Abstract. The kinetic parameters of methane oxidation in three mineral soils were measured under laboratory conditions. Incubations were preceded by a 24-day preincubation with 10% vol. of methane. All soils showed potential to the consumption of added methane. None of the soils, however, consumed atmospheric CH4. Methane oxidation followed the Michaelis-Menten kinetics, with relatively low values of parameters for Eutric Cambisol, while high values for Haplic Podzol, and especially for Mollic Gleysol which showed the highest methanotrophic activity and much lower affinity to methane. The high values of parameters for methane oxidation are typical for organic soils and mineral soils from landfill cover. The possibility of the involvement of nitrifying microorganisms, which inhabit the ammonia-fertilized agricultural soils should be verified.

Keywords: soil, methane oxidation, kinetic parameters, methanotrophic activity

INTRODUCTION

Methane (CH4) is the most abundant hydrocarbon in the atmosphere, and it is an important greenhouse gas contributing to an estimated 18-20% of postindustrial global warming (Knittel and Boetius, 2009). The atmospheric CH4 concentration has risen from the background level to 1 782 ppb in 2006 (IPCC, 2007). Soil is both emitter and consumer of greenhouse gases (Włodarczyk et al., 2011). The surface CH4 emissions is estimated to reach 643 Tg year⁻¹, while microbial CH4 oxidation in well-drained upland soils accounts for about 6% of the global atmospheric methane sink (Fowletr et al., 2009; IPCC, 2007).

Methane oxidizing bacteria (methanotrophs) play a significant role in the carbon cycling; they represent one of the largest biological sinks for methane in soils, and the only biological sink under aerobic conditions (Conrad, 2007). Methanotrophs are present in the aerobic soil layers, rhizo-sphere, roots and stem bases of dryland and flooded rice agroecosystems (Vishwakarma and Dubey, 2010). Numerous molecular methods have been developed to study the diversity, structure and abundance of methanotrophic communities in situ (Steenbergh et al., 2010). The key enzyme on methanotrophy is methane monoxygenase (MMO), which converts methane to methanol. The exact catalytic mechanism of methane oxidation is still hypothetical (Baani and Liesack, 2008). Methane monoxygenase, especially soluble sMMO, is remarkable in its broad substrate specificity, and catalyses oxidation of other alkanes, alkenes, alicyclic hydrocarbons, halogenated aliphatics, mono- and di-aromatics and others. This unique capability, catalyzing reactions of environmental importance, has attracted great attention for applied microbiologists and biochemical engineers, and a potential value of methanotrophs in environmental bioengineering for biodegradation of toxic compounds is studied (Jiang et al., 2010).

Knowledge about the kinetics of an enzyme reaction with its substrate is centrally important to biochemists. Information about kinetics of enzyme reaction is commonly used to interpret the role of particular enzyme in metabolic control of the biological process (Psillos and Niedderer, 2003). The kinetics of many enzyme-catalyzed reactions are described by Michaelis-Menten equation with the maximum reaction rate defined as $V_{\text{max}}$, and the Michaelis constant, $K_m$, defined as the substrate concentration at which the reaction achieves half the maximal rate ($V_{\text{max}}/2$); $K_m$ is independent on enzyme concentration, while $V_{\text{max}}$ increases with the enzyme concentration increase. Traditionally, $K_m$ is expressed in molarity, while several authors use such units as ppm and % vol. (Pawlowska and Stepiniewski, 2006). Kinetic constants are derived experimentally. An important point can be made about the physiological implications of...
enzyme kinetic constants. If the substrate concentration is known, then it is possible to predict from the $K_m$ value whether or not an enzyme is saturated. This, in turn, would indicate whether the enzyme was likely the rate-limiting step in a given pathway. Also, a knowledge of the values of $V_{max}$ for the various enzymes in a pathway would allow predictions to be made of the maximum obtainable flux of metabolites through a particular route (Smith and Wood, 1991).

Methane oxidation has been shown to follow Michaelis-Menten kinetics (Baani and Liesack, 2008; Saari et al., 2004; Steenbergh et al., 2010). Most known (cultured) methanotrophs have a low affinity for CH$_4$, and cannot maintain their growth on CH$_4$ at atmospheric (ambient) concentration, but need the higher CH$_4$ concentrations. Although there are no pure cultures of high affinity CH$_4$ oxidizers, these organisms are known to exist in upland soils where they account for about 10% of the annual global CH$_4$ sink. The observed capacity for both types of oxidation kinetics in saturated soils and sediments has been assumed to be due to the presence of mixed methanotrophic populations (Megonigal et al., 2004).

Because N fertilization, especially ammonium fertilizers, frequently reduces soil CH$_4$ consumption, agricultural soils have been regarded to be rather poor sink for methane. However, Chan and Parkin (2001) have shown that although the prairie and forest soils had the greatest potential to oxidize atmospheric concentrations of CH$_4$, but soils from many of the agricultural sites exhibited greater CH$_4$ oxidation activity when exposed to elevated CH$_4$ concentrations.

The aim of the paper was to study the kinetic parameters of methane oxidation in soils enriched with methane under controlled laboratory conditions.

**MATERIALS AND METHODS**

Three soils Mollic Gleysol, Haplic Podzol and Eutric Cambisol were collected in 2011 from a depth of 0-20 cm, air-dried and sieved to <2 mm. Soils were selected as characterized by a similar texture, which determines the in situ soil air-water conditions and, thus, strongly regulates soil methanotrophic activity. Basic soil properties are presented in Table 1.

The 3-g samples of air-dry soils were weighted into 20 cm$^3$ glass vessels and moistened with distilled water to obtain the field water capacity (FWC). Since aerobic methanotrophic bacteria require both oxygen and water, soils were incubated at field water capacity that optimize both air and moisture. In the conditions of Poland, FWC represents the water content which is bound in the soil at potential of 16 kJ m$^{-3}$ (pF 2.2) (Walczak et al., 2002). All the vessels were tightly closed with rubber stoppers and aluminium caps, and after adequate volumes of air from the vessels were removed to avoid overpressure, soils were enriched with methane. The samples were incubated at a constant temperature 25°C in the dark. The incubation was preceded by a 24-day preincubation of all samples under the same conditions with 10% methane in the headspace. When added CH$_4$ was consumed, all vessels were opened, and allowed to ventilate for 2 h. The bottles were then sealed and the procedure of soil enrichment was repeated; methane was added to obtain 0.5, 1.0, 1.5, 3.0, 5.0, and 10% (vol.), which corresponded to CH$_4$ range from 1.34 to 25.67 µmol CH$_4$ kg$^{-1}$ d.m. soil. Additionally, samples without CH$_4$ additions were prepared to study the possibility of soils to oxidize atmospheric CH$_4$ (1.8 ppm).

Determining the instantaneous reaction rate is not feasible in practice and the rate is determined instead based on the rate of change of CH$_4$ concentration over time (Gulledge et al., 2004). The initial methane oxidation rate was determined using the methane sampling times from 0 to 26 h (Steenbergh et al., 2010), and the rates were obtained by dividing the CH$_4$ oxidation with incubation time (Xu and Inubushi, 2009). Lineweaver-Burk equation was used to determine the kinetic parameters of methane oxidation:

\[
\frac{1}{v} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}},
\]

where: $K_m$ – the Michaelis constant, and $V_{max}$ – the maximum reaction rate, $v$ – CH$_4$ oxidation rate at a given substrate concentration [S]. Based on the values of the slope and intercept ($K_m/V_{max}$ and $1/V_{max}$, respectively) obtained for each soil by regression of $1/v$ vs. $1/S$, the apparent $K_m$ and $V_{max}$ were calculated. The $K_m$ and $V_{max}$ were expressed in µmol CH$_4$ and µmol CH$_4$ g$^{-1}$ h$^{-1}$, respectively.

Concentrations of CH$_4$ in the headspace was measured with gas chromatographs Shimadzu GC-14B (Japan) equipped with a flame ionization detector (FID) and GC-14A equipped with a thermal conductivity detector (TCD). Low, atmospheric CH$_4$ concentrations were detected by the FID; the gas components were separated on a column packed with a Porapak Q maintained at 80°C, and the temperature of the

**Table 1. Basic properties of tested soils**

<table>
<thead>
<tr>
<th>Soil types</th>
<th>Grain size distribution (%a, dia in µm)</th>
<th>$C_{org}$ (%)</th>
<th>pH (KCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 000-50</td>
<td>50-2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Mollic Gleysol</td>
<td>74.8</td>
<td>21.7</td>
<td>3.44</td>
</tr>
<tr>
<td>Haplic Podzol</td>
<td>74.6</td>
<td>22.3</td>
<td>3.04</td>
</tr>
<tr>
<td>Eutric Cambisol</td>
<td>71.6</td>
<td>25.1</td>
<td>3.23</td>
</tr>
</tbody>
</table>
injector was 150°C. For higher CH₄ concentrations, TCD
detector with a 2-m column (3.2 mm in dia) packed with
Porapak Q was used with He as a carrier gas flowing at a rate
of 40 cm³ min⁻¹; the temperature of the column and detector
were 40°C and 60°C, respectively (Brzezińska et al., 2011;
2012). The detector responses were calibrated using cer-
tified gas standards (Air Products) containing 10 ppm or 4%
CH₄ in He.

Particle size distribution (FAO Classification) was
measured using the Mastersizer 2000 (Malvern, UK) laser
diffractometer within the size range of 0.02 μm to 2 mm
(Bieganowski et al., 2010). The Hydro G dispersion unit was
used with 1750 r.p.m. of the pump and 700 r.p.m. of stirrer
(Sochan et al., 2012). Total organic carbon (C_organic) was de-
termined by TOC-VCPH analyzer (Shimadzu, Japan), and soil
pH was measured in 1 mol KCl (1:2.5 mass) after 24-h stabili-
zation at room temperature. All measurements were done in
triplicate.

RESULTS AND DISCUSSION

Tested soils differed in their ability to the oxidation of
added methane. During the preincubations, the 1- to 4-day
lag periods before the onset of CH₄ oxidation were observed
(Fig. 1). The lag phase typically occurs as a result of adapta-
tion of soil microorganisms to added substrate (Steenbergh
et al., 2010). The use of the preincubations allowed to reduce
this period during following incubations with different CH₄
concentrations (Fig. 2). Among the tested soils, the Mollic
Gleysol showed the highest methanotrophic activity. All
added CH₄ was taken up during seven days of the incubation
(Fig. 2a). Lower CH₄ enrichments (<1.5% vol.) were de-
pleted already after two days, and nearly linear decline of the
highest methane of 10% vol. was observed. The Haplic
Podzol soil showed some slower decline of CH₄ over time,
especially at elevated initial CH₄ in the headspace (5-10%
vol.) (Fig. 2b). In turn, the Eutric Cambisol incubated with
10% CH₄ showed the slowest CH₄ consumption among
tested soils, and needed 18 days to deplete high CH₄ amend-
ments (Fig. 2c). The Haplic Podzol and Eutric Cambisol
soils, contrary to the Mollic Gleysol, did not utilized all
methane added at the lowest 0.5% vol. treatment, and there
was still 0.2-0.3% vol. CH₄ in the headspace at the end of the
incubation. Moreover, none of tested soils showed ability to
consume the atmospheric methane.

The CH₄ oxidation rates were expressed in substrate sa-
turation curves as a function of initial methane concentra-
tions (Fig. 3a-c) and showed typical Michaelis-Menten cha-
teristics. Lineweaver-Burk plots based on regression of
1/v vs. 1/S are presented as insets in Fig. 3. The apparent $K_m$
and $V_{max}$ values obtained for tested soils, as calculated from
the slopes and intercepts of these linear equations are
summarized in Table 2.
Cultured methanotrophs, classified into different types I and II depending on the intracellular membrane arrangement, pathways of carbon assimilation, and phospholipid fatty acid composition, typically inhabit the aerobic interfaces of methanogenic environments, such as natural wetlands and rice paddies. Because of the usually high methane supply in these environments, methane is oxidized with low affinity and high $K_m$ values ($>1$ ìmol CH$_4$). These $K_m$ values are similar to those determined for cultured methanotrophs ($K_m = 2$-$12$ ìmol CH$_4$) (Baani and Liesack, 2008). In turn, in upland soils, methanotrophic bacteria take up CH$_4$ directly from the atmosphere. Methane consumption in these soils is a small but a significant part of the global methane budget, comparable in magnitude to the estimated excess of emissions over sinks in recent years (29 Tg year$^{-1}$). In contrast to those methanotrophs in the aerobic interfaces of methanogenic environments, the methanotrophs active in dry, well aerated upland soils, for example, forest soils, consume methane with high apparent affinity and low $K_m$ values of 0.01-0.28 ìmol CH$_4$ (Baani and Liesack, 2008; Megonigal et al., 2004). Recently, a type II methanotrophs able to adapt to $K_m$ values close to those measured in upland forest soil was isolated from a humisol suggesting that the affinity of methanotrophic bacteria for CH$_4$ can vary according to the environmental conditions (Saari et al., 2004). It also has been suggested that low- and high-affinity oxidations may be carried out by the same methanotrophs, in their case a type II methanotroph, but that affinity for CH$_4$ changes as a function of growth conditions. Moreover, it is well documented, that ammonia-oxidizing bacteria can also oxidize CH$_4$ as an alternative substrate for ammonia monooxygenase, perhaps making this group of microorganisms important in cycling of CH$_4$ in agricultural systems (Chan and Parkin, 2001).

All soils tested in our study showed the potential to methane oxidation. Relatively short lag phase of 1 to 4 days observed during preincubations indicated that methane oxidizers inhabiting these soils exhibit quite fast adaptation to high methane in the headspace. The Mollic Gleysol characterized by a high pH 7.71 showed the highest methanotrophic activity, while Eutric Cambisol characterized by pH 6.38 showed the lowest activity (Figs 1, 2). Such a difference in soil pH, however, probably can not be responsible for the difference in the CH$_4$ oxidation activity. Until recently, all cultured methanotrophs required a pH >5 and most were neutrophilic with optimum pH of 6.8-7.0. Yet, CH$_4$ oxidation at pH <5 has been demonstrated in acidic Sphagnum-bogs, and acidophilic methanotrophic bacteria have been isolated with an optimum activity between pH 4.5 and 5.5 (Megonigal et al., 2004). Saari et al. (2004) observed for several mineral and organic soils that the pH optimum for methanotrophic activity varied from 3.9 to 7.5. Nevertheless, based on the study of 12 different soils, Chan and Parkin (2001) reported that in soils with pH less than 5.0, CH$_4$ oxidation activity was nearly eliminated.
Methane oxidation in tested soils followed Michaelis-Menten kinetics. The Eutric Cambisol reached highest for this soil activity at relatively low CH4 concentrations, and no further increase of the activity was observed (Fig. 3). The apparent \(K_m\) value for this soil was 5.98 \(\mu\)mol, and \(V_{\text{max}}\) was 0.137 \(\mu\)mol g\(^{-1}\) h\(^{-1}\). These values are comparable with kinetic parameters measured in other mineral soils (Gulledge et al., 2004; Megonigal et al., 2004; and papers therein). In turn, Haplic Podzol and, especially, the Mollic Gleysol showed CH4 oxidation kinetics similar to those observed in organic soils (Bender and Conrad, 1992; Gulledge et al., 2004; Saari et al., 2004), as well as in some soils with agricultural origin and soils from landfill cover (Pawłowska and Stepniewski, 2006; and references therein). CH4 oxidation in these two soils may be described as low affinity activity with high \(K_m\) (19.8-30.7 \(\mu\)mol) and high \(V_{\text{max}}\) values (0.44-0.55 \(\mu\)mol g\(^{-1}\) h\(^{-1}\)). Similar activities with high \(K_m\) values up to 2.9% vol. in gaseous phase have been observed in a coarse sand purged with 99% methane (Pawłowska and Stepniewski, 2006). For comparison, if we express our results in the same unit, then the constants obtained in our experiment gave comparable values of \(K_m=2-3\%\) vol. Very high \(K_m\) values even up to 66 \(\mu\)mol CH4 were observed for forested peat soil and agricultural humisol (Gulledge et al., 2004; Saari et al., 2004).

As Bradford et al. (2001) pointed, the kinetic parameters for soil CH4 oxidation provide valuable information regarding the dynamics of CH4 oxidation and production within a soil profile. By quantifying the kinetics, it could be determined whether a soil was exposed only to atmospheric and sub-atmospheric concentrations of CH4, with high affinity methanotrophic activity then dominating. The additional presence of low affinity activity would imply that a significant CH4 source was present within the soil, possibly due to biological CH4 production, as has been observed for many peat systems (Bradford et al., 2001). The Mollic Gleysol tested in our study was characterized by a high C\(_{\text{org}}\) content of 3.93%. Typically, Mollic Gleysols are fertile soils rich in organic matter, and soils of high microbial biomass C and N contents show, as a rule, high biological quality (Gajda and Przewołoka, 2012). These properties may promote creation of anaerobic microsites in deeper soil layers, when soil get wet. As methanogenic archaea are present also in agricultural soils, and their growth is stimulated in periods of higher soils moisture content, they may be a source for CH4 diffusing through the soil profile. A surface layer, in turn, may be inhabited by populations of aerobic methanotrophic bacteria, adapted to CH4 concentrations higher than atmospheric. However, low affinity kinetics observed in the Haplic Podzol can not be explained by high fertility, as this soil showed only 0.43% C\(_{\text{org}}\). In fact, tested soils did not oxidize methane at ambient concentration, and did not express the high affinity activity which is typical for upland soils exposed to only atmospheric CH4 mixing ratios. However, Chan and Parkin (2001) showed that under an elevated CH4 atmosphere, N may actually stimulate CH4 oxidation activity and suggest that the CH4 oxidation activity carried out under elevated atmospheric CH4 levels may be predominately due to nitrifying bacteria, especially in the agricultural systems. Regular ammonia-N fertilizer application to agricultural soils may result in increased populations of nitrifying bacteria, which will coincidently metabolize CH4, and may also stimulate methanotrophic bacterial populations, as both methanotrophs and nitrifying bacteria can oxidize both CH4 and NH4\(^+\) (Chan and Parkin, 2001). Therefore, additional studies are necessary to better understanding the processes of methane oxidation in agricultural soils.

**CONCLUSIONS**

1. Tested soils, Mollic Gleysol, Haplic Podzol and Eutric Cambisol showed potential to oxidize added methane up to 10% vol. in the headspace.

2. None of the soils consumed atmospheric (ambient) methane.


4. The Eutric Cambisol showed higher than other soils' affinity to methane, characterized by relatively low \(K_m\) and \(V_{\text{max}}\) values of 5.98 \(\mu\)mol and 0.137 \(\mu\)mol g\(^{-1}\) h\(^{-1}\), respectively.

5. The Mollic Gleysol oxidized added methane most rapidly with a low affinity activity characterized by a high apparent \(K_m=30.7\) \(\mu\)mol and \(V_{\text{max}}=0.550\) \(\mu\)mol g\(^{-1}\) h\(^{-1}\).

**REFERENCES**

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