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Microbiological coal biogasification under laboratory conditions – biogas quantity and quality

The paper presents results of experiments consisting in biogasification of hard and brown coals. The process was carried out in closed containers using a microbiological consortium. The influence of various factors on the amount and composition of released gas was checked. Various degasification conditions were tested: hard and brown coal, three different fractions (from 1.4 to 5 mm, from 0.16 to 1.4 mm, and fraction below 0.16 mm), various temperatures (4°C, 20°C, and 40°C), the experiment duration (1, 2, 3, and 4 weeks). Nitrogen and carbon dioxide were the prevailing gas components during the experiments.

Key words: hard coal, brown coal, biogasification, microbiological consortium.

Mikrobiologiczne zgazowanie węgla w warunkach laboratoryjnych – ilość i jakość biogazu

W artykule przedstawiono wyniki eksperymentów polegających na biozgazowaniu węgli kamiennych i brunatnych. Proces ten był przeprowadzany w zamkniętych pojemnikach przy wykorzystaniu konsorcjum mikrobiologicznego. Sprawdzono wpływ różnych czynników na ilość i skład wydzielonego gazu. Przetestowane zostały różne warunki degazacyjne: węgiel kamienny i brunatny, trzy różne frakcje (od 1,4 do 5 mm, od 0,16 do 1,4 mm i frakcja poniżej 0,16 mm), różne temperatury (4°C, 20°C i 40°C), czas trwania eksperymentu (1 tydzień oraz 2, 3 i 4 tygodnie). W trakcie eksperymentów dominującymi składnikami gazu były azot i ditlenek węgla.

Słowa kluczowe: węgiel kamienny, węgiel brunatny, biozgazowanie, konsorcjum mikrobiologiczne.

Introduction

The undertaken study was aimed at the determination of optimum conditions to carry out biogasification of coal, both brown and hard. The influence of various factors on the amount and composition of released gas was checked. The gas amount was estimated based on the standard USBM (United States Bureau of Mines) degasification method developed by F.N. Kissel et al. in 1973. This is a volumetric method utilising a desorption canister and a graduated burette. Various degasification conditions were tested: hard and brown coal, three different fractions (from 1.4 to 5 mm, from 0.16 to 1.4 mm, and fraction below 0.16 mm), various temperatures (4°C, 20°C, and 40°C), the experiment duration (1, 2, 3, and 4 weeks). The analyses of chemical and isotopic composition of the gas combined with quantitative results of gas degasification allowed to determine the amount and quality of the produced gas.

Coal bed methane

Apart from hard coal, lignite is the most important energy raw material in Poland. Its role is also important in many industrialised countries of the world, such as Australia, China, Czechia, Greece, Germany, Russia, the United States, and Turkey. The resources of this raw material in Poland are significant and according to the updated data they amount to 29,814.7 million Mg, of which in documented deposits (resources proved in categories A + B + C1 + C2) – 13,851.2 million Mg, and

in preliminary explored deposits (estimate resources in category D) – 15,963.5 million Mg [18]. Despite substantial environmental damage accompanying the mining, the brown coal is still an attractive alternative to other energy sources due to the fact that energy generated from it is cheapest. This is one of the most important reasons making that both brown and hard coal are still the object of industrialised and developing countries interest. However, because of environmental reasons alternative methods for this valuable raw material are being sought. Biological gasification is one of such methods. The idea is similar to a classical idea of coal gasification, i.e. obtaining from lignite the gas, which is used as another energy raw material. The basic differences consist in the fact that this gas is methane, the process is to be carried out by microbes, and first of all it is to proceed in the deposit. The arguments in favour of carrying out research on the possibility of such technology application in practice are as follows:

1. Even up to 20% of global natural gas deposits are reservoirs containing methane originated as a result of methanogenic microbes activity [28]. These are usually young deposits, generally Tertiary or even Quaternary [21], created as a result of sudden tectonic movements closing the biomass, which was subject to further transformations in anaerobic conditions till the formation of methane, or as a result of processes occurring in deposits on the sea bottom. The biogenic origin is proved by the lack of C_2–C_4 hydrocarbons and by the isotopic composition.
2. Certain part of global oil resources contains oils degraded as a result of microbes activity, the process which resulted also in the origination of methane [15].
3. Methanogenic activity accompanies the processes of peat formation [3].
4. Methanogenic activity accompanies numerous gas deposits. This means that in this case we deal with the formation of methane in real time. This process proceeds also in waters accompanying the oil [17]. But the fact, that the formation of biological methane occurs also in waters accompanying coal deposits is most important [31, 32], and this means that the degradation of complex substances making the coal proceeds all the time.

In addition, the coal bed methane (CBM) is treated as a prospective source of natural gas, which can be acquired both from the operating and closed mines, and via boreholes at the application of hydraulic fracturing [30]. The methane existence in coal is related to the coal origination process. It is considered that in the coal of low coalification degree methane originates as a result of microbes activity, while in coals of higher coalification degree as a result of thermal processes of the organic matter [1, 13].

Methanogenic microorganisms (belonging to the Archaea domain and to the Euryarchaeota kingdom) responsible for the process of biogenic methane formation exist in various environments, such as bogs, sea deposits, animal alimentary systems, landfills, also extreme ones, e.g. oil deposits, hot springs, or hydrothermal chimneys [7]. Taxonomy distinguishes 3 groups of methonegens [2]. Methanogenesis is a form of energy acquisition by such microorganisms. The process of biogenic methane formation proceeds as follows:

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

So carbon dioxide or acetate can be the final electron acceptors. A part of microorganisms utilises one metabolic path, and another part the second path. Certain methanogens can use as electron acceptors also other C1 compounds, e.g. methanol or formaldehyde. Methonogens are considered to play an extremely important role in the environment (the last stage of organic matter decomposition processes); they generate during that approx. 400 million tonnes of methane a year [2, 12]. Converting this value into normal conditions we obtain the amount exceeding many times the annual demand for natural gas in Poland. As of today only a minute amount of originating biogenic methane is managed – mainly so–called biogas is used, which is formed as a by–product in sewage treatment plants or in landfills.

However, specific conditions are required to initiate the process of methanogenesis, i.e. appropriate humidity, appropriate C:N:P:S ratio, appropriate environment reaction and temperature, total lack of oxygen, very low redox potential (approx. 240 mV) and the existence of final acceptors. Most of those conditions can be met in brown coal deposits, while the degradation of complex chemical compounds – components of coal – is indispensable to obtain an appropriate amount of fine–molecular final acceptors. However, other microorganisms are required for that. A number of microorganisms was described, which degrade both hard and brown coal [6, 22, 24, 26], but only some of them can be applied in the deposit environment, namely those, which can carry out the degradation under anaerobic conditions. This substantially reduces the spectrum of available microorganisms, which are potentially fit for such application. Anaerobic fungi from the Neocallimastigales order seem to be promising here [9], living in the alimentary canal of herbivorous animals, which seem to be in symbiosis with methanogenic Archaea. The use of such fungi would be an alternative path to the solution suggested by the Arctech company, which uses anaerobic microorganisms obtained from termites belonging to *Zootermopsis* and *Nasutitermes* genera to degrade organic compounds. Experiments, checking the capability of methane formation from brown coal in laboratory conditions [5], have shown that the process proceeds, while its yield is relatively low if compared with methanogenesis processes utilising an input substrate other than coal.

Methodology

The microbiological consortium was obtained during the implementation of project "Studying a microbiological process of brown coal decomposition into methane, to determine possibilities of managing small non–commercial reserves" (application No UMO–2011/03/D/ST8/04467). The consortium composition was maintained on the level of 20% of Archaea and 80% of Bacteria. The dominating organisms among Archaea were species from the *Methanosarcina* genus (methanogenic microorganisms), while among bacteria species belonging to *Clostridium, Tindallia* and *Tepidibacter* (anaerobic bacteria). Anaerobic bacteria are responsible for the organic matter processing into acetate and CO_2 (also H_2), which are then substrates for methanogenesis.

Specimens preparation

Approximately 100 grams of hard and brown coal samples (after crushing and sieving into fractions – from 1.4 to 5 mm, from 0.16 to 1.4 mm and below 0.16 mm) were placed in degasification containers. A degasification container is a tight container equipped with two valves for perfusion (argon was used for perfusion, which minimised pollution with air), and with an additional GC septum for the gas collection.

Containers, in which the biogasification experiments were carried out, were sterilised by flushing many times with 70% ethanol. Coal specimens for testing were sterilised in an autoclave during 15 min. at 121°C.

An enriched substrate of the following composition was used for microorganisms culture: sodium acetate 2g/dm^3, beef extract 3g/dm^3, and bacteriologic peptone 5g/dm^3. Microorganisms were cultivated in 250 ml DURAN SCHOTT bottles with screwed on caps with a rubber plug fulfilling the function of a safety valve. Microorganisms were cultivated to the density of approx. $5 \cdot 10^8$ cells in 1 cm^3. The number of microorganisms was verified by direct counting under a microscope (Nikon Eclipse 50i) staining cells with a fluorescent dye DAPI (4',6–diamidino–2–phenylindole), excitation/emission 358/461 nm.

A coal specimen, substrate and microbiological consortium were placed in the container. After the time planned for specific version of the experiment the amount of gas was determined using a volumetric method and then the gas composition was analysed.

Measurement of gas amount

The volumetric determination of the gas amount from the container was made using a set consisting of a burette on a stand and a bottle with a bottom tubulure filled with brine. The burette was connected with the bottle via silicon hoses, and the degasification container was connected with the burette. Brine levels in the bottle and in the burette were made to equalise the zero of burette scale. After opening the degasification container valve the overpressure was pushing out the gas and its volume was read from the burette scale. Then the gas was collected and analysed by means of chromatography to determine the molecular and isotopic composition [4, 8, 19].

Results of the composition analysis had to be converted taking into account the gas volume (both that read from the burette, and of the free container space). Oxygen and related nitrogen and carbon dioxide (as the pollution with air) were deducted from the gas composition (assuming that all oxygen is contamination and that air composition is constant). After the oxygen and nitrogen deduction "excessive" amounts of compounds remained in the gas composition, i.e. gases released from the specimen. The released gas components were expressed in millilitres.

Analyses of chemical and isotopic composition

Chromatographic analyses of the molecular composition were carried out on two AGILENT 7890 A chromatographs, equipped with FID, TCD, and FPD detectors. A precise methodology and elements of validation of molecular composition determinations were presented in the paper "Elementy walidacji metody analitycznej (...)" [16]. Analyses of isotopic composition were carried out on a Delta V Advantage isotopic mass spectrometer combined with a Thermo Scientific Trace GC Ultra chromatograph.

Results and discussion

The first variant of the experiment was performed for two weeks lasting biogasification for different fractions (from 1.4 to 5 mm, from 0.16 to 1.4 mm, and below 0.16 mm) and temperatures (4°C, 20°C, and 40°C). Total amounts of gas released from hard coal and amounts of nitrogen, carbon dioxide, and total hydrocarbons are specified in Tables 1 and 2 and presented in Figures 1 and 3. Hydrocarbon composition was almost the same in each sample – methane was main component at level of approximately 99,9%. The gas amounts ranged from 21.2 to 46.7 ml. The maximum value was the highest amount of gas released in all experiment variants and it is a clear outlier as against the other values. The influence of fraction on gas amounts at a given temperature seems to be small. Pie charts (Fig. 3) were prepared for fraction from 0.16 to 1.4 mm due to a small variability of gas composition versus fraction. Nitrogen is the prevailing component, at very small amounts of hydrocarbons.

The presence of N_2 can be attributed to the first stages of coal degradation which require the oxidation of high molecular weight compounds. In anaerobic conditions the oxidation is coupled to reduction of nitrates. The influence of fraction and temperature on the gas composition is rather minute.

Table 1. Total amount of released gas – hard coal [ml]

Fraction	Temperature		
	4°C	20°C	40°C
from 1.4 to 5 mm	31.0	46.7	25.9
from 0.16 to 1.4 mm	24.4	27.6	31.3
below 0.16 mm	28.5	29.6	21.2

Table 2. Amount of released nitrogen, carbon dioxide, and total hydrocarbons – hard coal [ml]

Fraction	Temperature		
	4°C	20°C	40°C
from 1.4 to 5 mm	N_2 – 29.8 CO_2 – 0.9 ∑C – 0.3	N_2 – 43.5 CO_2 – 2.7 ∑C – 0.4	N_2 – 23.4 CO_2 – 2.4 ∑C – 0.1
from 0.16 to 1.4 mm	N_2 – 23.3 CO_2 – 0.9 ∑C – 0.2	N_2 – 24.8 CO_2 – 2.4 ∑C – 0.4	N_2 – 28.8 CO_2 – 2.4 ∑C – 0.1
below 0.16 mm	N_2 – 27.4 CO_2 – 0.9 ∑C – 0.2	N_2 – 27.5 CO_2 – 1.8 ∑C – 0.3	N_2 – 19.6 CO_2 – 1.5 ∑C – 0.0

Total amounts of gas released from brown coal and amounts of nitrogen, carbon dioxide, and total hydrocarbons are specified in Tables 3 and 4 and presented in Graphs 2 and 4. The gas amounts ranged from 11.0 to 36.6 ml. Pie charts (Fig. 4) were prepared for fraction from 0.16 to 1.4 mm to compare with charts for hard coal. Carbon dioxide prevails in the gas composition, at small amounts of hydrocarbons. Fractions < 0.16 and 1.4 to 5 mm at 4°C deviate from that, with dominating nitrogen. In the case of brown coal a low temperature resulted in smaller amounts of gas and in a different composition.

The isotopic composition of carbon in methane and in carbon dioxide for gases from hard coal biogasification is presented in Table 5, and from brown coal in Table 6. The coal fraction does not affect the isotopic composition (apart from individual exceptions: for hard coal methane from fraction 0.16–1.4 mm and for brown coal CO_2 from fraction 0.16–1.4 mm). In addition, clear differences are noticeable in the isotopic composition between gases from hard and brown coal at 20°C, while for 40°C such differences no longer exist. Temperature of 20°C seems to be the most efficient for microbiological consortium, when 4°C and 40°C are less appropriate. The isotopic carbon composition in methane confirms its microbiological origin.

Table 3. Total amount of released gas – brown coal [ml]

Fraction	Temperature		
	4°C	20°C	40°C
from 1.4 to 5 mm	21.8	25.4	24.3
from 0.16 to 1.4 mm	16.2	30.6	36.6
below 0.16 mm	11.0	35.0	18.6

Table 4. Amount of released nitrogen, carbon dioxide, and total hydrocarbons – brown coal [ml]

Fraction	Temperature		
	4°C	20°C	40°C
from 1.4 to 5 mm	N_2 – 14.2 CO_2 – 7.3 ∑C – 0.3	N_2 – 4.2 CO_2 – 19.9 ∑C – 0.3	N_2 – 6.0 CO_2 – 18.1 ∑C – 0.1
from 0.16 to 1.4 mm	N_2 – 6.7 CO_2 – 8.6 ∑C – 0.9	N_2 – 6.4 CO_2 – 23.2 ∑C – 0.3	N_2 – 10.5 CO_2 – 25.9 ∑C – 0.1
below 0.16 mm	N_2 – 6.8 CO_2 – 3.9 ∑C – 0.3	N_2 – 15.4 CO_2 – 17.9 ∑C – 0.2	N_2 – 6.5 CO_2 – 12.0 ∑C – 0.0

Table 5. Isotopic composition of carbon in methane and in carbon dioxide – hard coal [‰ PDB]

Fraction	Temperature	
	20°C	40°C
from 1.4 to 5 mm	$\delta^{13}C–C_1 = –55.0$ $\delta^{13}C–CO_2 = –31.8$	$\delta^{13}C–C_1 = –79.7$ $\delta^{13}C–CO_2 = –37.9$
from 0.16 to 1.4 mm	$\delta^{13}C–C_1 = –54.6$ $\delta^{13}C–CO_2 = –33.4$	$\delta^{13}C–C_1 = –72.7$ $\delta^{13}C–CO_2 = –37.1$
below 0.16 mm	$\delta^{13}C–C_1 = –55.8$ $\delta^{13}C–CO_2 = –29.1$	$\delta^{13}C–C_1 = –82.4$ $\delta^{13}C–CO_2 = –36.2$

Table 6. Isotopic composition of carbon in methane and in carbon dioxide – brown coal [‰ PDB]

Fraction	Temperature	
	20°C	40°C
from 1.4 to 5 mm	$\delta^{13}C–C_1 = –74.0$ $\delta^{13}C–CO_2 = –10.5$	$\delta^{13}C–C_1 = –82.7$ $\delta^{13}C–CO_2 = –20.1$
from 0.16 to 1.4 mm	$\delta^{13}C–C_1 = –74.5$ $\delta^{13}C–CO_2 = –10.8$	$\delta^{13}C–C_1 = –82.2$ $\delta^{13}C–CO_2 = –30.1$
below 0.16 mm	$\delta^{13}C–C_1 = –73.9$ $\delta^{13}C–CO_2 = –12.7$	$\delta^{13}C–C_1 = –82.7$ $\delta^{13}C–CO_2 = –20.7$

Comparing amounts of gas and its composition from hard and brown coal biogasification it is possible to notice different metabolic paths translating into domination of nitrogen or carbon dioxide in the released gas composition. The influence of coal type on the total amount of gas is not clear. This could

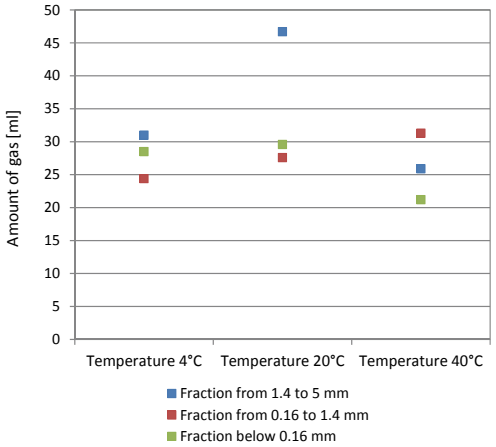

Fig. 1. Total amounts of released gas broken down into fractions and temperatures – hard coal [ml]

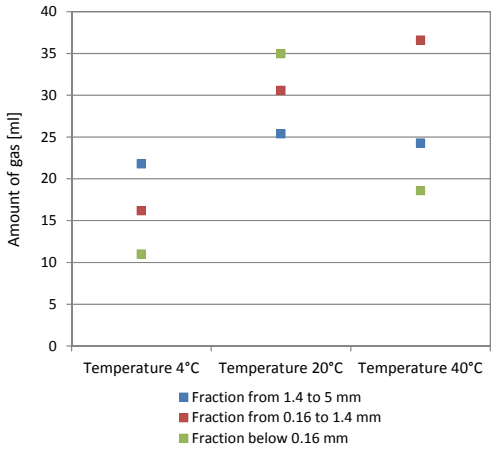

Fig. 2. Total amounts of released gas broken down into fractions and temperatures – brown coal [ml]

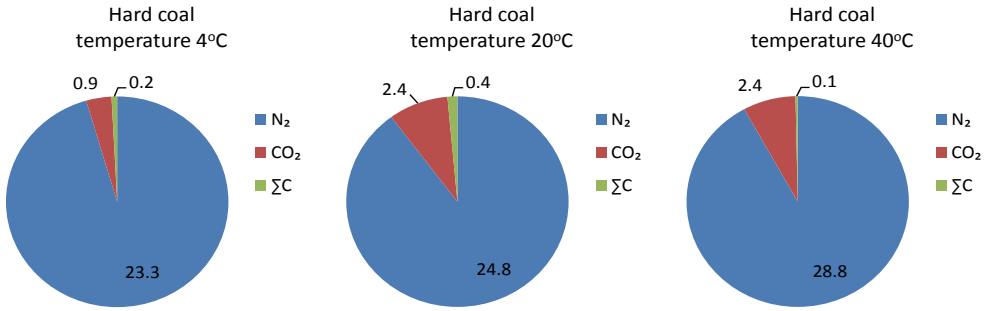

Fig. 3. Amounts of released nitrogen, carbon dioxide, and total hydrocarbons – hard coal, fraction from 0.16 to 1.4 mm [ml]

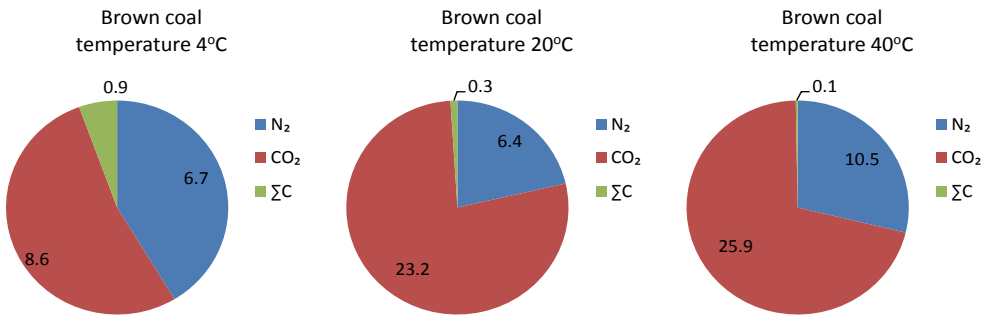

Fig. 4. Amounts of released nitrogen, carbon dioxide, and total hydrocarbons – brown coal, fraction from 0.16 to 1.4 mm [ml]

be related to a short – two week – duration of experiments. Many papers suggest a few months and even a few years as the duration of such studies [10, 11, 14, 20, 23, 25, 27, 29].

The second variant of the experiment (extended duration – 1, 2, 3, and 4 weeks, only one fraction – from 0.16 to 1.4 mm) allowed to collect cumulative amounts of gas (Table 7) and cumulative amounts of released nitrogen, carbon dioxide, and total hydrocarbons (Table 8). Results of isotopic composition analyses of carbon in methane and in carbon dioxide are collected in Table 9. Results are presented in the form of graphs (Figures 5 to 10). Total amount of gas released from hard coal

Table 7. Total amount of released gas [ml]

	1 week	2 weeks	3 weeks	4 weeks
Hard coal	18.8	16.4	33.6	47.7
Brown coal	28.3	31.7	82.3	65.1

Table 8. Amount of released nitrogen, carbon dioxide, and total hydrocarbons [ml]

	1 week	2 weeks	3 weeks	4 weeks
Hard coal	N_2 – 12.9 CO_2 – 5.6 ∑C – 0.3	N_2 – 11.4 CO_2 – 4.8 ∑C – 0.2	N_2 – 25.4 CO_2 – 5.9 ∑C – 2.3	N_2 – 38.2 CO_2 – 5.8 ∑C – 3.7
Brown coal	N_2 – 3.7 CO_2 – 21.7 ∑C – 0.8	N_2 – 7.5 CO_2 – 20.5 ∑C – 1.1	N_2 – 33.9 CO_2 – 43.7 ∑C – 0.9	N_2 – 17.6 CO_2 – 41.6 ∑C – 0.5

Table 9. Isotopic composition of carbon in methane and in carbon dioxide [‰ PDB]

	1 week	2 weeks	3 weeks	4 weeks
Hard coal	$\delta^{13}C–C_1 = –64.5$ $\delta^{13}C–CO_2 = –11.8$	$\delta^{13}C–C_1 = –67.7$ $\delta^{13}C–CO_2 = –14.3$	$\delta^{13}C–C_1 = –63.5$ $\delta^{13}C–CO_2 = –20.2$	$\delta^{13}C–C_1 = –62.7$ $\delta^{13}C–CO_2 = –18.4$
Brown coal	$\delta^{13}C–C_1 = –55.0$ $\delta^{13}C–CO_2 = –10.6$	$\delta^{13}C–C_1 = –55.4$ $\delta^{13}C–CO_2 = –12.2$	$\delta^{13}C–C_1 = –68.5$ $\delta^{13}C–CO_2 = –17.4$	$\delta^{13}C–C_1 = –68.2$ $\delta^{13}C–CO_2 = –14.0$

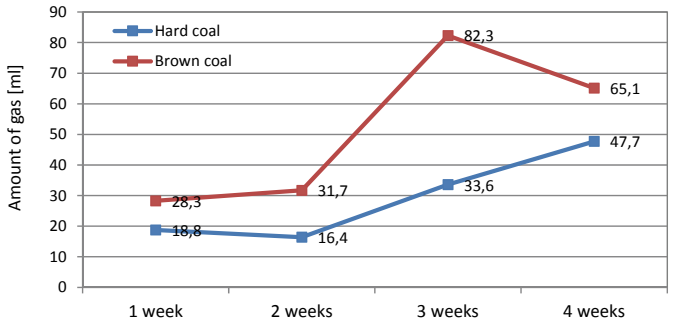

Fig. 5. Total amounts of released gas in consecutive weeks [ml]

increases in consecutive weeks (the value in the second week is slightly lower than in the first, but the difference is that small that it is rather related to the measurement error). In the case of brown coal the gas amount increases till the third week, achieving a high value, and in the fourth week it goes down. A trend exists in the gas composition in all weeks – for hard coal nitrogen is the prevailing gas component, and carbon dioxide for brown coal (albeit in week three and four to a smaller degree). More generated methane occurs for brown coal specimens in the first two weeks of the experiment, while in the next two – for hard coal.

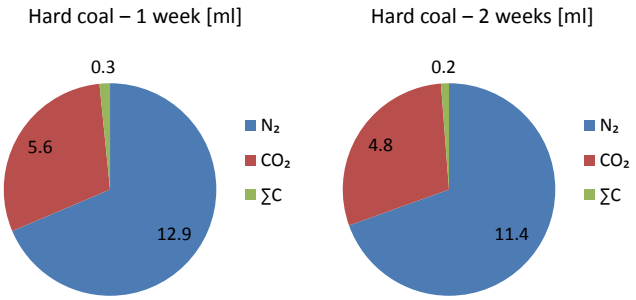

Fig. 6. Amounts of released nitrogen, carbon dioxide, and total hydrocarbons – hard coal [ml]

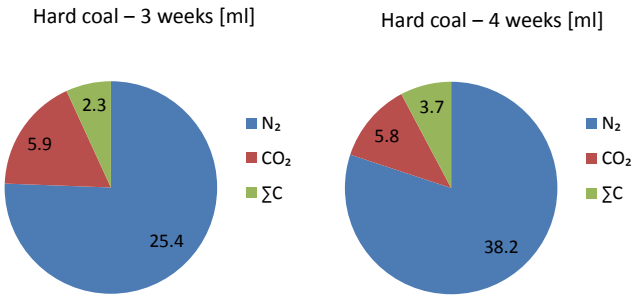

Fig. 7. Amounts of released nitrogen, carbon dioxide, and total hydrocarbons – hard coal [ml]

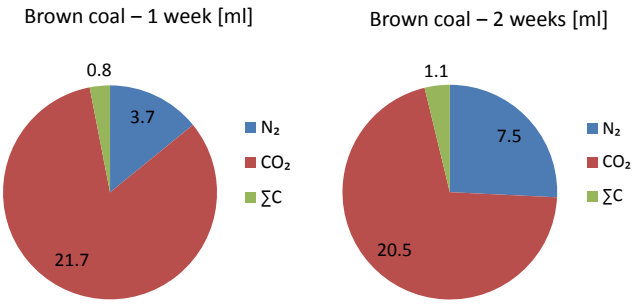

Fig. 8. Amounts of released nitrogen, carbon dioxide, and total hydrocarbons – brown coal [ml]

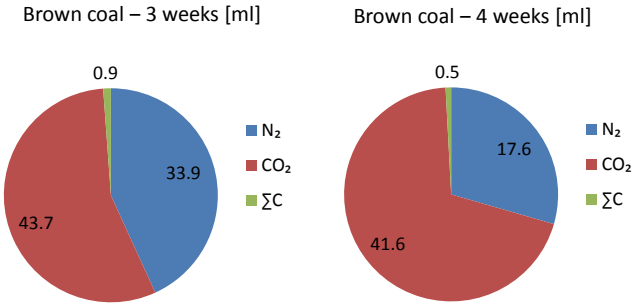

Fig. 9. Amounts of released nitrogen, carbon dioxide, and total hydrocarbons – brown coal [ml]

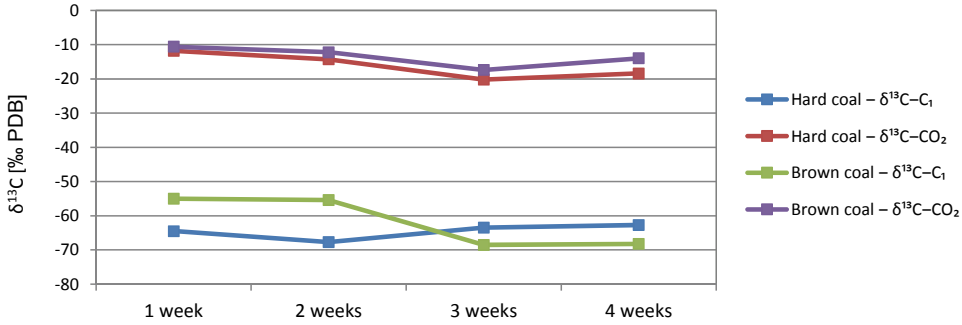

Fig. 10. Isotopic composition of carbon in methane and in carbon dioxide [‰ PDB]

The isotopic composition of carbon in methane and in carbon dioxide changes in consecutive weeks. Values of $\delta^{13}C–CO_2$ are similar for both coal types, which diverges from results of two–week experiments. Values of $\delta^{13}C–C_1$, initially differing, in the next weeks adopt close values. The isotopic composition confirms the biogenic methane origin. Additionally certain experiment results, deviating from the rest, can result from a specific nature of the microbiological consortium, which can be sensitive to changes of conditions due to a complex composition (many species).

Conclusions

Using a microbiological consortium under laboratory conditions the generation of biogenic methane from hard and brown coal was successful.

Nitrogen and carbon dioxide were the prevailing gas components during the experiments, but the duration of consortium action on coals was relatively short (comparing with literature examples of many months, and even years).

Certain experiment results, deviating from the rest, can result from a specific nature of the microbiological consortium. The consortium can be sensitive to changes of conditions due to a complex composition (many species). In addition, the determination of NGS composition has shown that approx. 30% of observed microorganisms were not identified to the level of species. Individual experiment variants allowed to notice that:

- For hard coal:
  - There is a small influence of the fraction on the gas amounts
  - Nitrogen is the prevailing component of the released gas
  - There is a small influence of the fraction on the gas composition
  - Total amount of gas released increases in consecutive weeks
- For brown coal
  - Carbon dioxide is the prevailing component of the released gas (fractions < 0.16 and 1.4 to 5 mm at 4°C are a deviation from that)
  - A low temperature (4°C) resulted in smaller amounts of gas and a different composition
  - Total gas amount increases till the third week, achieving a high value, and in the fourth week it goes down

The coal fraction (brown and hard) does not affect the isotopic composition, and the increased temperature does. The differences in the isotopic composition disappear with extended experiment duration.

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