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THE EFFECT OF OXYGEN CONCENTRATION ON THE ACTIVITY OF METHANOTROPHS IN SAND MATERIAL

The ability of certain soil microorganisms to oxidize methane significantly contributes to a reduction of this gas concentration in the atmosphere. This is due to the uptake of the methane present in atmospheric air as well as the limitation of the methane emission from various kinds of sources, for example: from wetlands, paddy fields or landfills. The obligate methanotrophs, responsible for methanotrophic processes, occur commonly in natural and artificial habitats but only under aerobic conditions. However, the effect of the oxygen on their activity in soils has not been sufficiently examined.

Our experiment showed a marked effect of oxygen concentration on methanotrophic activity of microorganisms introduced into a sand material (fraction of 0.5–1.0 mm) with a soil taken from a municipal landfill cover. An increase in oxygen concentration from 2.5% to 21% raised the rate of methane uptake. The shape of a curve representing the relation examined testified to the Michaelis–Menten kinetics of reaction ($V_{\max} = 5.88 \times 10^{-4} \text{ cm}^3\text{kg}^{-1}\text{s}^{-1}$ and $K_M = 10.52\%$). The comparison of the rates of oxygen and methane concentration losses showed an intensive oxygen consumption in processes different from methane oxidation. This could be explained by a large organic carbon content in soil samples.

1. INTRODUCTION

Atmospheric concentrations of some gases increased significantly during the last 200 years. This concerns, among others, methane, the gas being an effective absorber of long-wave radiation reflected from the Earth's surface, also the reductor of OH radicals in the upper layers of the atmosphere [1]. An increase in this gas concentration is caused mainly by anthropogenic emission, in which the municipal landfill sites play an important role [2], [3], [4]. The reduction in emissions of methane from these places is possible by an increase of the methanotrophic process efficiency in the soil cover at the landfill, where installation of the biogas-recovery system is not economical.

Methane oxidation in soil materials has a biochemical character. The population of methanotrophs is modified in terms of its quality and quantity by many physical

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and chemical factors in a direct or non-direct way. One of these factors is the accessibility of oxygen. All types of methane-oxidizing bacteria are aerobes; however, their oxygen demand is not identical. Obligate aerobes are not able to survive under anaerobic conditions. The second group, the aerobic bacteria, demonstrates the ability to survive an anaerobic period and to reactivate their growth under aerobic conditions. Most methanotrophs belong to obligatory aerobic microaerophiles, preferring the oxygen concentrations lower than the atmospheric level [5], [6]. However, there are many reports on the possibility of methane oxidation under anaerobic conditions, for example, in sea and lake sediments [7], [8], in paddy soils [9] or in the bottom part of landfills [10]. The examination carried out in these environments showed that the bacteria reducing sulphates [7], [10], nitrates [8], ferric iron compounds [11], and manganese (Mn^{4+}) compounds [9] are responsible for the process. But this matter is beyond the scope of this paper.

Our investigation deals with methane oxidation under aerobic conditions, in particular we have examined the effect of oxygen concentration on methanotrophic activity of methanotrophs. This problem has been studied by some authors. SCHNELL and KING [12] noticed that a decrease in oxygen concentration from its atmospheric level to 0.2% (v/v) caused a decrease in methanotrophic activity of microorganisms present in forest soil. The examinations carried out by HOEKS [13] on soil taken from the place near leakages of natural gas, by CZEPIEL et al. [14] and by STEIN and HETTIARATCHI [15] on soil taken from a landfill cover showed that the kinetics of the reaction could be described by the Michaelis–Menten equation. REN et al. [16] studied the influence of oxygen concentration on methane uptake in pure culture of *Methylosinus trichosporium* and *Methylobacter luteus*.

2. MATERIALS AND METHODS

2.1. THE MATERIAL EXAMINED

The study of the reaction kinetics was preceded by the examination of methane oxidation capacity. It was carried out in four columns (100 cm high and 15.2 cm ID) filled with sand and gravel materials (the sand mine in Rokitno, near Lublin) and purged of methane (ca. 99%) for 6 months at the rate of $5\text{ cm}^3\text{min}^{-1}$. It was a typical rate of CH_4 production derived from a 20 m-deep layer of the landfill [17]. In order to neutralize a natural acid reaction of sand, it was mixed with $CaCO_3$ in such a proportion that the latter constituted 1% w/w of the mixture. Then the sand was mixed with the landfill cover from Jawidz (near Lublin) in such a proportion that the cover constituted 2% w/w of the mixture. This allowed its inoculation with methanotrophic bacteria. The material for the kinetic experiment was taken from the depth of 20 cm of

the column filled with the fraction of a grain size ranging from 0.5 to 1.0 mm, defined as coarse sand [18]. The methanotrophs associated with this fraction showed to have a great capacity for methane oxidation [19].

2.2. EXPERIMENT DESCRIPTION

The moist (ca. 6% w/w) samples (2 g) were introduced into glass flasks (ca. 9 cm³) sealed with rubber stoppers. Adequate gas mixtures with oxygen concentrations of 21, 10, 5, 3 and 2.5% v/v were injected into the closed bottles. The gas mixture was obtained by the dilution of the atmospheric air with nitrogen. Next, the CH₄ was injected into the bottles as long as initial concentration of 8% was obtained. The composition of each gas mixture was controlled chromatographically (see below). The experiment lasted for 168 hours at the temperature of 22 ± 3 °C and it was repeated three times.

2.3. MEASUREMENT METHODS

The headspace samples (0.05 cm³) were withdrawn by syringes after 0, 3, 24, 48 and 168 hours from the beginning of the experiment. They were analysed in a gas chromatograph (Shimadzu 14B) with a thermal conductivity detector (TCD) fitted with glass packed columns. The Porapak Q column (2 m × 3.2 mm I.D.) was used to determine CH₄ and CO₂ concentrations. The oxygen concentrations were measured in the column (3 m × 3.2 I.D.) packed with Molecular Sieve 5A. The conditions were as follows: injector 40 °C, column oven 40 °C, detector 60 °C, current 150 mA. The carrier gas was helium (flow rate of 40 cm³ min⁻¹). Peak areas were determined by the computer integration program (CHROMA for Windows, 2.01, Pol-Lab). The loss of CH₄ and O₂ concentrations and the increase of CO₂ concentration with time were registered. Next, the methanotrophic activity of the particular samples at different initial concentrations of oxygen was calculated. The methanotrophic activity was defined as the quantity of CH₄ oxidized by the unit of mass or volume of the material examined per time unit. Kinetic parameters V_{\max} and K_M were estimated based on the linearization method of Lineweaver–Burke [20].

2.4. STATISTICAL ANALYSIS

The average values of CH₄, O₂ and CO₂ concentrations from three repetitions were taken for the calculations. The correlation coefficients r and the determination coefficients r^2 were calculated in the case of an examination of the relationship between the variables. The curves representing the correlations were obtained by applying the method of fitting least-squares. Microsoft Excel 97 was used for the statistical working.

3. RESULTS AND DISCUSSION

The loss of CH₄ concentration with time was a basic measure for the calculations of methanotrophic activity. The changes in CH₄ concentration in the headspaces of particular flasks with a different initial gas mixture were observed for 7 days. The rate of CH₄ concentration loss increased with the rise of the initial O₂ concentration (figure 1). CH₄ concentration decreased linearly in all cases, which was confirmed by very high values of the determination coefficients (table 1). It was observed that the values of these coefficients had risen with an increase in the initial O₂ concentration.

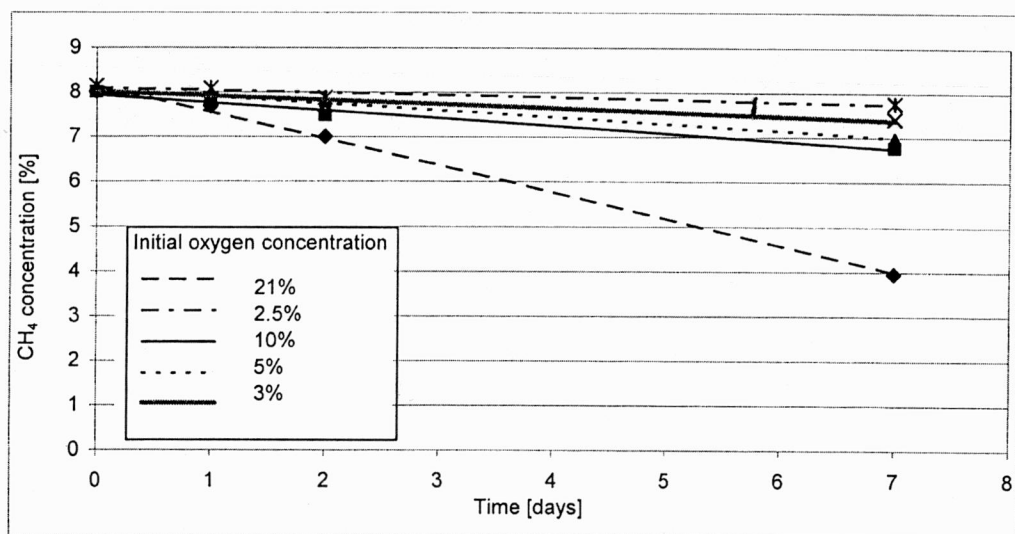


Fig. 1. Changes of CH₄ concentrations in the headspaces with time at different initial O₂ concentrations

Table 1

Statistical parameters and equations for the relations: the changes of CH₄ and O₂ concentrations, depending on the initial O₂ concentrations

| Initial O ₂ concentration (%) | Characteristics of the changes of CH ₄ concentrations | | Characteristic of the changes of O ₂ concentrations | |
|--|--|-------------------------|--|-------------------------------------|
| | Determination coefficient r^2 | Equation | Determination coefficient r^2 | Equation |
| 21 | 0.9901 | $y = -0.7006x + 8.2439$ | 0.9969 | $y = -0.0026x^2 - 2.6483x + 21.745$ |
| 10 | 0.9897 | $y = -0.2035x + 7.9628$ | 0.9894 | $y = 0.2433x^2 - 3.2906x + 12.337$ |
| 5 | 0.9861 | $y = 0.1798x + 8.0845$ | 0.8887 | $y = 4.2691x - 1.2425$ |
| 3 | 0.8909 | $y = -0.11x + 8.0324$ | 0.9704 | $y = 2.4344x - 1.1399$ |
| 2.5 | 0.9065 | $y = -0.0745x + 8.1225$ | 0.9882 | $y = 1.6617x - 0.9025$ |

The curve illustrating the influence of O_2 concentration on the activity of methanotrophs is presented in figure 2. The rate of CH_4 uptake increased with the rise of O_2 concentration, reaching a relatively stable value in the upper range of the concentrations examined. This shape of the plot indicated that we dealt with the Michaelis–Menten type of kinetics. The kinetic parameters, i.e. the maximum value of methanotrophic activity (V_{max}) and the apparent half-saturation constant (K_M), were determined by the Lineweaver–Burke linearization method. The V_{max} value was equal to $5.88 \times 10^{-4} CH_4 \text{ cm}^3 \text{ kg}^{-1} \text{ s}^{-1}$ and was about one order of magnitude smaller than the values obtained by GEBER et al. [21] in the expanded clay material flushed by landfill gas ($V_{max} = 8.52 \times 10^{-3} \text{ cm}^3 \text{ kg}^{-1} \text{ s}^{-1}$). In our experiment, the reaction proceeds at the rate equal to $\frac{1}{2} V_{max}$ when the O_2 concentration reached the value of 10.52% (v/v). A very high K_M , above twice as high as the highest K_{MO_2} , was found in the literature (GEBER et al. [21]). It equalled to 4.6% (v/v). The half-saturation constants reported by other authors were manifold smaller. The value of K_{MO_2} calculated by WATSON et al. [22] for peat soil was 3% (32 μM). HOEKS [13] recorded the K_M values for O_2 in the range from 0.2 to 1.2%, depending on the temperature of soil incubation which had been changed from 13.5 to 20 °C. His examinations were carried out on the soil taken in the vicinity of natural gas leakages. According to STEIN and HETTIARATCHI [15] K_S for O_2 measured in the soil taken from the depth of 36 cm of the column filled with loam from a landfill cover averaged 1.3%. The similar results, i.e. 1.24% for the simulated landfill cover, were obtained by de VISSCHER et al. [23]. A high value of K_M in our experiment suggested that the methane-oxidizing bacteria were no micro-aerophiles. The bacteria (*Methylosinus trichosporium* and *Methylobacter luteus*) responsible for methane oxidation in soil water did not belong to this group either [16]. The threshold value of O_2 concentration dissolved in soil water amounted to 0.53% v/v (5.7 μM). Below this value oxygen was the limiting factor of the methanotrophic process. GEBER et al. [21] did not detect any substantial methane-oxidizing activity in expanded clay material at the O_2 concentration below of 1.7–2.6% (v/v).

The results of the model laboratory experiments conducted previously for the gas profiles in a landfill cover show that oxygen concentration decreases rapidly with depth, approaching the value of K_M at the depth of 15 cm. Small concentrations of this gas were found at the depth of 80 cm [19], and even at the depth of 100 cm in field experiments [3]. Nevertheless, almost anaerobic conditions were obtained in a laboratory study at the depth of 30 cm [17]. The depth of a soil oxygenation layer predicted based on a theoretical model ranged from 20 to 100 cm, depending on the relative coefficient of gas diffusion and V_{max} value [24], [25].

A rapid decrease in oxygen concentration in a landfill cover profile is caused by a limited gas diffusion and by its intensive consumption in the processes of organic substrate oxidation. The lack of oxygen is an important reason for a decrease in the methanotrophic activity in deeper parts of the cover layer. A practical conclusion may be that aeration of the cover layer, which should play an important role in the reduc-

tion of methane emission from landfills, markedly increases the efficiency of methane oxidation. Practical aspects of this conclusion are not useful, because it seems rather troublesome to supply air to the bottom of the cover layer.

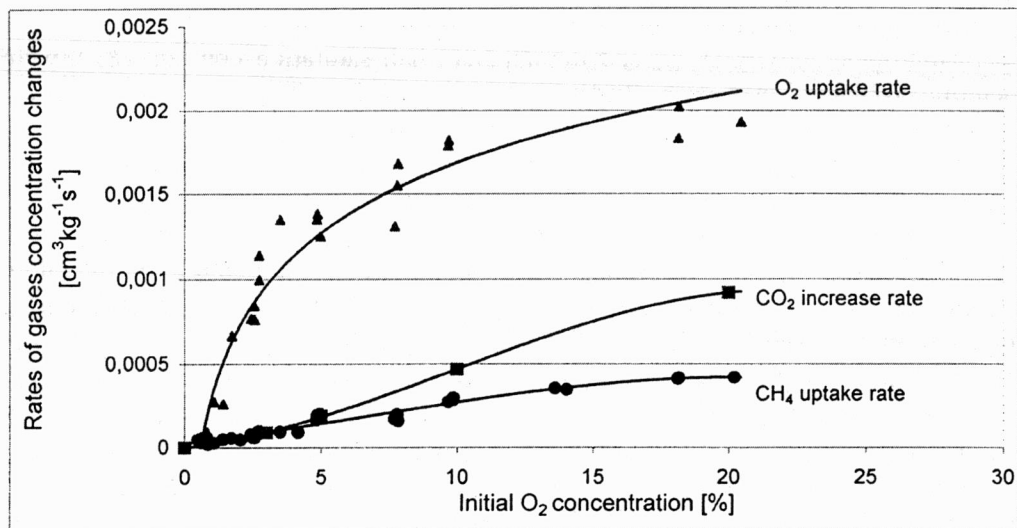


Fig. 2. The influence of O₂ concentration on the rate of CH₄ and O₂ consumption and CO₂ production

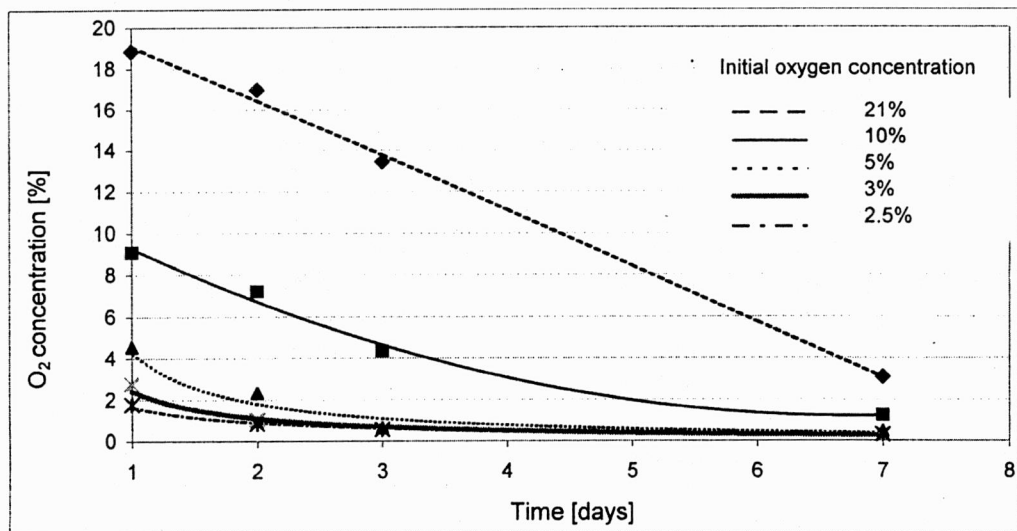


Fig. 3. The changes of O₂ concentrations in the headspaces, depending on the initial O₂ concentration

The rates of changes of O₂ and CO₂ concentrations were also measured. But they could not be used for the evaluation of methanotrophic activity because of the oxidation of organic compounds accumulated in soil material during 6 months of incubation. These compounds are worth analysing. They could act as a base for considering proportions between the rates of the changes of particular gases concentrations.

A significant increase of O₂ uptake rate with the rise of an initial O₂ concentration was observed (figure 3). The most fitted curve had the polynomial (in the upper range of O₂ initial concentration) character (table 1).

The plot illustrating the changes of the rate of O₂ uptake as a function of initial O₂ concentration resembles the curve representing the Michaelis–Menten equation (figure 2). The kinetic parameters of O₂ uptake were determined as before. The values of $V_{\max O_2}$ and K_{MO_2} were equal to 5% and $26.67 \times 10^{-4} \text{ cm}^3 \text{ kg}^{-1} \text{ s}^{-1}$, respectively.

The estimation of the influence of O₂ concentration on its consumption is based on the comparison of how many times the rate of O₂ uptake increased with the determined rise in the initial O₂ concentration. This influence was greater in a lower range of O₂ concentration. A twofold increase in this gas concentration, from 2.5% to 5%, caused a 1.5-fold increase in the rate of O₂ uptake, while a 2-fold change of O₂ concentration, from 10% to 20%, caused a 1.3-fold increase. This suggests that aerobes rapidly respond to the improvement of aerobic conditions in a lower range of O₂ concentration, but there is a certain limit value of the oxygen content which is responsible for slackening of their activity.

Table 2

The rates of the changes in CH₄, O₂ and CO₂ concentrations and their ratios, depending on an initial O₂ concentration

| Initial O ₂ concentration [%] | Rate of O ₂ uptake (V_{O_2}) [$\text{cm}^3 \text{ kg}^{-1} \text{ s}^{-1}$] | Rate of CH ₄ uptake (V_{CH_4}) [$\text{cm}^3 \text{ kg}^{-1} \text{ s}^{-1}$] | Rate of the increase in CO ₂ concentration (V_{CO_2}) [$\text{cm}^3 \text{ kg}^{-1} \text{ s}^{-1}$] | V_{O_2}/V_{CH_4} | V_{CO_2}/V_{CH_4} | V_{O_2}/V_{CO_2} |
|--|--|--|---|--------------------|---------------------|--------------------|
| 21 | 19.9×10^{-4} | 4.2×10^{-4} | 9.2×10^{-4} | 4.47 | 2.2 | 2.16 |
| 10 | 15.2×10^{-4} | 2.9×10^{-4} | 4.7×10^{-4} | 5.24 | 1.6 | 3.23 |
| 5 | 13.4×10^{-4} | 1.9×10^{-4} | 1.9×10^{-4} | 7.05 | 1.0 | 7.05 |
| 3 | 10.3×10^{-4} | 0.9×10^{-4} | 0.9×10^{-4} | 11.44 | 1.0 | 11.44 |
| 2.5 | 9.0×10^{-4} | 0.7×10^{-4} | 0.7×10^{-4} | 12.86 | 1.0 | 12.86 |

The comparison of the rates of methane and oxygen uptake, depending on their initial concentrations (table 2), showed that the rates of O₂ consumption manifold exceeded relative rates of CH₄ oxidation. As it could be concluded from the stoichiometry of methane utilization (equation (1)), a decrease in O₂ concentration occurred twice as fast as a decrease in CH₄ concentration. In our calculation, however, the ratios ranged from 4.74 to 12.86, depending on an initial O₂ concentration. The lower this concentration, the higher the value of the V_{O_2}/V_{CH_4} ratio (V_{O_2} is the rate of O₂ consumption, and V_{CH_4} is the rate of CH₄ consumption).



In contrary to our observations, HOEKS [13] noticed that the quantity of O_2 necessary for oxidizing 1 mole of CH_4 was less than 2 mole and ranged from 0.2 to 1.8 mole. In his opinion, a certain amount of carbon from CH_4 is fixed by microorganism cells (assimilation of CH_4). Our results can be explained as follows: the proportions of reactants are higher than those resulting from the reaction stoichiometry, because CH_4 oxidation is not the main cause of a decrease in O_2 concentration. Probably the oxygen is consumed in the oxidation of other compounds present in the soil material. A increase in the values of $V_{\text{O}_2}/V_{\text{CH}_4}$ ratios with a decrease in an initial O_2 concentration suggests that the methanotrophs are less successful in the competition for oxygen than other aerobic bacteria. This confirmed our earlier conclusion that their affinity with O_2 is weaker.

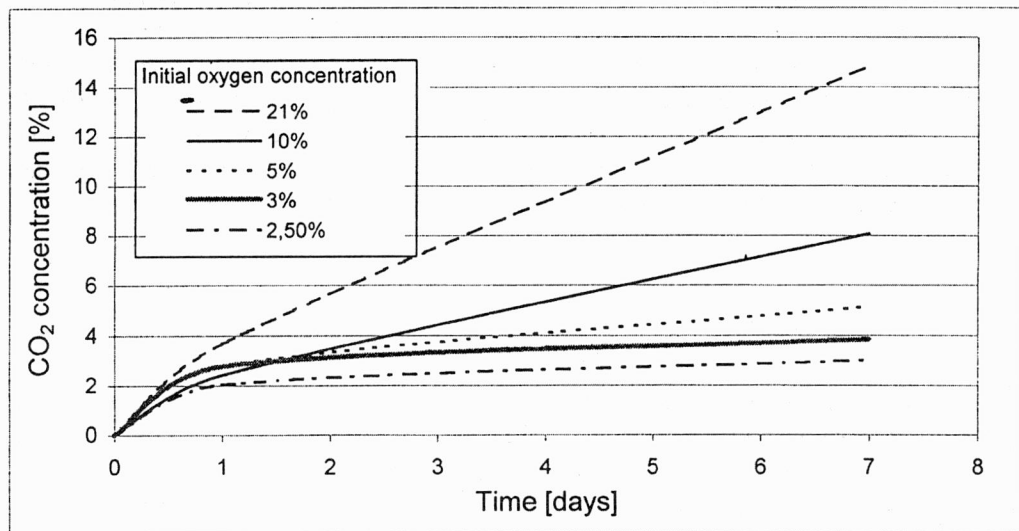


Fig. 4. The changes of CO_2 concentrations in the headspaces, depending on the initial O_2 concentration

The measurements of CO_2 concentration in the headspaces were carried out on the first and the seventh days of the experiment, hence the curves which represented an increase in the CO_2 concentration with time had only an approximate character (figure 4). The rates of the increase in CO_2 production rose with an increase in initial O_2 concentration (figure 2). The rates of an increase in CO_2 concentration within the range of initial O_2 concentrations from 2.5 to 5% were similar to the rates of CH_4 uptake (table 2). This suggested that CH_4 oxidation was the main source of CO_2 (the stoichiometric ratio of $\text{CH}_4:\text{CO}_2$ is 1:1). However, it was possible that it was not its only

source, because a part of carbon derived from CH_4 could be assimilated by the methanotrophs. Hence, a certain amount of CO_2 could be produced in other processes. The rate of an increase in CO_2 concentration exceeded the rate of CH_4 consumption at higher initial O_2 concentrations. Presumably, other processes running with CO_2 evolution were initiated. The $\text{CH}_4:\text{CO}_2$ ratios reported by HOEKS [13] never reached the stoichiometric value and ranged from 0.2 to 0.9, which was probably connected with the carbon assimilation from CH_4 by the microorganisms.

The ratios of $V_{\text{O}_2}/V_{\text{CO}_2}$ were 2.16 and 12.86 for 21 and 2.5% of the initial O_2 concentration, respectively. A fast O_2 consumption at its concentration is difficult to explain. The values of $V_{\text{CO}_2}:V_{\text{CH}_4}$ ratio proved that oxygen was not used in the processes which proceeded with the production of CO_2 . Possibly, the oxygen is built into the cell of microorganisms or used for oxidizing non-carbon compounds.

4. CONCLUSIONS

From our experiment the following conclusions can be drawn:

- Oxygen concentration affects significantly methane oxidation. In the examined range of O_2 concentration (2.5–21%), the rate of CH_4 uptake was six times higher (an increase from 0.7×10^{-4} to $4.2 \times 10^{-4} \text{ cm}^3 \text{ kg}^{-1} \text{ s}^{-1}$).

- This shape of the curve which represented the analysed relation testified to the Michaelis–Menten type of the kinetics. The kinetic parameters were: $V_{\text{max}} = 5.88 \times 10^{-4} \text{ cm}^3 \text{ kg}^{-1} \text{ s}^{-1}$ and $K_M = 10.52\%$.

- The consumption of oxygen was higher than it could be expected based on the stoichiometry of methane oxidation. The ratios of $V_{\text{O}_2}/V_{\text{CH}_4}$ exceeded the stoichiometric value of 2 in each case (they ranged from 4.47 to 12.86 for the respective 21 and 2.5% initial concentrations of oxygen).

- The ratios of $V_{\text{CO}_2}/V_{\text{CH}_4}$ exceeded the stoichiometric value of 1 only in the cases where initial O_2 concentrations were 10 and 21% and reached the values of 1.6 and 2.2, respectively.

- The ratios of $V_{\text{O}_2}/V_{\text{CO}_2}$ always exceeded the stoichiometric value of 2 (they ranged from 2.16 to 12.86, respectively, at 21 and 2.5% of the initial O_2 concentration).

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WPLYW STĘŻENIA TLENU NA AKTYWNOŚĆ METANOTROFICZNĄ
MIERZONĄ W MATERIALE PIASZCZYSTYM

Zdolność mikroorganizmów glebowych do utleniania metanu w znaczący sposób przyczynia się do zmniejszenia stężenia tego gazu w atmosferze. Bakterie metanotroficzne, które należą do tlenowców, mają zdolność wychwytywania metanu z powietrza atmosferycznego, mogą również nie dopuszczać do jego wydostawania się ze źródeł, np. na terenach podmokłych, polach ryżowych czy składowiskach odpadów. Wyniki przeprowadzonych doświadczeń wskazują, że stężenie O_2 w zakresie 2,5–21% wyraźnie wpływa na aktywność metanotroficzną mikroorganizmów w materiale piaszczystym (frakcja 0,5–1,0 mm), którym zaszczerpiono glebę z nadkładu składowiska odpadów. Kształt krzywej obrazującej ten wpływ wskazuje na kinetykę Michaelisa–Menten. Wartości parametrów kinetycznych są następujące: $V_{\max} = 5,88 \times 10^{-4} \text{ cm}^3 \text{ kg}^{-1} \text{ s}^{-1}$, $K_M = 10,52\%$. Zaobserwowano, że zużycie tlenu było dużo większe niż metanu, co świadczy o pobieraniu tlenu do procesów innych niż utlenianie metanu. Prawdopodobnie tlen został zużyty w procesach utleniania substancji organicznej zawartej w próbkach.



