

Environmental Factors Influencing Attenuation of Methane and Hydrochlorofluorocarbons in Landfill Cover Soils

Charlotte Scheutz* and Peter Kjeldsen

ABSTRACT

The influence of different environmental factors on methane oxidation and degradation of hydrochlorofluorocarbons (HCFCs) was investigated in microcosms containing soil sampled at Skellingsted Landfill, Denmark. The soil showed a high capacity for methane oxidation resulting in a maximum oxidation rate of $104 \mu\text{g CH}_4 \text{g}^{-1} \text{h}^{-1}$ and a low affinity of methane with a half-saturation constant of 2.0% v/v. The hydrochlorofluorocarbons HCFC-21 (dichlorofluoromethane) and HCFC-22 (chlorodifluoromethane) were rapidly oxidized and the oxidation occurred in parallel with the oxidation of methane. The maximal HCFC oxidation rates were 0.95 and $0.68 \mu\text{g g}^{-1} \text{h}^{-1}$ for HCFC-21 and HCFC-22, respectively. Increasing concentrations of HCFCs resulted in decreased methane oxidation rates. However, compared with typical concentrations in landfill gas, relatively high HCFC concentrations were needed to obtain a significant inhibition of methane oxidation. In general, the environmental factors studied influenced the degradation of HCFCs in almost the same way as they influenced methane oxidation. Temperature had a strong influence on the methanotrophic activity giving high Q_{10} values of 3.4 to 4.1 over the temperature range of 2 to 25°C. Temperature optimum was around 30°C; however, oxidation occurred at temperatures as low as 2°C. A moisture content of 25% w/w yielded the maximum oxidation rate as it allowed good gas transport together with sufficient microbial activity. The optimum pH was around neutrality (pH = 6.5–7.5) showing that the methanotrophs were optimally adapted to the in situ pH, which was 6.9. Copper showed no inhibitory effect when added in relatively high concentrations (up to 60 mg kg^{-1}), most likely due to sorption of copper ions to soil particles. At higher copper concentrations the oxidation rates decreased. The oxidation rates for methane, HCFC-21, and HCFC-22 were unaltered in ammonium-amended soil up to 14 mg kg^{-1} . Higher ammonium concentrations inhibited the oxidation process. The most important parameters controlling oxidation in landfill cover soil were found to be temperature, soil moisture, and methane and oxygen supply.

ANAEROBIC DECOMPOSITION OF organic matter in refuse generates landfill gas consisting of methane (55–60% v/v) and carbon dioxide (40–45% v/v). Atmospheric emissions include methane and trace gases that were originally present in the waste or that were formed during decomposition. Trace gases include halogenated and aromatic hydrocarbons and sulfur- and oxygen-containing compounds. Typical gas concentrations are in the range of 10 to 250 mg m^{-3} (Rettenberger and Stegmann, 1996; Allen et al., 1997). Emission of trace gases is a potential risk to human health and the global environment as some trace gases are toxic while other compounds contribute to stratospheric ozone depletion and the greenhouse effect (Christensen and Kjeldsen, 1995; Wallington et al., 1994). Currently, focus has been on

methane emission from landfills, since methane absorbs terrestrial radiation in the atmosphere and contributes to global climate change.

Microbial oxidation of methane plays a significant role in reducing the emission of methane to the atmosphere (Lelieveld et al., 1998; Oremland and Culbertson, 1992). Much interest has focused on the role of aerobic soils as sinks for methane and on the ecological and land use practices such as agriculture that affect its magnitude. Rates of methane uptake in soils have been determined for a wide range of natural environments including agricultural soils (Hütsch et al., 1994; Hütsch, 1998), forest soils (Bender and Conrad, 1993; King and Schnell, 1998; King and Adamsen, 1992), tundra soils (Whalen and Reeburgh, 1990), and peatlands (Sundh et al., 1995; Dedysh and Panikov, 1997).

Landfill cover soils can develop a high capacity for methane oxidation through methanotrophic bacteria. A defining characteristic of the methanotrophic bacteria is the enzyme methane monooxygenase (MMO), which facilitates the conversion of methane to methanol, the first step on the pathway for methane utilization. Methanotrophs are divided into two general classes (Type I and Type II) based primarily on the structure of their internal membranes (Whittenbury et al., 1970; Hanson and Hanson, 1996). Both classes can express a membrane-bound or particulate methane monooxygenase (pMMO). Some Type II methanotrophs can, however, also express a soluble methane monooxygenase (sMMO) that has a broader substrate range compared with pMMO and catalyzes faster cometabolic degradation of a large number of compounds including some halogenated hydrocarbons (Henson et al., 1989; Hanson and Hanson, 1996; Alvarez-Cohen and McCarty, 2001; Oldenhuis et al., 1989). Recent research confirms that a high number of halogenated compounds can be cometabolized in landfill cover soils (Scheutz et al., 2004). Several research projects have tried to quantify the methane oxidation in landfill soil covers. Observed oxidation rates range between 10 to 100% (Czepiel et al., 1996; Liptay et al., 1998; Christophersen et al., 2001; Börjesson and Svensson, 1997; Boeckx et al., 1996), making this a very uncertain parameter when estimating the emission of methane from landfills. Methane oxidation is controlled by a number of environmental factors (e.g., soil texture, temperature, water content, methane and oxygen concentrations, and nutrients) that partly explain the variability in observed methane oxidation rates. In landfill soil covers temperature and soil moisture are very important parameters controlling methane oxidation (Whalen et al., 1990; Czepiel et al., 1996; Figueroa, 1993; Boeckx et al., 1996; Christophersen et al.,

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: HCFC, hydrochlorofluorocarbon; MMO, methane monooxygenase; pMMO, particulate methane monooxygenase; sMMO, soluble methane monooxygenase.

2000). Thus, the climatic conditions are of importance for the actual methane oxidation rate. Copper might have an important influence on the degradation rates of trace gases as methanotrophs only express sMMO at very low copper concentrations (Tsien et al., 1989). Ammonia is known to inhibit methane oxidation in soils as a result of competitive interaction of NH_4^+ with methane for the active sites of the MMO enzymes (Hanson and Hanson, 1996). However, it has also been suggested that the inhibition of methane oxidation by NH_4^+ is not always the direct result of its concentration but rather of its nitrification rate (Sitaula et al., 1995) or N turnover (Hütsch et al., 1994). The presence of trace components in landfill gas would be expected to have an effect on methane oxidation due to competitive inhibition, although this effect and the factors that influence its magnitude are not well-studied.

Compared with other nonpoint terrestrial sources of CH_4 (e.g., wetland, rice fields) landfills function as a more closed system (burial of biodegradable waste in a limited area) and thus offer possibilities to control gas emission through both engineered and natural controls. If favorable conditions enhancing methane oxidation can be maintained, microbial attenuation can be an alternative inexpensive method to control gas emissions from landfills. Natural attenuation could be especially favorable at older or smaller landfills where gas extraction is not economically feasible or as an add-on to gas extraction systems.

The aim of this study was to investigate the physico-chemical parameters controlling methane oxidation and degradation of selected trace gases in landfill soil covers. The investigations have been performed in soil microcosms incubated with methane and trace gases under different environmental conditions. The following factors were studied: temperature, soil moisture, pH, ammonium, and copper, as well as the inhibitory effect of selected trace components on methane oxidation. The selected trace components were HCFC-21 and HCFC-22, which are among the most frequently occurring fluorinated hydrocarbons in landfill gas (Deipser et al., 1996).

MATERIALS AND METHODS

Field Location, Soil Sampling, and Soil Characteristics

Soil samples were collected at Skellingsted Landfill south of Holbæk, Denmark at a location emitting methane. The landfill gas migration has been intensively studied due to a gas explosion accident in 1991 (Kjeldsen and Fischer, 1995). The soil sampling procedure together with the soil characteristics are described in Scheutz et al. (2004).

Short-Term Response to Increasing Hydrochlorofluorocarbon Concentrations, Soil Moisture Content, Temperature, pH, and Copper and Ammonium Concentrations

The microcosms and the analytical procedures are previously described in Scheutz et al. (2004). In general, the batch experiments were all conducted with soil sampled at 15 to 20 cm below the surface incubated with an initial methane concentration of 15% v/v at room temperature (22°C). The soil moisture content was 25% w/w. All soil concentrations

are expressed as mass of dry soil. A mixture of HCFC-21 and HCFC-22 (250 $\mu\text{g L}^{-1}$ each) was added to the test vessels. Gas samples withdrawn from the headspace were sampled periodically and analyzed by gas chromatography. The gas chromatographic setup and the procedure for data evaluation are described in Scheutz et al. (2004). We obtained HCFC-21 and HCFC-22 in high purity from Fluorochem Limited (Old Glossop, England).

Methane Oxidation Kinetics

The methane oxidation kinetics were determined by incubation of soil under a range of methane concentrations varying from 0 to 23% v/v.

Hydrochlorofluorocarbon Oxidation Kinetics

The HCFC oxidation kinetics were determined by incubation of soil under a range of HCFC concentrations varying from 0 to 800 $\mu\text{g L}^{-1}$. The soil had been pre-incubated for 24 h with methane to ensure microbial activity. The inhibition of methane oxidation by HCFCs was investigated by measuring the initial methane oxidation rates at various HCFC concentrations ranging from 0 to 1600 $\mu\text{g L}^{-1}$. Similarly, the inhibition of HCFC oxidation by methane was investigated in batch experiments containing 500 $\mu\text{g L}^{-1}$ of HCFC incubated with methane ranging from 0 to 25% v/v.

Temperature

The effect of soil temperature on oxidation activity was determined by incubating soil microcosms under different temperatures ranging from 2 to 50°C. The soil microcosms were acclimated to the different temperatures for two hours before addition of methane and trace gases.

Soil Moisture Content

The effect of soil moisture content on the oxidation rate was examined by adjusting the moisture content in soil microcosms before incubation. Low soil moisture contents (<15%) were obtained by air-drying soil samples in the laboratory before the start of the experiment. The soil moisture content was adjusted in each microcosm by adding small drops of distilled water. The soil microcosms were allowed to equilibrate after moisture addition overnight (approximately 12 h) before addition of methane and trace gases. The soil moisture content ranged from 6 to 50% w/w. Soil moisture contents were determined gravimetrically by drying soil samples for 24 h at 104°C at the end of the experiment.

Acidity

The influence of acidity on the oxidation rate was determined in soil water suspensions (20 g soil + 30 mL H_2O). Variation of pH in soil suspensions was accomplished by addition of HCl and NaOH solutions. Due to high buffer capacity of the soil, approximately 24 h was needed to obtain stable pH values. At the end of the experiment pH was measured again. The pH in the soil microcosms varied between 2.6 and 9.9.

Ammonium and Copper

The NH_4^+ and Cu^{2+} soil concentrations were created by adding one of a range of solution concentrations to each soil microcosm. Ammonium chloride was added to soil samples in quantities ranging from 2.3 to 1210 mg N kg^{-1} . The effect of copper (added as CuCl_2) on the oxidation rate was studied

in soil microcosms with copper concentrations ranging from 4.7 to 1680 mg Cu kg⁻¹.

Control Experiments

To check if any disappearance could be due to nonmicrobial processes (abiotic degradation, sorption, and volatilization), sodium azide (25 mg kg⁻¹ soil) was added to avoid microbial growth in the control batches. To verify that the degradation of HCFCs was not due to the presence of anaerobic bacteria in the soil, anoxic batch experiments flushed with methane or nitrogen were performed. In addition to the sterilized control experiments, other experiments were performed where acetylene (known to inhibit MMO) was added instead of sodium azide to batches containing HCFC-21 and HCFC-22.

RESULTS AND DISCUSSION

Methane Oxidation Kinetics

In all active soil microcosms methane and oxygen concentrations declined over time while carbon dioxide increased, showing that methane oxidation was taking place (Fig. 1A). Lag phases were never observed, which indicated that the bacteria were well adapted to oxidizing methane. The methane oxidation followed zero-order kinetics, indicating that the oxidation was not methane limited. High initial oxygen concentrations ensured that the oxidation was never O₂ limited. Without oxygen in the gas phase no methane oxidation was detectable (results not shown). The same was the case when the soil had been sterilized by sodium azide (Fig. 1A) or was incubated with acetylene (10% v/v) (results not shown).

The methane oxidation kinetic parameters for the

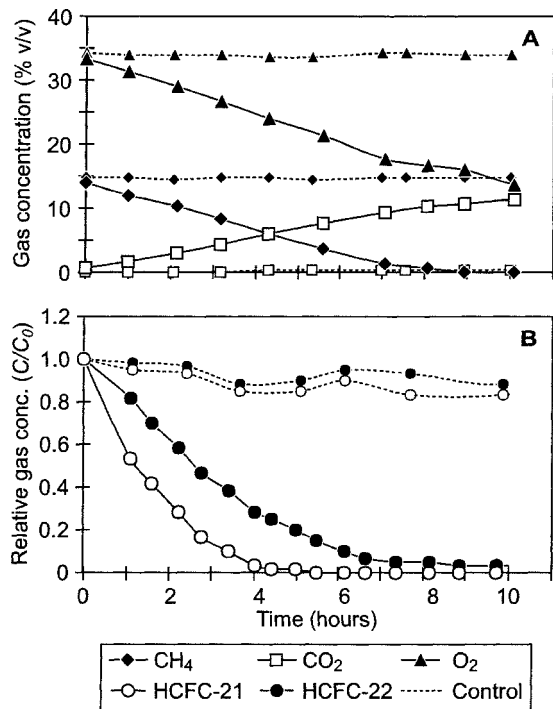


Fig. 1. Headspace concentration of gas components in a soil microcosm experiment as function of time. (A) Methane, oxygen, and carbon dioxide. (B) Hydrochlorofluorocarbons HCFC-21 and HCFC-22.

soil were determined by supplying varying quantities of methane to the microcosms. The resulting oxidation rates were used to calculate the maximum rate of methane oxidation (V_{max}) and the apparent half-saturation constant (K_m). Oxidation rate data were expressed in substrate saturation curves as a function of initial headspace methane concentrations and showed typical Michaelis-Menten characteristics (Fig. 2A). A Lineweaver-Burk plot ($1/V_{max}$ vs. $1/CH_4$) was used to linearize the data from which V_{max} and K_m were calculated ($R^2 > 0.95$). The soil showed a high capacity for methane oxidation resulting in a maximum oxidation rate of 104 $\mu\text{g CH}_4 \text{ g}^{-1} \text{ h}^{-1}$, comparable with results obtained by Figueroa (1993) (between 40 and 86 $\mu\text{g CH}_4 \text{ g}^{-1} \text{ h}^{-1}$) and Czepiel et al. (1996) (up to 42 $\mu\text{g CH}_4 \text{ g}^{-1} \text{ h}^{-1}$). The soil showed a low affinity for methane, with a half-saturation constant of 2.0% v/v, which is comparable with K_m values obtained by Czepiel et al. (1996) (0.6% v/v) and Bogner et al. (1997) (2.5% v/v) for landfill cover soils. On the contrary, soils exposed to ambient CH₄ concentrations often exhibit kinetics with high affinity (low K_m) and low activity (low V_{max}) (Bender and Conrad, 1993).

Some evidence in the literature exists that methane concentration is selective to Type I or II methanotrophs. Amaral and Knowles (1995) examined the growth of methanotrophs in methane and oxygen countergradients and found that Type I appeared to be best adapted to growth in low CH₄ concentrations while Type II dominated under high CH₄ concentrations. Their hypothesis has been supported by observations that a Type I methanotroph (*Methylomonas albus* BG8) out-competed a Type II methanotroph (*Methylosinus trichosporium* OB3b) in continuous cultures under methane-limiting conditions (Graham et al., 1993). Type II methano-

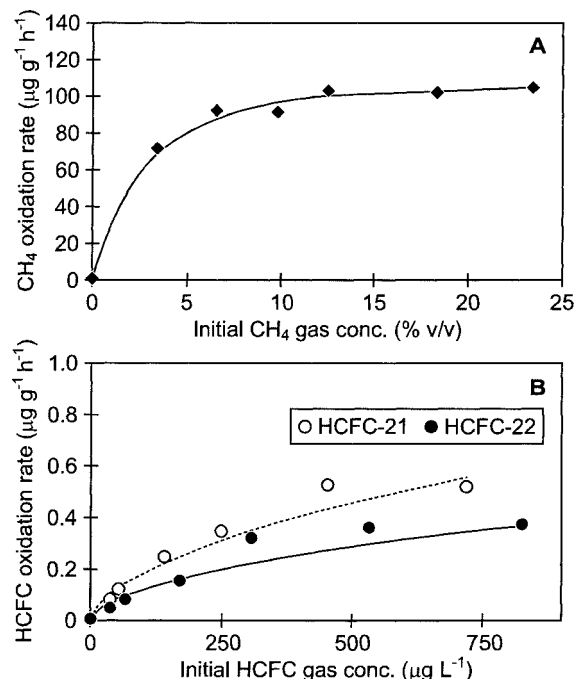


Fig. 2. Oxidation rates as a function of initial gas concentrations. (A) Methane. (B) Hydrochlorofluorocarbons HCFC-21 and HCFC-22.

troths expressing sMMO were dominating in the Skellingsted landfill soil at depths of 20 to 25 cm; 15 isolates of Type II (where 10 carried the genes for sMMO) were identified and only one Type I (Svenning et al., 2004). The predominance of methanotrophs expressing sMMO might be particularly valuable in attenuation of trace gases in landfill covers due to the broader substrate specificity of sMMO compared with pMMO.

Oxidation of Hydrochlorofluorocarbons and Inhibition of Methane Oxidation by Hydrochlorofluorocarbons

The hydrochlorofluorocarbons HCFC-21 and HCFC-22 were rapidly oxidized in soil pre-incubated with methane (Fig. 1B). The oxidation was microbially mediated as seen from comparison with the sterilized control batch (Fig. 1B). Methane oxidation and degradation of HCFC-21 and HCFC-22 was totally inhibited in batches containing approximately 10% v/v acetylene in headspace (results not shown). This indicates that the oxidation of methane and HCFCs observed in the active batches was due to the activity of methanotrophic bacteria, as acetylene is known to bind to the MMO and inhibit its activity (Prior and Dalton, 1985). Figure 2B shows the HCFC oxidation rate as a function of initial HCFC concentrations. A Lineweaver–Burk plot ($1/V_{\max}$ vs. $1/\text{HCFC}$) was used to linearize the data from which V_{\max} and K_m were calculated ($R^2 > 0.98$). The maximal HCFC oxidation rates were 0.95 and $0.68 \mu\text{g g}^{-1} \text{h}^{-1}$ for HCFC-21 and HCFC-22, respectively, whereas the K_m values were 416 and $513 \mu\text{g L}^{-1}$, respectively.

Increasing concentrations of the HCFCs resulted in decreased methane oxidation rates. The methane oxidation rate decreased by approximately 30% when the total HCFC concentration was increased from 0 to $1600 \mu\text{g L}^{-1}$. Figure 3A shows the methane oxidation rates versus total initial HCFC gas concentration. The inhibition of methane oxidation by HCFCs is probably a combination of competition for MMO and of accumulation of toxic intermediates that inhibit the microbial activity. Matheson et al. (1997) observed irreversible inhibition of methane oxidation by HCFC-21 and HCFC-22 in a study with *Methylococcus capsulatus* (Bath). Furthermore, the HCFCs also proved inhibitory to the methanol dehydrogenase (driving the second step in the methane oxidation pathway) suggesting that the HCFCs also disrupt other aspects of C_1 catabolism in addition to MMO activity. Typical HCFC concentrations in landfill gas will be less than $250 \mu\text{g L}^{-1}$. In soil covers the trace gas concentration will be even lower due to dilution in the upper soil with atmospheric air, mitigating the inhibitory effect on methane oxidation. However, other trace components present in landfill gas like trichloroethylene, chloroform, and 1,1-dichloroethylene might have an inhibitory effect on the methanotrophic bacteria due to the toxicity of the compounds themselves or accumulation of toxic degradation products (Alvarez-Cohen and McCarty, 1991; Alvarez-Cohen and Speitel, 2001). The inhibition of HCFC oxidation by methane was more pronounced, with HCFC oxidation rate decreasing 90% when the methane concentration increased from 0 to 23% v/v (Fig. 3B). These results would predict that maximum

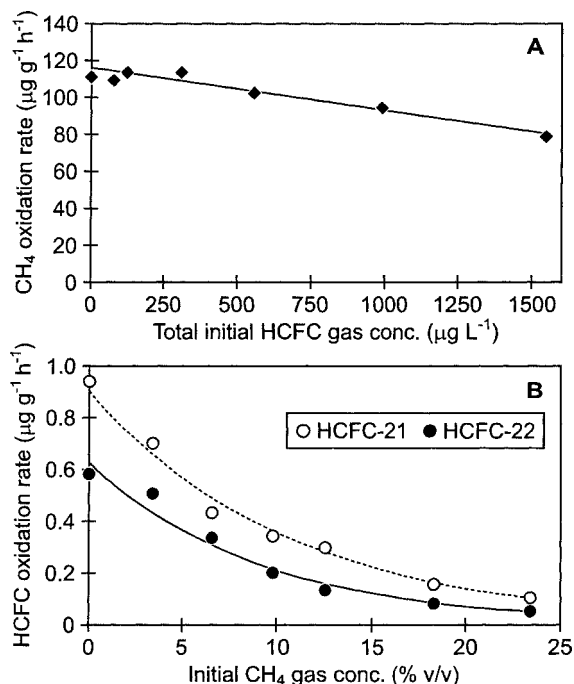


Fig. 3. (A) Influence of increasing initial hydrochlorofluorocarbon (HCFC) concentrations on the methane oxidation rate. (B) Influence of increasing initial methane concentrations on degradation rate of HCFCs.

HCFC oxidation is expected in zones with lower methane concentrations. Furthermore, resting methanotrophs in the upper part of the soil profile, which are only periodically exposed to higher methane concentrations, may contribute to rapid oxidation of trace gases.

Temperature

Figure 4 shows the zero-order oxidation rates in relation to temperature. Temperature had a profound influence on the methanotrophic activity in oxidizing methane, HCFC-21, and HCFC-22. In general, the response to temperature was similar for methane and HCFCs indicating that the methane oxidizers mediated the degradation of the HCFCs. The oxidation rates increased exponentially ($R^2 > 0.91$) from 2 to 25°C (resembling the Arrhenius relationship), reaching maximum rates around 30°C . A further increase in temperature to 40°C

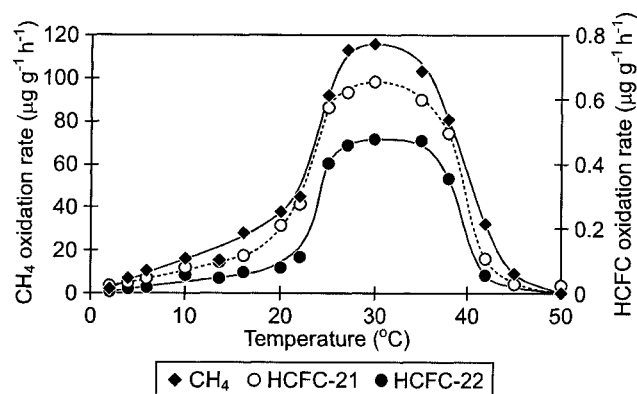


Fig. 4. Influence of temperature on the oxidation rate of methane and hydrochlorofluorocarbons (HCFCs).

resulted in a steep decline in oxidation rates and at 50°C the activity of the microorganisms was totally inhibited. However, the bacteria were active and oxidized methane and HCFCs even at 2°C, which implies that even during winter some methanotrophic activity might be expected to reduce the emission of methane and trace compounds from landfills. Several researchers (e.g., Whalen et al., 1990; Stein and Hettiaratchi, 2001) have also reported optimum temperatures for methane oxidation around 30°C. The Q_{10} values ranged between 3.4 and 4.1 when calculated for the oxidation over the temperature range of 2 to 25°C. The term Q_{10} is the value for how many times the degradation rate increases when temperature is increased 10°C at temperatures below the optimum temperature. The Q_{10} values for methanotrophs in soil environments reported in the literature are generally lower, around 2 (Whalen et al., 1990; Boeckx et al., 1996; Czepiel et al., 1996). However, most of these investigations were conducted at low initial methane concentrations in the range of 1×10^{-4} to 10×10^{-4} % v/v. At high initial methane concentration, as in this study, the methane oxidation is not phase-transfer limited, but more likely enzymatically limited. Consequently a pronounced temperature response is expected, as also suggested by Christophersen et al. (2000), who found high Q_{10} values (4.1–7.3) in landfill covers soils incubated with high methane concentrations (18% v/v). Börjesson and Svensson (1997) investigated the seasonal as well as the diurnal variation in methane emissions from a small Swedish landfill and found temperature to be the controlling factor. Methane emissions were negatively correlated with soil temperature, indicating that microbial oxidation was an important regulating factor. Christophersen et al. (2001) also found higher methane emissions during winter, while no methane was emitted during summer at Skellingsted Landfill in Denmark, which was attributed to temperature.

Soil Moisture

Figure 5 shows the oxidation rates of methane, HCFC-21, and HCFC-22 as a function of soil moisture content. Microcosms with soil moisture contents between 18 and 24% w/w produced maximum methane oxidation rates. The response of soil moisture to degradation of HCFC-21 and HCFC-22 was similar but

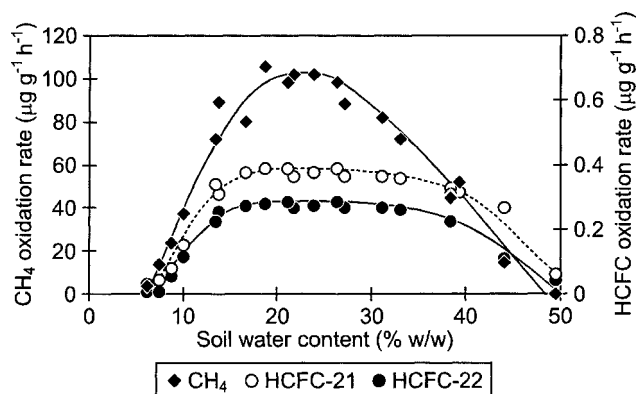


Fig. 5. Influence of soil moisture content on the oxidation rate of methane and hydrochlorofluorocarbons (HCFCs).

yielded a broader optimum range compared with methane (17–33% w/w). A decrease in moisture content reduced the oxidation rates significantly, probably due to microbial water stress resulting in desiccation and reduced activity. In experiments with air-dried soil it also seemed difficult to recover the oxidation activity of the bacteria when rewetting the soil (results not shown), implying that after very dry periods with low methanotrophic activity, a lag period could be expected for the methanotrophs to reproduce or regain their activity. An increase in soil moisture from 30% w/w resulted in reduced oxidation rates. At a soil moisture content higher than 35% w/w, the soil was waterlogged, most likely resulting in transport limitation due to the much lower molecular diffusion in water compared with air (10^4 -fold less rapid). The broader optimum range observed for HCFC-21 and HCFC-22 could be explained by the higher water solubility and lower Henry's law constants for HCFC-21 and HCFC-22 compared with methane. Optimal soil moisture contents for methane oxidation in landfill cover soils reported in the literature often range between 10 and 20% w/w (Whalen et al., 1990; Czepiel et al., 1996; Figueroa, 1993; Boeckx et al., 1996). However, the oxidation activity is significantly reduced when soil moisture is below 5% w/w (Czepiel et al., 1996; Whalen et al., 1990; Stein and Hettiaratchi, 2001; Christophersen et al., 2000). Boeckx et al. (1996) found that the methane emission was controlled by soil moisture content in a field experiment conducted at a small landfill in Belgium. Likewise, Jones and Nedwell (1990) measured the highest methane emissions from a landfill in England during the warmest and driest periods.

pH

Figure 6 shows the influence of pH on oxidation rates of methane, HCFC-21, and HCFC-22. In general the change in pH during the trials was less than 0.5 units. The optimum pH was around neutral (pH = 6.5–7.5) showing that the methanotrophs were optimally adapted to the in situ pH, which was 6.9. The generally lower oxidation rates obtained in the experiment are due to the fact that the experiment is conducted in a soil–water slurry, which limits substrate diffusion. Bender and Conrad (1995) found optimum pH between 6.7 and 7.5 in four different soils. However, one soil

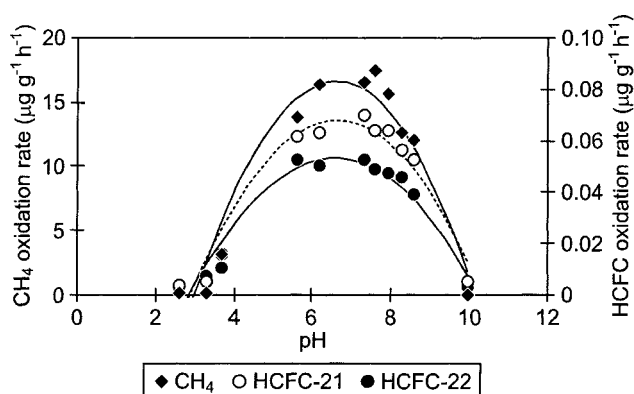


Fig. 6. Influence of soil acidity on the oxidation rate of methane and hydrochlorofluorocarbons (HCFCs).

(forest luvisol) showed a lower in situ pH (4.5) while another soil (cultivated cambisol) showed a higher in situ pH (8.1), indicating that not all methanotrophs were adapted to pH in their environment. Similar observations have been made by others (Dunfield et al., 1993; Hütsch et al., 1994) and confirm that the pH optima for growth of most known methanotrophs is between pH 6.6 and 6.8 (Whittenbury et al., 1970). Changes in pH were observed in soil columns permeated with methane, with a trend toward more acidic conditions near the top of the columns. Addition of lime raised the pH and enhanced methane oxidation (Hilger et al., 2000). In “natural” landfill cover systems it is questionable whether significant pH gradients will develop as the dynamics of the system (infiltrating water, changes in soil gas concentrations) will mitigate the accumulation of acidifying oxidation products (H^+ , methanol, formic acids, CO_2). Furthermore, surface soils often have high buffer capacities and it is likely that the pH of most landfill covers will be around neutral. Consequently, the oxidation capacity for methane and trace gases will be less affected by pH.

Copper

The copper content of the soil in situ was 4.7 mg kg^{-1} . The oxidation rates of this soil were compared with oxidation rates of soils where different amounts of copper were added (up to 1680 mg kg^{-1}). The oxidation rates for methane, HCFC-21, and HCFC-22 were unaltered in copper-amended soil up to 60 mg kg^{-1} (Fig. 7). At higher concentrations the oxidation rates decreased; however, a very high copper concentration (850 mg kg^{-1}) was needed before total inhibition was obtained. The intercellular location of MMO (soluble or particulate) is dependent on the availability of copper in the growth medium. In wild-type organisms soluble MMO is only produced at very low copper concentrations ($<16 \text{ } \mu\text{g L}^{-1}$) (Tsien et al., 1989). Methanotrophs grown under conditions of copper excess express pMMO but switch to sMMO in response to copper stress (Stanley et al., 1983). In conditions with rising copper concentrations the shift from soluble to particulate MMO will induce a higher bacterial growth rate (Joergensen and Degn, 1987). The methanotrophic culture in the Skellingsted soil consisted mainly of Type II methanotrophs holding the MMO gene coding for the soluble form of methane

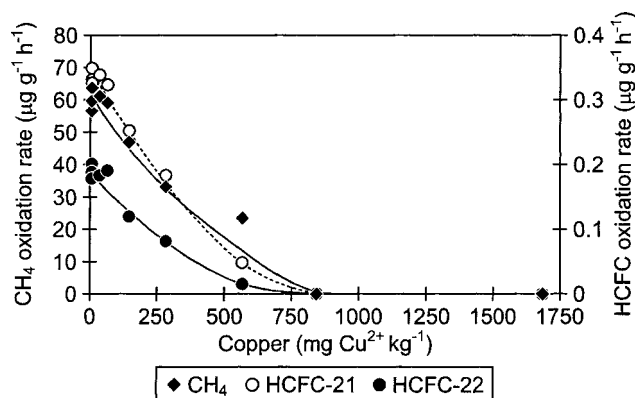


Fig. 7. Influence of copper on the oxidation rate of methane and hydrochlorofluorocarbons (HCFCs).

(Svenning et al., 2004). Thus increasing copper concentrations were expected to inhibit the cometabolism of the HCFCs, as observed here. The influence of copper in soil studies is not directly comparable with studies conducted in aqueous solution due to the strong sorption of copper ions to soil particles (K_d values of 1000 L kg^{-1} [McLaren et al., 1983]), which in soil systems would lower the water concentration significantly. De Visscher (2001) found no influence on methane oxidation kinetics between soil having a copper content of $26 \text{ } \mu\text{g kg}^{-1}$ and copper-amended soil having a total copper content of $78 \text{ } \mu\text{g kg}^{-1}$, which made them suggest that methanotrophs expressing pMMO were active already at the lowest copper content ($26 \text{ } \mu\text{g kg}^{-1}$). However, a total soil concentration of $78 \text{ } \mu\text{g kg}^{-1}$ would result in a soil water concentration of approximately $0.08 \text{ } \mu\text{g L}^{-1}$ (using a soil-water distribution coefficient of 1000), suggesting that the methanotrophs expressing sMMO were active, and that insufficient copper quantities were added to obtain a shift to pMMO or a toxic effect. Bender and Conrad (1995) observed that low copper concentrations up to 4.2 mM in soil water had a slightly stimulating effect on the induction of the oxidation process. At copper concentrations of $>4.2 \text{ mM}$ methane oxidation activity was inhibited. These observations are quite consistent with our results, since inhibition was observed in soils with copper concentrations higher than 60 mg kg^{-1} , which corresponds to an approximate water concentration of 2.5 mM in our soil system. The strong sorption of copper ions to soil particles will mitigate the effect of high copper concentrations on methanotrophs in soil environments compared with methanotrophs in aquatic environments. It is unlikely that copper will limit the activity of methanotrophic bacteria in soil cover systems, since this would require unrealistically high copper concentrations.

Ammonium

The ammonium content of the soil in situ was 2.3 mg kg^{-1} . The oxidation rates of this soil were compared with oxidation rates of soil where different amounts of ammonium were added ($2.3\text{--}1200 \text{ mg kg}^{-1}$). The oxidation rates for methane, HCFC-21, and HCFC-22 were unaltered in ammonium-amended soil up to 14 mg kg^{-1} (Fig. 8). At higher ammonium concentrations the oxida-

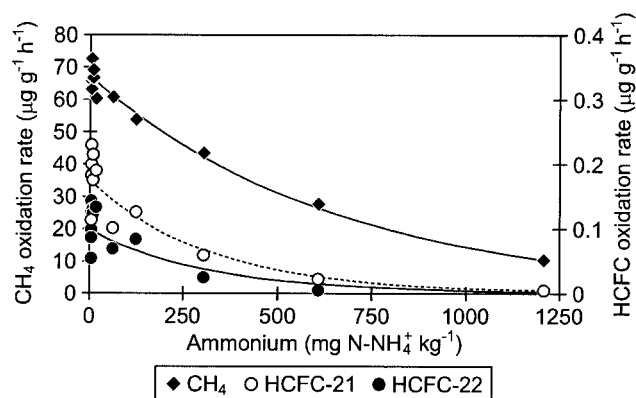


Fig. 8. Influence of ammonium on the oxidation rate of methane and hydrochlorofluorocarbons (HCFCs).

tion rates decreased. Bender and Conrad (1995) found that low NH_4^+ concentrations ($14\text{--}62 \mu\text{g N g}^{-1}$) stimulated methane oxidation during induction, but that higher concentrations were inhibiting. Boeckx and Van Cleemput (1996) observed inhibition of methane oxidation in landfill cover soil amended with 25 mg N kg^{-1} , where the methane oxidation rate decreased linearly with the initial NH_4^+ content of the soil. Similar results were obtained by Hütsch (1998) who found that application of 40 mg N kg^{-1} [added as NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$] to an arable soil caused a strong instantaneous inhibition of CH_4 oxidation by up to 96%. After nitrification of the added N the inhibitory effect was not fully reversible, resulting in a residual inhibition of up to 21%. King and Schnell (1998) observed inhibition of methane consumption in soil with added nonammonium salts. Even though the inhibition mechanisms remain uncertain, it cannot be excluded that part of the inhibitory effect observed in this study with addition of NH_4Cl is due to changes in the ion composition of the soil water. Even though ammonium inhibition might be very important for methanotrophic activity in fertilized agricultural soils, ammonium is less important in controlling the emission of methane in landfill cover soils, as no difference in oxidation rates between nonamended soil and soil containing up to 14 mg N kg^{-1} was observed. However, if landfill covers are fertilized to promote plant growth (e.g., to control erosion, or at golf courses), ammonium inhibition might become a very important factor increasing the emission of methane and trace gases to the atmosphere. Also, in landfill covers amended with nitrogen-rich material as compost or sewage sludge, N turnover rates might strongly affect the methanotrophic activity. Humer and Lechner (1999) observed significantly reduced methane oxidation rates in sewage sludge compared with soil and fully matured compost, which they attributed to the high content of nitrate (1070 mg kg^{-1}) and ammonium (1200 mg kg^{-1}).

CONCLUSIONS

The landfill soil showed a very high capacity for methane oxidation resulting in a maximum oxidation rate of $104 \mu\text{g CH}_4 \text{ g}^{-1} \text{ h}^{-1}$. The hydrochlorofluorocarbons HCFC-21 and HCFC-22 were rapidly oxidized, and the oxidation occurred in parallel with the oxidation of methane. The maximal HCFC oxidation rates were 0.95 and $0.68 \mu\text{g g}^{-1} \text{ h}^{-1}$ for HCFC-21 and HCFC-22, respectively. Inhibition of methane oxidation by HCFCs played a minor role, especially at lower concentrations typical for landfill gas. The inhibition of HCFC oxidation by methane was more pronounced, as the oxidation rate decreased by 90% when the methane concentration increased from 0 to 23% v/v. Zones near the surface with lower methane concentrations might favor HCFC oxidation. Methane oxidation and degradation of HCFCs was influenced by both physical-microbial and chemical-microbial interactions. In general, the environmental factors studied influenced the degradation of HCFCs in almost the same way as they influenced methane oxidation. Temperature had a profound influence on the methanotrophic activity giving high Q_{10} values of

3.4 to 4.1. Temperature optimum was around 30°C . However, oxidation occurred at temperatures as low as 2°C at significant rates, which implies that even during winter, some methanotrophic activity might be expected to reduce the emission of methane and trace compounds from landfills. A moisture content of 25% w/w yielded the maximum oxidation rate, most likely because it allowed rapid gas transport together with sufficient microbial activity. The optimum pH was around neutrality ($\text{pH} = 6.5\text{--}7.5$) showing that the methanotrophs were optimally adapted to the in situ pH. Relatively high concentrations of copper and ammonium ($60 \text{ mg Cu}^{2+} \text{ kg}^{-1}$ and $14 \text{ mg N NH}_4^+ \text{ kg}^{-1}$) were needed to obtain an inhibitory effect, indicating that these parameters are less important in unfertilized landfill soil covers.

Factors influencing methane oxidation in landfill top covers have been intensively studied to evaluate the contribution of landfills to global warming. This study shows that the degradation of HCFCs in landfill cover soil is controlled by the same factors controlling methane oxidation, with the most important parameters being temperature, soil moisture, and methane and oxygen supply. However, short-term incubation studies may not be representative of long-term, on-site performance; therefore, experiments better simulating the long-term operation of landfill cover systems are needed. Environmental conditions that favor growth of methanotrophs producing sMMO may be important as these bacteria may be particularly effective in improving trace gas attenuation in landfill covers. Finally, to develop better biostrategies for remediation of landfills, future design and engineering of landfill soil covers should not only focus on methane but also on the additional specificity of trace component degradation.

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