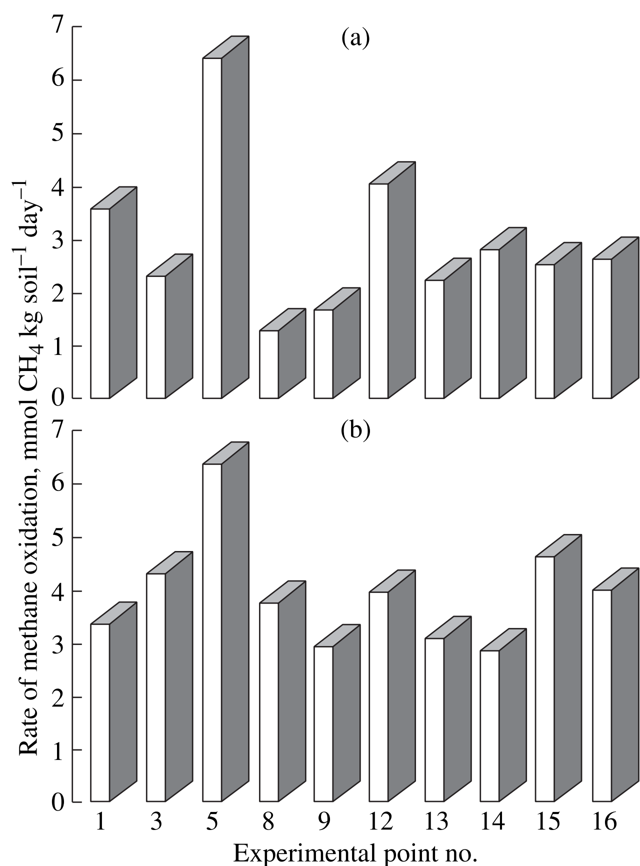

Fig. 2. (a) Initial and (b) maximal rates of methane oxidation in the samples of cover soil (5–35 cm) from the experimental site of the Khmet'evo SMW landfill (April 2002).

were lower. They increased with depth and peaked in the 40–60 cm layer of cover soil. This distribution of methanotrophs was probably caused by the anomalous weather conditions during the first half of summer 2002. The high temperature and low humidity of the soil during that unusually hot and dry summer resulted in suppressed bacterial activity and, consequently, in decreased bacterial number in the upper soil layer (0–40 cm) as compared to spring 2002. It is worth mentioning that, in June and early July 2002, average methane emission from the experimental site was higher than in April. In contrast to the anomalously hot and dry summer of 2002, both the methane-oxidizing activity of the cover soil and the cell number of methanotrophs usually increased in summer, while methane emission decreased (manuscript in preparation).

In general, the initial rate of methane oxidation in the soil suspension correlated with the cell number of culturable methanotrophic bacteria. High methane-oxidizing activity and high number of culturable methanotrophic bacteria were usually, although not always, characteristic of the points with low methane emission (Table 4).

The numbers of culturable methanotrophic bacteria determined in the present study using the serial tenfold dilution method were lower than those previously reported for other landfills [2, 13]. This distinction is probably the result of the growth conditions that we employed in the present study, including the relatively low initial methane concentration in the gas phase, lower temperature, and relatively short incubation time (2.5 months). The numbers of culturable methanotrophic bacteria were also considerably less than the

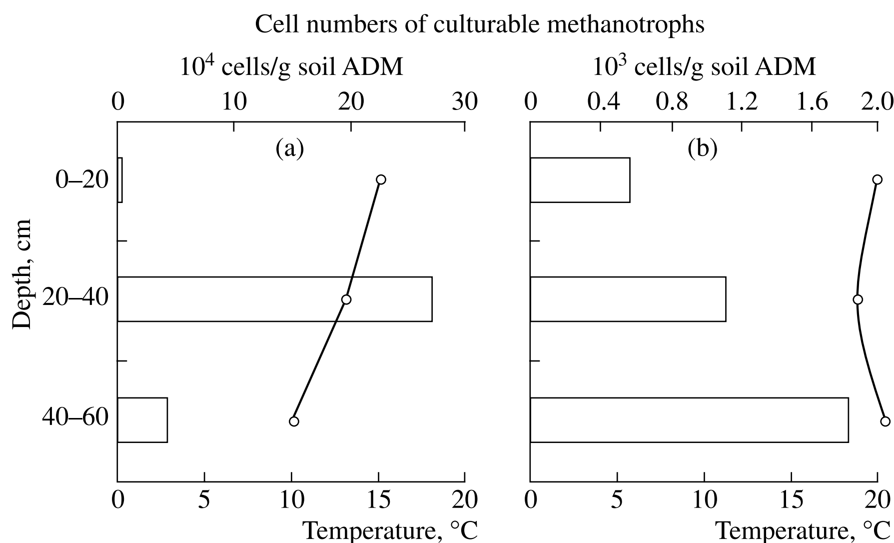

Fig. 3. Most probable numbers of culturable methanotrophic bacteria (□) as dependent on the depth and temperature (○) of the cover soil. Soil samples were collected at point 2 of the experimental site of the Khmet'evo SMW landfill in (a) April and (b) June 2002.

Table 4. Initial rate of methane oxidation and cell numbers of culturable methane-oxidizing bacteria in the cover soil of the experimental site of the Khmet'evo SMW landfill in April 2002

Point	2	4	d9
Depth, cm	20–40	0–20	40–60
Initial rate of methane oxidation, μmol g soil ADM^{-1} day^{-1}	166.2	20.3	57.6
Cell numbers of culturable methanotrophs, cells g soil ADM^{-1}	2.7×10^5	4.2×10^4	Not determined

total cell numbers of methanotrophs as determined by in situ hybridization (manuscript in preparation).

Identification of methanotrophic bacteria in enrichment cultures obtained from the cover soil of the SMW landfill. Enrichment cultures of methane-oxidizing bacteria were isolated from the samples of the cover soil of Khmet'evo SMW landfill at 10 and 20°C. The cultures differed with respect to their growth patterns (dispersed, with sediment formation, or with film formation), the color of the microbial biomass, and their cell morphology.

Methane-oxidizing bacteria from the enrichment cultures were identified using the indirect immunofluorescence method. Ten methanotrophic species were identified: *Mm. methanica, Mb. bovis, Mb. chroococcum, Mcs. echinoides, Mcs. methanolicus, Mcs. pyriformis, Mcs. parvus, Ms. sporium, Ms. trichosporium,* and *Mcp. acidiphila.* The identified species have previously been detected in other SMW landfills and sludge checks [13, 17, 18] with the exception of the acidophilic *Mcp. acidiphila,* isolated from an acidic peat bog [19] and not previously found in SMW landfills. In the enrichment cultures isolated from the soil samples collected in early spring at point 2, methanotrophs of the identified species constituted 0.4 to 0.8% of the total microbial number and were present mostly in cultures incubated at 20°C. None of the tested species occurred in the enrichment cultures obtained from the soil samples taken at point 4; other unidentified species presumably predominate at this point. Unlike the spring season, methanotrophs of the identified mesophilic species predominated in the microbial population in summer; in some enrichments, they constituted over 50% of the total microbial number. The majority of the identified mesophilic methanotrophic species were capable of growth at the decreased temperature characteristic for the climate of Moscow oblast. Some species, like *Msc. pyriformis* and *Ms. trichosporium*, were found in enrichments incubated both at 10 and 20°C; at 10°C, their cell numbers were even higher. These results enable us to suggest the presence of psychrotolerant strains of the known mesophilic species, as well as of unknown psychrotolerant methane-oxidizing bacteria, in the cover soil of the experimental site of the Khmet'evo landfill.

To conclude, it should be noted that the density and activity of the methanotrophic microbial population present in the cover soil at an SMW landfill depend on a number of environmental factors (humidity, temperature, soil composition, and available substrates). The level of methane-oxidizing activity determined by incubation of soil samples at native humidity is a more reliable measure of the methane-oxidizing ability of the landfill soil. We suggest that both the initial and the maximal rates of methane oxidation should be used for estimation of the methane-oxidizing activity of soil. The initial rate of methane oxidation probably correlates with the actual activity of the methanotrophic population at the landfill, while the maximal one correlates with the potential ability of the cover soil to oxidize methane. The high diversity of methanotrophs in the cover soil and the presence of psychrotolerant and acidophilic methanotrophs indicates the adaptation of the methanotrophic population of the landfill to different environmental conditions.

ACKNOWLEDGMENTS

We thank V.F. Gal'chenko, who helped us to master the indirect immunofluorescence method, and S.N. Dedysh, who provided a pure culture of *Mcp. acidiphila* for the preparation of an immune serum.

The initial stage of this work was supported in part by INCO-COPERNICUS, grant ICA2-CT-20001-10001. The subsequent stages were supported by the Moscow Government, treaty no. 12-E/04.

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