ARCHIVES OF ENVIRONMENTAL PROTECTION

vol. 34 no.3 pp. 183 - 191 2008

PL ISSN 0324-8461

METHANOTROPHIC ACTIVITY OF COALBED ROCKS FROM „BOGDANKA" COAL MINE (SOUTH-EAST POLAND).

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Abstract: Methane is an atmospheric trace gas, which is estimated to contribute about 20% to global warming. Coal mining used to be regarded as attributing considerably to the anthropogenic emissions of that potent greenhouse gas. Recently discovered methanotrophic abilities of coalbed rocks brought a new argument to the discussion about the environmental impact of the mining industry. In the present work, we determined the methanotrophic activity and maximum capacity (V_{max}) of methane oxidation originating from rocks surrounding seam 385/2 of the "Bogdanka" coal mine. Methane oxidation rates ranged from 0.231μM CH_4 $g^{-1}day^{-1}$ in the rock from the middle of the seam to 0.619 μM CH_4 $g^{-1}day^{-1}$ in the bottom rock (4.4 m depth). Methanotrophic activity and V_{max} increased with the distance to the coal body and with decreasing TOC content. Initial and terminal redox conditions (Eh>320 mV, pH 7.60-8.62) confirmed the oxic character of the methane oxidation process.

Key words: Methanotrophy, coalbed rocks, IC, TOC

INTRODUCTION

Methane, one of the most important greenhouse gases (Lelieveld, 1993) is, molecule for molecule, about 23 times more effective in trapping IR radiation than CO_2. Therefore, in spite of its relatively low mixing ratio (c.a. 1774 ppb) (IPCC, 2007), its contribution to global warming is estimated to be around 20% (EPA, 2006). Data show that methane's current mixing ratio is more than twice that in the pre-industrial era and remains in strong correlation with the growth of human population, the development of industry and agriculture. About 60% of overall methane sources is considered to be of anthropogenic origin (coal mining is estimated to contribute about 8% of this) (Bosquet, 2006; IPCC, 2001, 2007; EPA, 2006). Human activity influences the methane global budget through enlarging emissions (Crutzen, 1991; IPCC, 2001, 2007) as well as limiting natural sinks of that potent greenhouse gas (Hutsch, 2001; King, 1994).

The great majority of methane is removed from the atmosphere through reaction with OH radicals but a variety of biochemical reactions is its second most important (Crutzen, 1991; IPCC, 2001; Lloyd, 1995; Wuebbles, 2002). Methanotrophs, responsible for those processes, are a group of organisms diverse in phylogeny and ecology, constituting a subset of the physiological group of organisms known as methylotrophs (Hanson, 1996). They are widespread in nature and inhabit soils (Bender, 1995; Bodelier, 2004; Dunfield, 1999; Knief, 2003; Le Mer, 2001; Regina, 1994), wetlands (Watson, 1997), fresh and marine waters and sediments (Boetius, 2000; Waekham, 2003). Recent findings revealed that methanotrophs are also well adapted to extreme conditions such as high or low temperature, pH and salinity (Trotsenko, 2002; 2005).

Methanotrophs are known to utilize two patterns of substrate oxidation: oxic, which has been extensively investigated in soils, and anoxic, which was confirmed to take place in deep marine sediments (Boetius, 2000; Raghoebarsing, 2006) and places of geochemical CH_4 leaking (Niemann, 2006; Raghoebarsing, 2005). Thanks to the variety of adaptations, microbial, methane-oxidizing consortia not only reduce atmospheric CH_4 levels via high affinity metabolism but also prevent the huge amounts of that potent greenhouse gas produced in anoxic environments from leaking into the atmosphere (Lloyd, 1995).

The growth of methane consuming bacteria has been confirmed and extensively investigated in many methane-rich environments, as mentioned above, but little attention was paid to habitats connected with coal seams (Pokorný, 2000; Stępniewska, 2004). In contrast, numerous studies of subsurface areas have shown that microbial life is far more widespread in the Earth's crust and influences global processes much more than previously thought. (Amend, 2005; Kotelnikowa, 2001; Pedersen, 2000).

The presence and activity of methanotrophic bacteria in coalbed rocks collected from the dumping area of the "Bogdanka" coal mine, has already been confirmed by Stępniewska (2006). Therefore, the primary objective of the present study is to recognize methanotrophic activity in the samples of "fresh" rocks as well as the spatial distribution of microbial CH_4 oxidation rates in coalbed rocks of the currently exploited seam of the "Bogdanka" coal mine, (seam 385/2). We also investigate the influence of location with respect to the coal body as well as the content of particular forms of carbon on methane oxidation rates.

MATERIAL AND METHODS

Site description - "Bogdanka" coal mine

"Bogdanka" ($51^{0}18'$N; $23^{0}01'$E) is the only coal mine exploiting resources of the Lublin Coal Basin. It has an average annual net coal production rate of 15 000 Mg (waste rock constitutes about 30 % of gross weight). The roof of the Carboniferous coal-bearing strata roof is located at the depth of ca. 700m. Seam 385/2 (1.7m thick), lying at the depth of approximately 760 m, is one of two currently exploited veins of the "Bogdanka" coal mine. In order to determine the spatial (vertical) distribution of methanotrophic activity around the seam, samples were taken from the bottom and roof as well as from the thin (ca. 0.12 m) rock layer that developed within the coal body at the height of ca. 1.1 m of the coal layer. Rocks surrounding the investigated seam are mostly mudstones and claystones. The mineral composition of coalbed rocks surrounding seam 385/2 is presented in tab. I. The average temperature of rocks in Bogdanka coal mine is ca. 28 ^{0}C. The moisture content measured in excavated rocks ranged from 1.6% to 4.1%. Coalbed gas in the "Bogdanka" mine, unlike in other Polish coal mines, is only present in inconsiderable amounts, unable to cause a threat to miners, though projections for the future suggest that this may change with the beginning of exploitation of deeper coal seams (Mazurkiewicz, 2004).

Tab.1. Mineral composition of coalbed rocks from geological surrounding of the seam 385/2 of "Bogdanka" coal mine (mean values n=4).

Macroelements [%]							
CaO	Na_2O	K_2O	P_2O_5	SiO_2	Al_2O_3	Fe_2O_3	MgO
0,15	0,29	2,76	0,09	57,38	23,68	2,93	1,01
Microelements [ppm]				S speciation			
Cu	Zn	Cr	Ni	S SO_4	S pir	S org	S s
27,5	110	120	45	<0,005	0,01	0,01	0,03

Sampling

Samples of rock lying closest to the coal body (C-0, I-S and B-0) were hammered manually, from the distance of ca. 0.2 m from the surface of the excavated wall. Samples of rocks from layers distant from the seam (C-1, C-2, B-1, B-2) were obtained from cores that were drilled both in the bottom and in the roof of the investigated seam (Tab.II).

Preparation of rock material

Samples representative of particular levels of rock profile (Tab.II.) were crushed into pieces and ground in a mill (Testchem, Poland) to 2 mm maximum grain diameter. The total water capacity of each rock was determined gravimetrically in plastic cylinders by saturating ground rock material with deionized water up to a constant weight.

pH and Eh measurements

pH and Eh values were determined for each sample before and at the terminal day of incubation, after complete methane depletion, using pIONeer 65 multichannel analyzer (Radiometer Analytical, Lyon, France) with a combined electrode for pH and a platinum electrode for redox potential measurements with Ag/AgCl reference electrode (Radiometer Analytical, France).

Total carbon, organic carbon, inorganic carbon

Total carbon, organic carbon and inorganic carbon amounts in each rock were determined using TOC-VCSH equipped with a solid sample analysis kit SSM-5000A (Shimadzu, Kyoto, Japan,). Before measurements, rocks were fine ground, homogenized and dried for several days at ambient temperature in a desiccator. The total carbon (TC) content was determined using a dry combustion method at 900 ^{0}C with V_2O_5 used as an additional oxidizing factor. Inorganic carbon (IC) was detected after acid (25% H_3PO_4) and high temperature (200 ^{0}C) treatment of rocks. Total organic carbon (TOC) content was calculated from the difference of TC and IC.

Assessing methanotrophic activity and maximum capacity of methane oxidation (Vmax) of coalbed rocks

Incubations, aiming to determine the methanotrophic activity of the investigated rocks, were commenced within 5 days of collecting the samples.

The methanotrophic activity of bacteria inhabiting coalbed rocks was determined at oxic conditions at a temperature of 30 ^{0}C. Triplicate samples of each rock (15 g) were placed in dark bottles (60 cm^3), filled with deionized water to obtain sample moisture adequate to 100% of total water capacity than closed with rubber septa, capped with an aluminum cap and sealed with paraffin. For experiments an initial concentration of ca. 10 % (v/v) CH_4 in was obtained by replacing an appropriate volume of air with high purity (99.99%) methane (Praxair, Poland) using a gastight syringe (5 ml, SGE, Australia). The headspace concentrations of gases (CH_4, CO_2, O_2) were determined using a gas chromatograph (3800 GC Varian, USA) equipped with flame ioninization (FID) and thermal conductivity (TCD) detectors. Gases were separated on Molecular Sieve 5A, 0.53 mm ID, 30 m length and Poraplot Q, 0.53 mm ID, 25 m length columns (Varian, USA) using helium as the carrier gas. The analyses were carried out under the following conditions: injector temperature 120 ^{0}C, oven temperature 40 ^{0}C, temperature of detectors: 120 ^{0}C and 200 ^{0}C for TCD and FID respectively. Oxygen levels during incubation were controlled and periodically readjusted to saturating conditions, depending on the current methane mixing ratio. Incubation times varied depending on the activity of the sample and included 8 to 10 measurement points. Conditions under which the rocks were incubated were previously confirmed as optimum for induction of methanotrophic activity by Stępniewska et Szmagara (2004) who found that further increase of substrate concentrations (above 10%) did not result in elevated consumption rates. Water content (100% total water capacity) in experimental treatments was chosen based on preliminary experiments.

The specific methanotrophic activity of particular rocks [μM CH_4*g $^{-1}$day $^{-1}$] was defined as the mean CH_4 consumption through the experiment, including the time required for activation. Maximum methanotrophic capacity of rocks (V_{max}) [μM CH_4*g $^{-1}$day $^{-1}$] was calculated from the slope of the regression line of the measured CH_4 molar amounts vs. time.

Statistical analysis

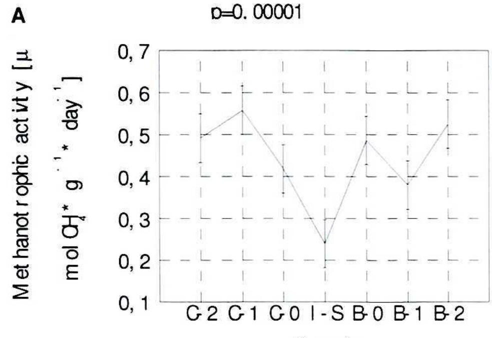
All data were statistically evaluated with Statistica 7 software (STATSOFT, USA). One way ANOVA was used to investigate significant ($p<0.05$) differences between particular rocks, their position in relation to coal seam as well as changes in pH and Eh in all experimental treatments before and after incubation. Homogeneity of variances was tested with Brown-Forsyth's test and post-hoc comparisons were made using the Tukey HSD test. Pearson correlation coefficients were calculated wherever possible.

RESULTS

Methanotrophic activity and maximum capacity (V_{max})

Methanotrophic activity, observed in all tested rock samples, ranged between 0.231 (rock I-S) and 0.619 (rock B-2) [μM CH_4 g $^{-1}$day $^{-1}$] and maximum capacity (V_{max}) was in the range of 0.337 to 1.578 [μM CH_4*g $^{-1}$day $^{-1}$] (Tab. II.). The difference between methanotrophic activity and capacity resulted from the lag phase that could be observed at the beginning of the incubation, however, there was a significant correlation between those parameters ($r=0.88$) implying that the time required for activation of methanotrophy was similar for all samples and the disparities in methane oxidation rates observed between particular rocks were due to other factors. Complete depletion of methane was observed in all samples after 36 to 70 days.

There was no correlation between the depth (below surface) and methanotrophic activity and the differences between the bottom and roof rocks were insignificant ($p=0.499$). However, there were significant differences between samples, with rock originating from the internal part of the seam showing the lowest rates of methane consumption (Fig. 1.). Methanotrophic activity as well as V_{max} were positively correlated with the distance to the seam ($r=0.775$ and $r=0.674$, respectively) and presented similar patterns of distribution both in the roof and the bottom.



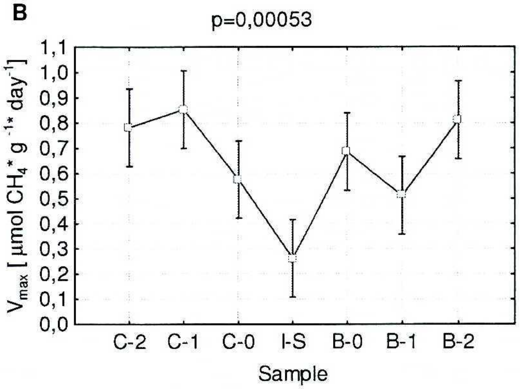


Fig.1. Differences in methanotrophic activities (A) and capacities (B) of particular rocks. Error bars represent 0.95 confidence intervals.

Tab. 2. Spatial distribution (distance to the coal body [m], depth below surface [m]) of methanotrophic activity and capacity (V_{max}) of particular coalbed rocks from the seam 385/2 of "Bogdanka" coal mine.

	Sample	Distance to the coal [m]	Depth [m]	Methanotrophic activity*	±SE	V_{max}*	±SE	pH_{ini}*	Eh_{ini}
Roof rocks	C-2	3,94 m	-755,3	0,500	0,00	1,22	0,03	8,62	424,4
	C-1	2 m	-757,2	0,528	0,01	1,21	0,01	8,38	356,5
	C-0	c.a. 0,2 m	-759,1	0,434	<0.01	0,82	0,09	7,60	361,9
Internal rocks	I-S	-	-761	0,287	0,00	0,47	0,02	7,60	361,7
Bottom rocks	B-0	c.a. 0,2 m	-763	0,457	0,01	0,81	0,10	8,22	353,9
	B-1	0,5 m	-763,9	0,406	0,01	0,86	0,12	8,08	373,0
	B-2	4,4 m	-767,8	0,524	0,04	1,27	0,16	8,12	371,1

*-mean value (n=3)

**-initial values measured directly in experimental treatments.

Total carbon, total organic carbon and inorganic carbon contents

Total carbon (TC) and total organic carbon amounts in investigated rocks were variable and ranged from 1.56% to 37.43%. In some samples, due to their extremely low inorganic carbon (IC) contents (<0.01% (detection limit)), TC was identical with TOC. The maximum measured IC was 0.85% (Fig. 2.). The total amount of carbon (TC) as well as the contents of particular forms (IC and TOC) in rocks changed with the distance to the seam (Fig.2.). TC and TOC contents were highest in the rock excavated from in within the coal body (I-S) and decreased with increasing distance to the coal, whereas the tendency in IC distribution was reverse. TC and TOC correlation with methanotrophic activity was negative (r= -0.85 and r=-0.86) whereas IC was positive (r=0.67).

pH and Eh conditions

At the beginning of incubation the pH varied between 7.6 and 8.68 and correlated positively with methane oxidation rates (r=0.69). Measured pH values were significantly (p<0.05) lower after incubation in comparison with values measured at the beginning of the experiment. Terminal pH values were not correlated with methane oxidation.

Redox conditions were oxic during the whole incubation as measured Eh never dropped below 320 mV (minimum observed 351.46 mV), which was suggested by Stepniewska (Stępniewska, 1988) as border value between oxygenated and reduced soil conditions in the transformation of iron.

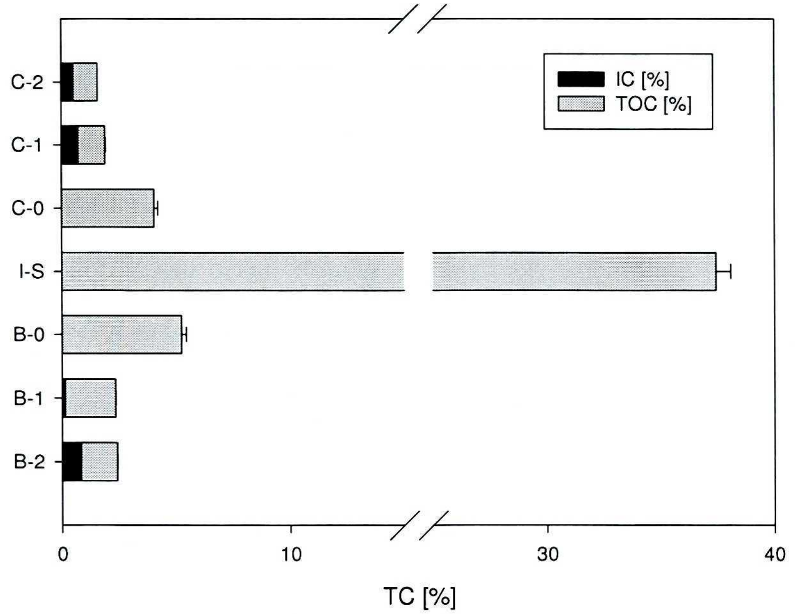


Fig.2. Occurrence of total carbon (TC) and particular forms of carbon: total organic carbon (TOC) and inorganic carbon (IC) in coalbed rocks. Error bars represent SD values.

DISCUSSION

In nature, methanotrophs are present wherever stable methane emission takes place. Several studies confirmed the presence of methane utilizing microbial consortia both in the surface (Hanson et al.,1996) and subsurface (Kotelnikova, 2001) environments, therefore, the occurrence of methane oxidizing bacteria accompanying coal seams should not come as a surprise. However, the fact that microbial communities inhabiting rocks from the "Bogdanka" coal mine oxidize methane with the use of molecular oxygen is intriguing as surveys suggest that the investigated rock layers were isolated from the atmosphere for a geologically long period of time (Rożkowski and Rudzińska-Zapaśnik, 1983, referred to in Kotarba, 2001). Therefore, the age, origin as well as metabolic pathways utilized by those microorganisms are uncertain and require further investigations.

A similar issue was recently discussed by Pokorný (2005), who characterized organisms inhabiting lignite excavated from Záhorie coal mine. Pokorný suggested two possible scenarios for the appearance of coal. The first one suggested that microbial life was buried with remnants of plants and managed to survive coalification and oxygen starvation in cryptobiosis (Clegg, 2001). The second scenario linked the presence of microbial life in coal with secondary colonization that might occur in recent times during temporary disintegration of impervious layers surrounding seams. In the case of rocks from "Bogdanka" coal mine the second scenario would be hard to realize as it has been proven that Carboniferous strata of Lublin Coal Basin has been isolated from meteoric supplies of water (potential carrier of microorganisms) and nutrient before the Pleistocene (Rożkowski and Rudzińska-Zapaśnik, 1983, referred in Kotarba, 2001). Additionally, geological surveys found that the coal-bearing strata of Lublin Coal Basin coal mine have a continuous character (the biggest fault found during mining works was of 2.6 m). This is mostly due to the plasticity of rocks in the Lublin Coal Basin that results in deformation of rocks, which prevents water and gases from moving through seams. Therefore the most credible (though somewhat controversial) explanation seems to be the suggestion that the microorganisms responsible for the observed methane oxidation are those which were present in the primary sediments that covered anaerobically decomposing, methane exuding layers of organic matter that later gave rise to coal or colonized the rocks at the very beginning of their formation. Methanotrophs are currently commonly found in the vicinity of oxic-anoxic interfaces (Brune et al., 2000). The survival of microorganisms in the deep subsurface may be attributed to the formation of resting stages. Such forms have already been reported as methanotrophs adaptation for survival in oxygen and nutrient depleted media (Hanson and Hanson, 1996).

Additional information, confirming the presence and activity of microbial life (including methanotrophs) at different stages of coal seam formation, may be derived from the isotopic composition of coal-bed gases, which is different for gases of geochemical and biological origin (Clayton, 1998). It was suggested by Kotarba (2001) that methane and carbon dioxide from the "Bogdanka" coal mine originate both from thermogenic and microbial processes. Production of those gases, and therefore microbial activity was suggested to take place Ma ago as their recent genesis was excluded due to nutrients depletion (Kotarba, 2001).

If we accept the possibility that the microorganisms responsible for the observed methane oxidation were entrapped in coal-accompanying rocks at the stage of their formation, the relation between TOC content and the observed methane oxidation rates could be attributed to the oxygen limitation (due to the decomposition of organic matter) at the early stages of the rocks formation. In the investigated area, there are some amounts of methane accumulated in the roof and bottom of the seam 385/2 (estimated to be 7000 and 32000 m^3 CH_4, respectively), but within the coal body they are close to zero (Mazurkiewicz, 2004). Therefore, there is another question to be answered - is this lack of substrate also one of the factors limiting methanotrophic activity within the seam and its closest surroundings.

The mechanism of TOC influence may also be physical and connected with unfavourable changes in the conditions of the micro-habitat. Priemé and co-workers (1996) found that methanotrophs responsible for methane oxidation in soils are tightly connected with the soil particles and lose activity very fast when dissociated. This finding indicates that the structure of the habitat may play a key role for these organisms. Coal itself as habitat for microorganisms has already been investigated by Pokorný (2005), who isolated from samples of lignite numerous species of bacteria and fungi diverse in ecology and phylogeny. None of them however, was methanotrophic.

The function and presence of inorganic carbon (IC) in the examined rocks may be perceived twofold. Firstly, IC may be regarded as an integral ingredient of the rock and then the correlation between methanotrophic activity and IC content would have to be understood as a positive (in contrast to TOC) impact of its presence on the micro-scale habitat mentioned above. Secondly, the IC presence in the most active samples may be suspected as being an effect of bacteria-mediated methane oxidation processes. Rocks containing carbonates formed from CO_2 that was released during microbial CH_4 oxidation, were reported by Sarkar (1996) and Ivanov (1993) although, to answer the question of whether the IC found in coalbed rocks of the "Bogdanka" coal mine is of microbial origin will need isotopic investigations..

Environmental approach

Methane oxidation rates, observed in the coalbed rocks of seam 385/2 were comparable to those reported previously by Stępniewska et al. (2004) for rocks collected from the dump area (0.53 [μM CH_4 $g^{-1}day^{-1}$]). Slag heaps of dump rocks were formerly considered to create a significant environmental hazard (Ledin, 1996). The proven ability of dump rocks from "Bogdanka" coal mine however, to utilize methane with the use of atmospheric oxygen changes the outlook on their impact on the environment and creates new possibilities for their disposal, for example as an effective, methane capturing, landfill cover.

The problem of methane is inseparable from mining due to its explosivity. Elevated CH_4 levels create considerable danger to miners and influence the composition of the atmosphere as substantial amount of coalbed methane is removed through ventilation systems directly to the atmosphere (Su, 2005). In the presence of uncertainties about the atmospheric methane budget (Keppler, 2006; Schiermeier, 1996), information about new, uninvestigated potential sinks of this greenhouse gas cannot be discarded without further research. Currently, due to the lack of data, possible relationships between methane levels in mining atmospheres and the presence of methane oxidizing bacteria in coalbeds cannot be confirmed and therefore the comparison of coalbed rocks from different mines and mining areas are needed.

CONCLUSIONS

- Rocks surrounding the seam 385/2 of the "Bogdanka" coal mine revealed methanotrophic activity in the bottom and roof as well as in a layer located within the coal body.
- Methane oxidation observed increased with the distance to the coal body both in the bottom and in the roof of the seam.
- Methanotrophic activity revealed a negative correlation with TOC ($r = -0.86$) and a positive one with IC ($r = 0.67$).
- Further investigations are necessary to evaluate the age, origin and the phylogeny of the microorganisms responsible for the observed methane oxidation as well as their influence on the methane levels in mining atmospheres.

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Received: September, 2007; accepted: June, 2008.