**Biotic methane oxidation within an instrumented experimental
landfill cover**

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1 **Abstract**

2 An experimental landfill cover composed of a mixture of sand and compost was
3 installed at the St-Nicéphore landfill in Québec (Canada). The mixture was evaluated as
4 potential substrate to promote methane (CH₄) oxidation by methanotrophic bacteria. One
5 of the objectives of this field project was to assess the efficiency of the cover in reducing
6 CH₄ emissions. For this, both CH₄ abatement and the extent to which methanotrophic
7 bacteria developed were followed over time and space in relation to environmental and
8 physico-chemical variables. The results obtained during the first year of monitoring
9 indicated that precipitation and air temperature had a great impact on CH₄ oxidation.
10 Over the study period, different patterns in CH₄ oxidation through the cover soil were
11 observed; in certain cases, oxidation was observed at various depths, although the zone of
12 optimum oxidation occurred mostly near the surface. However, in the second half of this
13 first year of monitoring, almost no oxidation was observed at depths greater than 10 cm,
14 presumably because of lack of oxygen reaching deeper zones within the cover. In the 0-
15 10 cm zone high numbers and important variations of particulate methane
16 monooxygenase gene (*pmoA*) copy number and counts of methanotrophic bacteria were
17 also observed. It is concluded that the adopted substrate has proved to be satisfactory in
18 sustaining and promoting growth of methanotrophic bacteria. However, the water content
19 of the soil seems to be a key factor influencing CH₄ oxidation, to an extent that requires
20 further investigation. The uppermost 0–10 cm layer seems to play a critical role in CH₄
21 abatement; one important challenge will be to explore this in more detail by conducting
22 more measurements within this zone.

1 **Introduction**

2 Methane (CH_4) is a potent greenhouse gas. It absorbs infrared radiation more
3 effectively than CO_2 (Crutzen, 1991) and its atmospheric concentration is increasing at a
4 rate of 0.6% per year (IPCC, 2001). This increase has been linked to global climate
5 change. It is now estimated that as much as 70% of CH_4 emissions are due to human
6 activities, and as much as 19% of the increase can be attributed to landfill emissions
7 (IPCC, 2001). Therefore, management practices that could help reduce emissions from
8 landfills are of great importance in connection with the atmospheric CH_4 budget. Gas
9 extraction systems, which are now widely adopted in the developed world, are considered
10 the principal means of achieving such reductions. However, CH_4 reduction can also be
11 regulated by methanotrophic bacteria that develop in the aerobic zone of the landfill
12 cover. It is well established that methanotrophs are capable of efficiently converting CH_4
13 to CO_2 and biomass (Conrad, 1996). If a technology – and a methodology - is developed
14 to harness this ability of methanotrophs, it could be applied to improve CH_4 emission
15 abatement from landfills. However, the quantitative significance of the reduction, which
16 depends on the availability of CH_4 (Pawlowska and Stepniewski, 2006), as well as on
17 several environmental factors, needs to be determined in the field.

18 Composted organics promote good microbial growth and appear to make an effective
19 medium for landfill cover (Humer and Lechner, 2001), as it offers a good mixture of
20 porosity, water holding capacity, pH, and nutrient supply (Hilger and Humer, 2003). Both
21 field observations at landfills and results from laboratory studies have demonstrated that
22 organic cover soils have a high capacity to mitigate CH_4 emissions (Borjesson et al.,
23 1998b; Humer and Lechner, 2001), and the CH_4 oxidation potential in mineral soils can

1 be enhanced by adding organic material, e.g. sewage sludge (Kightley et al., 1995) and
2 compost (Humer and Lechner, 2001; Barlaz et al., 2004; Maurice and Lagerkvist, 2004).
3 Research so far indicates that compost should be capable of oxidizing CH₄ at rates two to
4 three times higher than that of mineral soils (Wilshusen et al., 2004). However, use of
5 compost alone, or other high organic contents material may not constitute an ideal
6 substrate: the material can undergo significant compaction over time and can become
7 easily saturated, causing a reduction in oxygen diffusion into the cover to promote CH₄
8 oxidation.

9 It seems logical that a landfill cover could be designed to promote optimum growth of
10 methane oxidizing bacteria and thus enhance biological oxidation of CH₄. Various
11 landfill covers or biofilter designs, using different configurations and substrates to
12 support growth of methanotrophs have been evaluated elsewhere (Hilger and Humer,
13 2003; Gebert and Groengroeft, 2006). However, results from efficiency tests under field
14 conditions are mostly available for covers or biofilters working under temperate climatic
15 conditions. To the best of our knowledge, no such attempts have been made so far under
16 the cold Nordic conditions. This type of cover, referred to here as a passive methane
17 oxidation barriers (PMOB), might provide an alternative method of emission abatement
18 for old sites, where flaring or energy recovery is not economically feasible, or as a
19 complementary strategy to gas collection systems. Indeed, it has been reported that even
20 at sites with gas collection systems, significant amounts of biogas can still escape as
21 fugitive emissions (e.g. Spokas et al., 2006; Börjesson et al., 2007). In order to evaluate
22 the potential of PMOB in Nordic climatic conditions, this multidisciplinary project
23 considered the geotechnical, hydraulic, physico-chemical, environmental, climatic and

microbiological aspects in assessing the efficiency of landfill cover materials that would act as a PMOB. Some of these issues are addressed herein.

Within the framework of this multidisciplinary project, 3 PMOBs were designed and constructed at the St-Nicéphore landfill, Quebec, Canada, a waste disposal facility covering approximately 65 hectares that receives mainly domestic waste. The cover includes a layer constituted of a mix of compost and sand as substrate. Both methane abatement through the designed PMOB and the extent to which methanotrophic bacteria developed in response to the flow of CH_4 were followed over time and space and in relation to environmental and physico-chemical variables. This paper reports the results obtained over the first year of monitoring.

Material and methods

Description of the field experimental plots

Three experimental plots measuring 2.75 m (W) x 9.75 m (L) were constructed during the summer of 2006. Each of the plots included an 80-cm thick layer of substrate underlain by a 10-cm thick transitional layer consisting of 6.4-mm net gravel and a 20-cm thick gas distribution layer (GDL) consisting of 12.7-mm net gravel. In this paper, only details pertaining to one of the plots, PMOB-1, are presented. This plot was fed directly by biogas coming from the 3.5-year old buried waste mass (Figure 1). Given the fact that the substrate layer thickness was fixed at 80-cm, a 200-cm thick GDL was necessary in order to reach the waste mass, at this particular location of the landfill.

The substrate layer consists of a mixture of sand and compost, composed of 5 volumes of compost (before sieving) and 1 volume of coarse sand ($D_{10} = 0.07$ mm; $D_{85} =$

1 0.8 mm; Cu= 4.3). The 2-year old compost, was provided by a local producer that
 2 composted a mix of municipal sewage sludge and sludge from pulp and paper and agri-
 3 food industries. The sand-compost mixture was sieved using an industrial mesh so that
 4 the end product would contain particles no greater than 12 mm. The organic matter
 5 content of the mixture was 17.8% ($\frac{\text{g_volatile matter}}{\text{g_dry soil}}$). Respirometry tests were
 6 performed according to the BNQ 0413-205-art 9.5 standard (BNQ, 1997) on duplicate
 7 samples of the mixture. The following results were obtained: 268 and 230 $\text{mgO}_2/\text{kg.v.s.h}^{-1}$.
 8

9 The substrate layer was placed in four 20 cm layers and compacted with a vibrating
 10 plate to obtain layers with an average density of 838.8 kg/m^3 (or 85% of the optimum
 11 Proctor standard). The walls around each of the cells were thermally shielded from the
 12 outside environment by 15 cm thick polystyrene panels. The goal was to prevent lateral
 13 migration of moisture due to thermal gradients.

14 **Environmental, physico-chemical and microbiological variables**

15 Probes for measurement of water content (θ), suction (ψ), temperature and gas
 16 concentration were installed at four separate downgradient points and at 4 different
 17 depths (6 in the case of gas probes) in each profile (Figure 1b). Meteorological data,
 18 including precipitation and atmospheric pressure, were recorded continuously by a
 19 weather station installed near the experimental plot. Suction data is neither presented nor
 20 discussed in the present paper. The temperature (HOBO U12, from Onset) and water
 21 content (ECH₂O EC-5, from Decagon) probes were connected to data loggers.

22 On a weekly basis, vertical concentration profiles of CH₄ and O₂ in the pore volume
 23 of the PMOB were manually obtained. For this, gas samples were collected from gas

1 probes installed permanently at 6 different depths within the PMOB (Figure 1b). Samples
2 were analysed *in situ* using a portable infrared gas analyzer (Columbus Instruments). Gas
3 emissions at the surface of the experimental plot were also measured using a dynamic
4 chamber, as described by Fécil et al. (Fécil et al., 2003).

5 Samples were collected from the uppermost part of the substrate (0-10, 10-20, 20-30
6 and 30-40 cm) using PVC coring tubes (internal diameter = 5 cm). The cores were kept at
7 4°C until analyzed in the laboratory within 24-hours. The analyses included pH
8 (determined in distilled water 1:3 v/v) (Page et al., 1982), counts of methanotrophs using
9 the most probable number method, and quantitative detection of methanotrophs by
10 targeting the particulate methane monooxygenase gene (*pmoA*) using quantitative-PCR
11 (Q-PCR). For methanotroph counts, soil slurries were serially diluted in 96-well plates
12 (microliter) containing ammonium mineral salts medium. The plates were incubated for 4
13 weeks at 25°C in gastight jars containing 18% CH₄ in air.

14 Q-PCR is a relatively novel methodological approach to quantifying bacterial
15 abundance in the environment. This method is used to determine the concentration of
16 target DNA in environmental extracts. In this study, *pmoA* was the target gene since it is
17 present in almost all known methanotrophs, with the possible exception of members of
18 the genus *Methylocella*, which are generally isolated from acidic environments (Dedysh
19 et al., 1998; Dedysh et al., 2000; Dunfield et al., 2003; Theisen et al., 2005). For Q-PCR
20 analysis, total DNA was extracted directly from our samples using mechanical shaking
21 and an enzymatic lysis procedure as described in Fortin et al (2004). Genomic DNA was
22 then purified using a MicroSpin column containing polyvinylpolypyrrolidone (PVPP)
23 (Berthelet et al., 1996). Total genomic DNA was quantified by staining with pico green

dye using a Spectrafluor fluorometer (Tecan) and lambda phage DNA as a standard. Q-PCR was performed on DNA template using the *pmoA* primer set A189/mb661.

Results and discussion

Throughout the present study, with the exception of the period between late July and early August 2006, the barometric pressure (p_{bar}) ranged from 99.34 to 102.90 kPa (mean $\pm$ SD = 101.12 ± 0.69 kPa; Figure 2) and fluctuated daily. This fluctuation was mainly opposite to the wind speed with a significant negative correlation ($r = -0.5$, $p < 0.05$) found between these two variables. Nastev et al (2001), Czepiel et al. (2003) and Gebert and Gröengröeft (2006) found an inverse linear relationship between landfill CH_4 emissions and p_{bar} . Accordingly, it can be expected that the fluctuation observed during this study affected the outward flow of biogas. However, since gas sampling and surface measurements of gas emissions were performed on a weekly basis, whereas meteorological data was recorded on an hourly basis, a clear relationship between outward gas flow and meteorological data could not be established in the present study.

Besides external controlling factors such as barometric pressure, wind speed and engineered controls (gas collection system), the water content of the cover soil has been identified as an important factor controlling CH_4 emissions from landfills (Bogner, 1992; Bogner et al., 1995; Kjeldsen, 1996). The water content of the soil depends mainly on atmospheric precipitation and on the capacity of the material to retain or release moisture. During this study, precipitation varied between 0 and 1.12 mm d^{-1} with a mean $\pm$ SD = $0.06 \pm 0.17 \text{ mm d}^{-1}$, whereas the air temperature ranged from 2.3°C to 22.7°C , with a

1 mean equal to 12.86 ± 5.09 °C. Both variables fluctuated with time, but the trend in air
2 temperature showed a gradual decrease as Fall approached (Figure 2).

3 The volumetric water content (θ) of the substrate varied from 51% to 64% and was
4 obviously sensitive to precipitation. Near the surface of the PMOB, where significant
5 variations in the water content occurred, values of θ were close to the lower range ($\theta =$
6 52%) only on two occasions, July 20 and September 25. Both occasions corresponded to
7 periods of high temperature (Figure 2) and prolonged absence of precipitation.
8 Otherwise, most of the water content profiles exhibited variable trends that were related
9 to episodic events of precipitation spread out over a variable number of days during the
10 study. For example, changes observed in the water content profiles on August 2,
11 September 5 and October 2 matched with periods of significant precipitation (Figures 2
12 and 3). Furthermore, from October 2 until the end of the study, the water content
13 remained high in the uppermost part of the cover (Figure 3), while the air temperature
14 remained relatively low at $\sim 8^\circ\text{C}$ (Figure 2).

15 As expected, the temperature profiles (Figure 3) exhibited variations with depth and
16 time, the highest thermal amplitude (25 to 10°C) being associated with the uppermost part
17 of the cover, which is most affected by weather conditions. At 82 cm below the surface,
18 the temperature varied from $\sim 22^\circ\text{C}$ during the hottest period to $\sim 15^\circ\text{C}$ in October.
19 Temperature has a direct impact on biological reactions, such as the oxidation of CH_4 by
20 methanotrophs; it has been shown that CH_4 consumption by methanotrophs increases
21 with temperature (Whalen et al., 1990; Czepiel et al., 1996; Gebert et al., 2003; Borjesson
22 et al., 2004; Park et al., 2005; Jugnia et al., 2006), when the CH_4 concentration, among
23 other factors, is not limiting (Pawlowska and Stepniewski, 2006).

1 With the exception of the CH₄ concentration measured on July 20, concentrations of
2 CH₄ at the bottom of the PMOB (82 cm) were generally close to 55% (Figure 4), a
3 common value for CH₄ concentration in biogas from landfills (Bogner et al., 1995;
4 Borjesson et al., 2001; Kallistova et al., 2005). This indicates that the supply of CH₄ was
5 not a limiting factor during the study period. The decrease in CH₄ concentration as it
6 diffused through the sand-compost substrate exhibited different patterns over time. As
7 shown in Figure 4, during the first five sampling campaigns (July 20 to Sept 1), the
8 concentration of CH₄ in the upper most gas probe (at 10 cm) decreased to much lower
9 values (from below detectable to approximately 18%) than the 55% found at the base.
10 The abatement was gradual throughout the profile for the first two sampling dates.
11 However, for the other dates, the oxidation front (indicated by the steep decline in CH₄
12 concentration) seems to be located between 30 and 20 cm from the surface (Figure 4).

13 In a controlled laboratory environment, Berger et al. (2005) observed an upward
14 displacement of the oxidation zone as infiltration was supplied to their test cell.
15 Furthermore, factors such as temperature, moisture content and availability of O₂ may
16 influence the depth of optimum oxidation, which can vary significantly. For example,
17 Whalen et al. (1990) found that this zone is situated between 3 and 12 cm, while
18 Visvanathan et al. (1999) located it between 15 and 40 cm. Humer and Lechner (2001)
19 found in their field test, where sewage sludge and MSW compost was used as substrate,
20 that the zone of maximum CH₄ oxidation extended from 40 and 90 cm. The conditions
21 under which these covers were placed, as well as climatic and other limiting conditions,
22 such as precipitation, pH, O₂ supply, etc., affect oxidation rates and, ultimately, the
23 location of the optimal oxidation zone.

1 The pH of the soil was not a limiting factor in this study, as it varied only slightly (7.0
2 to 7.3; mean $\pm$ SD = 7.2 ± 0.1) over time and depth throughout the monitoring period
3 (Figure 5). Growth of methanotrophs is generally optimal in this pH range (Whittenbury
4 et al., 1970; Dunfield et al., 1993; Hütsch et al., 1994; Bender and Conrad, 1995), which
5 indicates that fluctuations observed for the activity and abundance of methanotrophs were
6 governed by other factors. The oxygen supply below 10 cm varied from below detectable
7 to 9.7% during the first five sampling campaigns, but during the subsequent sampling
8 (September 5 onwards), the O₂ concentration remained very low ($\sim$ 1%) below 30 cm.
9 From September onwards the water content and temperature within the PMOB began to
10 decrease with depth. The high water content through the depth profile acted as a physical
11 barrier to O₂ penetration (Boeckx and Van Cleemput, 1996; Czepiel et al., 1996;
12 Christophersen et al., 2000; Kallistova et al., 2007). Studies of CH₄ oxidation kinetics
13 have shown that the oxidation rate plummets when the gaseous O₂ concentration is lower
14 than $\sim$ 3% (Czepiel et al., 1996; Stein and Hettiaratchi, 2001; Gebert et al., 2003).
15 Accordingly, the temperature and water content within the substrate might have
16 simultaneously affected CH₄ oxidation during this study.

17 Starting on September 5, and with the exception of the profile obtained on September
18 11, the reduction in CH₄ concentrations started to be negligible below 10 cm from the
19 surface. Surprisingly, surface emissions of CH₄ below the detection limit were obtained
20 during this same period. In general, the low reductions in CH₄ concentrations across the
21 profile should be accompanied by high surface fluxes; it can thus be hypothesized that,
22 during this period, a significant proportion of the CH₄ was oxidized within the first 10
23 cm of the PMOB. Although this interpretation should be taken with caution (gas

measurements were not performed within the 0 to 10 cm superficial layer), this hypothesis is substantiated by Q-PCR analyses, which showed that the number of copies of the *pmoA* gene decreased 10 folds between the 0–10 and 10–20 cm sublayers (Figure 5). Similar to the observation by Kolb et al. (2003) that cell numbers determined by Q-PCR analysis in spiked soil samples showed a good match with the numbers of added cells, this trend paralleled that observed with methanotroph counts that were highest in the upper most sublayer (Figure 5). Some studies (Jones and Nedwell, 1993; Bender and Conrad, 1995; Borjesson et al., 1998b; Kallistova et al., 2007) reported a correlation between CH₄ oxidizing activity and the number of cultivable methanotrophs.

On average, the number of methanotrophs (range = 1.50×10^6 to 1.83×10^8 CFU g⁻¹ DW (dry weight of soil); mean $\pm$ SD = $3.40 \pm 2.68 \times 10^7$ CFU g⁻¹ DW) decreased somewhat with depth, from $7.83 \pm 5.42 \times 10^8$ CFU g⁻¹ DW near the surface (0-10 cm) where –it is hypothesized– CH₄ oxidation mainly occurred. A similarly high population of methanotrophs (1.8×10^6 to 3.2×10^7 cells per g) was detected in the upper layer of a mature compost material (Jäckel et al., 2005), whereas population sizes of $n \times 10^6$ cells g⁻¹ soil were detected previously in wetland and landfill soils using the MPN method (Jones and Nedwell, 1993; Svenning et al., 2003; Kallistova et al., 2007).

Temporal evolution of depth profiles of methanotrophs counts could be divided into two phases: the lag phase and the growth phase itself (Figure 5). The lag phase followed the construction and included the first three sampling campaigns, with abundance profiles more or less identical. The length of the lag phase in a medium to which the cells require adaptation varies with the history of the culture (Dean, 1957). Usually, the increase in total mass precedes any division, the majority of the cells swelling towards the end of the

lag period. Therefore, any measured increase of the CH₄ oxidation activity during this lag phase was due to an increase of the activity of the resident population rather than the size of the methanotrophic population. Kightley et al (1995) reported that under laboratory conditions (19°C), it took 1 month to establish the steady state conditions needed for growing methanotrophs in a soil column. Our results indicated that the establishment of methanotrophs probably takes much longer under field conditions. This observation corroborates a report from a previous study in a landfill cover soil by Borjesson et al. (1998a). During the second phase, which extended from August until the beginning of October 2006, counts of methanotrophs increased with time, particularly at the surface, to reach the maximum values observed during this study.

Conclusions

From the results presented, the substrate (or soil) used to construct the PMOB (mixture of sand and compost), was able to sustain and promote growth of methanotrophic bacteria. However, the water content of the soil seems to be a key factor influencing deep CH₄ oxidation, the extent of which requires a better understanding. The uppermost 0-10cm layer appears to be the most important layer in CH₄ consumption, and the next challenge will be to explore this in more detail by conducting more measurements within this zone.

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1 **Figure legends**

2 Figure 1 – (A) Lateral cross-section of PMOB-1 and (B) profile of the installed probes

3

4 Figure 2 - Temporal evolution of the barometric pressure, wind speed, precipitation and
5 air temperature during the monitoring period

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7 Figure 3 - Temporal evolution of the profiles of temperature and water content within the
8 PMOB

9

10 Figure 4 - Temporal evolution of the profiles of CH₄ and O₂ concentrations within the
11 PMOB

12

13 Figure 5 - Temporal evolution of pH, colony forming unit (CFU) of methanotrophs and
14 number of *pmoA* copy within the PMOB

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Figure 1

Figure 2 — Temporal evolution of the barometric pressure, wind speed, precipitation and air temperature during the monitoring period

—●— Temperature

—△— Water content

Figure 3 - Temporal evolution of the profiles of temperature and water content within the PMOB

Figure 4 - Temporal evolution of the profiles of CH₄ and O₂ concentrations within the PMOB

Figure 5 - Temporal evolution of pH, colony forming unit (CFU) of methanotrophs and number of *pmoA* copy within the PMOB