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METHANOTROPHIC BACTERIA AND THE IMPACT OF SOIL PHYSICAL CONDITIONS ON THEIR ACTIVITY

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Abstract. Occurrence of methane in the soil is accompanied by the development of methanotrophic microorganisms with structural and functional adaptation to its oxidation. Methanotrophs, microaerophilic organisms which are widely spread in aerobic soils and sediments, oxidise methane to derive energy and carbon for biomass production. Thus, they play an important role in reducing methane emission from the soil to atmosphere. Several physico-chemical factors influence the rate of methane oxidation in the soil, including soil diffusivity, water potential and levels of oxygen, methane, ammonium, nitrate, and copper. Methane-oxidising organisms inhabit the aerobic portion of the soils. The depth of the aerobic zone of the soil and gaseous composition of the soil air, which are primarily governed by gas diffusivity, are important parameters in determining population density and growth rate of aerobic methane-oxidising bacteria.

Consequently, identification of the factors influencing methane flux into atmosphere is becoming increasingly important. In this paper, the effects of the above mentioned parameters on the methanotrophic activity in the soil are discussed on the basis of the pertinent literature.

Keywords: methanotrophs, methane oxidation, soil conditions

INTRODUCTION

Soil is one of the most important sources of methane in the atmosphere. This radiatively active gas is produced as a result of biodegradation of organic matter in the soil in anaerobic conditions e.g., on rice paddy fields, natural wetlands and landfills as well as in the saturated horizons of soils with variable moisture regime. An important role in the reduction of methane emission from the soil to atmosphere is assigned to its microbial oxidation during its flow through the soil.

Occurrence of methane in the soil is accompanied by the development of methanotrophic microorganisms with structural and functional adaptations to its oxidation. They are characterised by an ability to utilise methane as a source of energy and carbon. Most of these microorganisms are obligate methanotrophs, i.e., methylotrophic organisms capable of consuming methane and the products of its partial oxidation such as methanole, formaldehyde, and methyl formate, i.e., of the compounds without C-C bonds. They are obligatory aerobes and their methanotrophic activity depends on the soil aeration status.

The aim of the paper was to review the pertinent literature on the topic and to discuss the activity of methanotrophs in relation to the aeration status expressed in terms of oxygen concentration, oxygen availability and of soil redox potential.

CLASSIFICATION OF METHANOTROPHS

Methanotrophs can be functionally divided into three types based on their carbon-assimilation pathway, or pathways, as well as intracytoplasmic membrane arrangement, cell morphology, and guanine and cytosine content of their DNA (Table 1) [11].

Phylogenetic classification based on the analyses of 5S and 16S RNA sequence analyses suggests that the type I methanotrophs clusters within the γ-subgrup of the proteobacteria, the type II seems to cluster with the α-subgrup. Phylogenetic status of the type X methanotrophs has not been well established with respect to these groupings.

CONDITIONS AFFECTING METHANOTROPIC BACTERIA ACTIVITY

Effect of pH

The optimum pH values for methane oxidation and growth of methanotrophs in soils are generally consistent with the optimum pH values for the growth of the best known methanotrophs in pure cultures. Similar methane oxidation rates were observed in the soils from pH 3.5 to 8.0 by Borne *et al.* [4]. Dunfield *et al.* [6] demonstrated that the pH optimum (pH 6.0 to 7.0) for methane oxidation in acidic peats was at least 2.5 pH units higher than the pH in the native peat (pH 3.5) from the Hudson Bay lowland. They concluded that the methane oxidising microflora in these acidic environments consists of neutrophilic methanotrophs.

Effect of soil water content

Changes in the soil water content have profound effects on the microbial activity that in

T a b l e 1. Characteristics of methanotroph types

Characteristic	Type		
	I	X	II
Cell shape	Short rods	Cocci	Rod or pear shaped
Growth at 45 °C	No	Yes	No
GC content of DNA (mol %)	50-64	62.5	62.5
Membrane arrangement			
Bundles of vesicular discs	Yes	Yes	No
Paired membranes aligned to cell periphery	No	No	Yes
Resting stages			
Azotobacter-like cysts	Present	Present	Absent
Exospores or "lipid" cysts	Absent	Absent	Present
Rosettes formed	No	No	Yes
HMP pathway- 3-hexulose phosphate synthase	Present	Present	Absent
Serine pathway- hydroxypyruvate reductase	Absent	Present	Present
Ketoglutaratedehydrogenase and a complete tricarboxylic acid cycle	Absent	Absent	Present
Ability to fix elemental nitrogen and presence of nitrogenase	Absent	Present	Present
Predominant phospholipid fatty acid chain length	16	16	18
Autotrophic CO_2 fixation and presence of ribulose bisphosphate carboxylase	Absent	Present	Absent
Isocitrate dehydrogenase type			
NAD related only	Absent	Present	Absent
NADP related only	Absent	Absent	Present
Both NAD and NADP utilised	Present	Absent	Absent
Example of the named species	*Methylomonas methanica*	*Methylococcus capsulatus*	*Methylosinus trichosporium*

turn alters composition of the soil microbial populations. The optimum conditions for the growth of methanotrophic bacteria and induction of methane oxidation activity were water contents of 0.20-0.35 kg kg^{-1}. Boeckx and Van Clemput [3] reported the maximum rate of methane oxidation (2.36 ng CH_4 h^{-1} g^{-1} soil) at the water content of 0.15 kg kg^{-1} (Fig. 1). A decrease of methane oxidation in the water-saturated soil (0.40-0.50 kg kg^{-1} H_2O) probably resulted from a slower diffusion of methane and oxygen through the soil to microorganisms. A decrease in the water content to 0.05 kg kg^{-1} resulted in dramatic decrease in methane oxidation rates as a result of the physiological requirement of methanotrophs for water. A physiological response to water stress caused a lower microbial activity manifested by 40% loss of CO_2 produced [3].

Effect of temperature

Most methanotrophs available in pure cultures are mesophiles, although *Methylococcus capsulatus* Bath and related strains are capable of growing at temperatures up to 50 °C. Also psychrophilic methylotrophs have been described recently [12].

Boeckx and Van Cleemput [3] reported that methane oxidation reached the maximum between 25 and 30°C with a steadily increasing rate at the temperature level from 12 to 25 °C (Fig. 2). The optimum temperature for methane oxidation was 25 °C in most of the peat soils, although oxidation occured also at 0 to 10 °C and at 35 °C. It is similar to 31 °C optimum reported by Whalen *et al.* [13] for a landfill cover soil. It is also in the range (19-38 °C) reported by King and Adamsen [1] for pure cultures of the methanotroph *Methylomonas rubra.*

Effect of oxygen availability and methane concentration

Nearly all methanotrophic bacteria are obligate aerobes, preferring subatmospheric oxygen concentrations. Microaerophilic methane-oxidizing organisms inhabit the aerobic portion of soils. The depth of oxygen penetration into typical landfill soil has been calculated to be approximately 1 m [5]. There some papers (e.g., [11]) indicating that short periods of anoxic conditions cause a loss of methanotrophic activity.

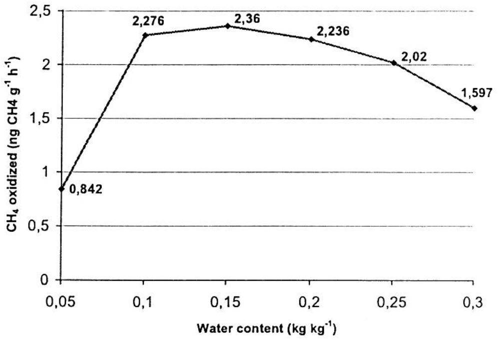

Fig. 1. Influence of water content on the CH_4 uptake rate in the landfill cover soil with the following granulometric composition: 9.3% clay, 14.5% silt, 76.2% sand. The soil contained 1.79% organic matter. According to the USDA textural classification that it was a sandy loam soil. (After Boeckx and Van Cleemput [3]).

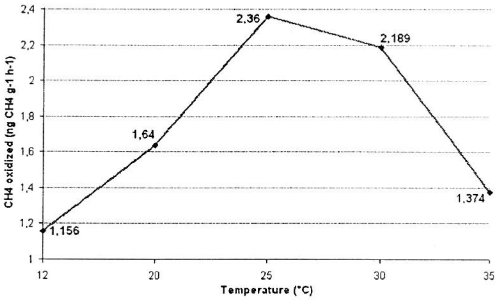

Fig. 2. The influence of soil temperature on the CH_4 uptake rate (According to Boeckx and Van Cleemput [3]).

It is considered that two different populations of methanotrophs are present in the soils exposed to different methane concentrations. One population hypothetically oxidises methane at low concentrations and has high affinity for methane. Soils exposed to higher concentrations of methane contain populations of methanotrophs with constant affinity for methane similar to the values observed in pure cultures of the well-known methanotrophs [2].

The results of some experiments [7] indicated that the growth of type II methanotrophs was favoured by low-oxygen, high-methane conditions. Type I methanotrophs dominated most frequently close to the top of the soil surface [7].

Studies of methane oxidation in the soils and sediments suggest that the minimum threshold value for methane oxidation in the soils is much lower than that in the sediments. For example, threshold values of $2\text{-}3 \cdot 10^{-6} m^3 m^{-3}$ have been reported for sediments, whereas the threshold values for the soil are lower, ranging from 0.1 to $0.4 \cdot 10^{-6} m^3 m^{-3}$ [11]. These variations may be related to the differences in methanotroph population types, in the binding efficiency of the two types of methane monooxigenases (MMOs) to methane at its low partial pressure levels.

The effect of methane concentration was determined by Bender and Conrad [2]. Increases in the populations of methanotrophs were detectable only at methane concentrations exceeding $7 \cdot 10^{-3} m^3 m^{-3}$. At methane mixing ratio of $0.2\ m^3 m^{-3}$, a 100-fold increase in methane oxidation by the soils and 10- to 100-fold increases in the populations of methanotrophs were observed.

THE FLUX OF METHANE BETWEEN SOIL AND ATMOSPHERE

Wetland soils

Wetland soils are generally anoxic. The depth of the oxic surface layer is a result of balance between the influx of O_2 from the atmosphere and the O_2 consumption by the soil microflora. In general, oxygen is depleted rapidly with the increasing distance from the soil surface resulting in the redox stratification. Theoretically speaking, this stratification creates relatively well-known habitats for different groups of trace gas metabolising microorganisms. Various redox zones are characterised by the dominance of different electron acceptors such as: O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2+}, and CO_2.

Most wetland soil is devoid of O_2 and other electron acceptors with the exception of CO_2 and H^+. This zone is dominated by methanogenesis [5].

Upland soils

Upland soils are generally well aerated. Gases are transported by diffusion or convection within the soil gas phase rather than within the water phase. Microorganisms in the soil crumbs or on sand grains are the actual units of trace gas metabolism. Their activities may be quite heterogeneus and result in different redox gradients.

In most upland soil, CH_4 production is usually absent or marginal and the CH_4 flux is dominated by CH_4 oxidation [5].

CONCLUSIONS

1. It was stated that temperature optimum for methanotrophic activity in soil is 25-31 °C.
2. It was confirmed experimentally that methanotrophic activity shows maximum at moderate water content decreasing at lower water contents due to limitation by water availability and the higher - due to limitation of substrate diffusion.
3. There is a need of further research on the effect of such physical properties as soil compaction, gas diffusion capacity and soil water potential in order to optimise methanotrophic activity of soil.

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