

# Microbial metabolic activity of drilling waste

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**Keywords:** respiration, oxygen consumption, drilling fluid, drilling waste, spent drilling mud.

**Abstract:** Operations conducted by petroleum industry generate an entire range of drilling waste. The chemical composition of drilling waste and its toxicity depend primarily on the geological and technological conditions of drilling, the type of drilled rock deposits and on the type and composition of the drilling mud used. In the course of drilling operations, drilling fluids are in constant contact with bacteria, fungi and other organisms infecting the mud. Pioneer species, capable of surviving and using the resources of this specific environment, are selected. For this reason, the effectiveness of microbiota survival on different types of spent drilling muds and in different dilutions with brown soil was measured. Spent drilling muds samples came from drilling operations in various regions of Poland, e.g. Subcarpathia, the Polish Lowland and Pomerania regions. Oxygen consumption after 96 h was around 20  $\mu\text{g}\cdot\text{g}^{-1}$  dry mass in soil or soil/drilling water-based mud mixture. Soil mixes contained 10 wt% synthetic base, mud had a higher oxygen consumption – 38  $\mu\text{g}\cdot\text{g}^{-1}$  dry mass. Oxygen consumption decreases sharply as the content of the spent synthetic base mud fraction increases. A higher concentration of spent SBM (35 wt%) reduced the aerobic metabolism by slightly more than 50%. A high concentration of reduced carbon decreased the respiratory quotient (RQ) value to 0.7. All the researched drilling waste shows microbiological activity. At the full concentration of drilling fluids and non-dilution options, the chemical composition (salinity, inhibitors, etc.) strongly inhibits microbiota development and consequently, respiration.

## Introduction

Operations conducted by the petroleum industry generate an entire range of drilling waste materials. The chemical composition of drilling wastes and their toxicity depend primarily on the geological and technological conditions of drilling, the type of drilled rock and on the type and composition of the applied drilling mud (Babaei et al. 2019).

The chemical compositions of drilling mud were aimed at obtaining optimum parameters, i.e. acceptance of cuttings entrained in the mud flow and carried to the surface, good lubricating properties and many others. In compliance with their function, drilling muds are polymeric/mineral microcomposites of varying chemical, mineral and phase composition. They contain different organic compounds, and also a colloidal solid phase dispersed in the aqueous (water-based mud, WBM) or non-aqueous drilling fluids (NADF) environment (Jamrozik et al. 2019). The organic matter in drilling mud should be protected against bacterial decomposition. This is done by antimicrobial substances. The spent and contaminated mud is problematic when talking about environmentally-friendly utilization.

Most investigations concerning the toxicity of drilling mud usually focus on dilutions or concentrations at level  $\text{LC}_{50}$  when drilling fluids or spent mud are environmentally acceptable. Analogous estimations of the toxicity of drilling mud or its components on selected species are frequently made. Different

measures are used for estimating the harmfulness effect – enzymatic level, metabolic level of species or populations (Edwards et al. 2011), cellular level, or entire organisms.

Most of these investigations concern oil-based drilling fluids (OBM) or synthetic-based mud (SBM) used in marine drillings or sometimes marine oil spills like *Deepwater Horizon* in the Gulf of Mexico (Edwards et al. 2011, Imarhiagbe and Atuanya, 2013). These SBMs are composed of linear alpha olefins, internal olefins, esters, or paraffins. Numerous bacteria are capable of aerobic degradation of polycyclic aromatic hydrocarbons (PAH) – *Pseudomonas* (Tagger et al. 1990), *Flavobacterium* (Okpokwasili et al. 1984), *Moraxella* (Tagger et al. 1990), *Marinobacter* (Gauthier et al. 1992), and *Vibrio* (Roberts and Nguyen, 2006). Other drilling fluids used in land drilling have a water-base and a lot of organic compounds like acrylic polymer compounds, modified lignosulphonates, modified carboxymethyl cellulose derivatives or organic and inorganic polymers. Almost all of these compounds are degradable. Acrylamide creates anaerobic conditions, resulting in the release of  $\text{NH}_4^+$  (Abdelmagid, 1982). Acrylamide degrading can be carried out by *Enterobacter aerogenes* (Buranasilp and Charoenpanich, 2011). Lignosulphonate is slowly degraded by *Streptomyces viridosporus* (Hernandez-Pérez et al. 1998). Side-chain sulfonation reduces the availability of simple aromatics to bacteria, carbon-to-carbon bonding was resistant to all bacteria tested by Watkins in 1970.

Some organic polymers (starch, carboxymethyl cellulose) are easily decomposed by bacteria or fungi (Behera et al. 2014).

In the course of drilling operations, drilling fluids and then spent drilling mud are in constant contact with bacteria, fungi and other organisms infecting this mud. This biological contamination is accidental. Only strong microorganisms which can adapt to the existing conditions will survive in a given drilling fluid. In this sense, the spent mud environment predetermines the organisms. Pioneer species, capable of surviving and using the resources of this specific environment, are selected. The colonization of soil-spent drilling mud mixes containing semi-natural and natural polymers e.g. starch, partially hydrolyzed acrylamide, guar gum, and cellulose, has been rarely analyzed. For this reason, we measured the effectiveness of microbiota survival on different types of spent drilling mud and in different dilutions.

## Experimental analysis

### Materials

The following spent drilling mud types were analyzed:

1. WBM: bentonite (1), chloride-polymeric (2), polymeric-triple inhibited (3), potassium-polymeric (4), polymeric-chloride-inhibited (5), salt-barite (6), and
2. SBM (7).

*Bentonite mud – K-1/B (1)* is an aqueous suspension containing minimum 5wt.% bentonite API. Prior to adding bentonite to water, it was softened with 0.15wt.% sodium carbonate, and 0.3wt.% low-viscosity carboxymethyl cellulose (CMC LV) was also added.

*Polymeric-chloride mud – K-1/P-Cl (2)* was applied in a depth interval 300 – 3,280 m. It was based on 30wt.% bentonite mud from the previous interval. The filtration of the mud was lowered with 1.0wt.% low-viscosity carboxymethyl cellulose (CMC LV) and 0.3wt.% low viscosity polyanionic cellulose (PAC LV). The rheological parameters were regulated with 0.3wt.% biopolymer Xhantan Gum (XCD Polymer) and 0.5wt.% high viscosity polyanionic cellulose (PAC R). The hydration of drilled clayey rocks was reduced with potassium chloride (~7.0wt.%) and partly hydrolyzed polyacrylamide PHPA (max 0.5wt.%). 0.1wt.% of triazine derivative was used as a biocide.

*Triple inhibited polymeric mud – W-2 (3)* – clayey rock hydration was inhibited by KCl (4.5wt.%) and polyglycol (2.0wt.%). Moreover, the mud consisted of the following polymers: PHPA (0.3wt.%), XCD Polymer (0.2wt.%), PAC (0.6wt.%), CMC LV (1.0wt.%), carboxymethyl starch

(1.0wt.%), biocide (0.1wt.%), sodium hydroxide (0.45wt.%), and biocide (~0.1wt.%).

*Potassium-polymeric mud – KRAM-1/K (4)* consisted of KCl, NaCl, PHPA (0.3wt.%), liquid polyamine (3.0wt.%), low-molecular-weight acrylamide copolymer (0.65wt.%), polyglycol (max. 3.0wt.%), and biocide (~0.1wt.%).

*Inhibited polymeric-chloride mud – W-1/Cl-P (5)*, was based on potassium-polymeric mud with KCL, and PHPA (0.55 wt.%) as inhibitors. Filtration was regulated with PAC (0.6wt.%), CMC (1.2wt.%), XCD Polymer (0.2wt.%) and starch agents. Mud also consisted of sodium hydroxide, caustic soda, sodium carbonate and acidic sodium carbonate and barium (as a weighing agent) and 0.1wt.% of triazine derivative was used as a biocide.

*Saline-barite mud – L-2K (6)* based on NaCl, consisted of barite, high temperature-resistant synthetic polymers (0.5wt.%), XCD Polymer (max. 0.6wt.%), modified starch (1.2wt.%) and salts in the form of sodium carbonate and acidic sodium carbonate, caustic soda and calcium, biocide (~0.1wt.%).

*SBM – PO (7)* – inverse emulsion ('water in oil'), based on paraffin oil containing hydrocarbons C<sub>13</sub>–C<sub>17</sub> (57wt.%) Mud also contained organophilic clays, gilsonite, calcium hydroxide, calcium chloride, emulsifiers, cellulose (1.0wt.%), barite (30–60wt.%) and water.

The spent drilling mud samples came from drilling operations in various regions of Poland, e.g. Subcarpathia, the Polish Lowland and Pomerania regions. They were collected from various wells and at various depths (90 m to 5040 m). The general characteristics of spent drilling mud are given in Table 1.

The content of dry mass varied from 37.38 to 83.2%. The reaction (pH) ranged from 8.1 to 8.7, excluding sample W-2, for which the reaction was 9.90. The salinity of the samples characterized by electrolytic conductivity was high, 110 to 240 mS/cm, excluding sample K-1/B with 6.43 mS/cm.

SBM, sample P-O, was low in Cl<sup>-</sup> ions in the water extract – 1.443 g/dm<sup>3</sup>, and pH equaled 8.86. A majority of chlorides in the organic phase suspension were not extracted by water. Only after demulsification the Cl<sup>-</sup> concentration in eluate grew to 28,500–55,000 mg/dm<sup>3</sup> (Jamrozik et al. 2017).

The microbiological activity of the spent muds was tested on samples with a brown soil admixture. The parameters of brown soil used in the dilution were as follows: phosphorus (P) 0.207 mg·g<sup>-1</sup>, total nitrogen (Kjeldahl methods) 1.60 g·kg<sup>-1</sup>, total carbon 13.6 g·kg<sup>-1</sup> (0.11 %), and, the C/N ratio was 8.5. Brown soil used as a reference sample was not sterilized but used in the air-dry state, then sprinkled with water as other samples.

Table 1. Spent drilling mud and its basic parameters

Sample code	Type and origin of drilling mud	Specific gravity [g/cm <sup>3</sup> ]	Content of dry mass [%]	Electrolytic conductivity of filtrates [mS/cm]	pH of filtrates	Concentration of Cl <sup>-</sup> , [g/dm <sup>3</sup> ]
K-1/B	Bentonite	1.28	74.44	6.43	8.31	0.8
K-1/P-Cl	Chloride-polymer	1.23	69.74	112	8.32	26
W-2	Polymer inhibited	1.26	68.25	90.2	9.90	40
KRA-1/K	Potassium-polymer	1.51	55.86	204	8.44	9.1
W-1/Cl-P	Chloride-polymer inhibited	1.28	68.87	171	8.10	90
L-2K	Saline-barite	2.20	37.38	240	8.70	202
P-O	SBM	2.34	53.00	5.14	8.86	1.4

## Methods

For identifying mineral components in the spent drilling mud samples and checking for the presence of organic admixtures, analyses were conducted with use of the Fourier absorption spectroscopy in infrared (FTIR) with a spectrometer produced by the Bio-Rad Analytical Instrument, model FTS 165. The device operated in the following conditions:

- measurement range 4,000 do 400  $\text{cm}^{-1}$ ,
- 4  $\text{cm}^{-1}$ , spectral resolution
- transmission – pills with KBr.

Mineral phases were identified by ascribing particular absorption bands to minerals, based on the reference spectra listed in the H. Moehke catalog.

The microbiological activity was measured using the Columbus Instruments Micro-Oxymax flow respirometer, at room temperature.

The samples with a dry weight of 7 g were placed into a 50 ml glass chamber, sprinkled with distilled water and exposed in a thermostated water bath at 20°C for 96 h. The sampling interval was 1 hour. The duration of the measurements was limited to 96 hours as the rate of respiration slowly dropped with the depletion of the organic substrate.

All samples were naturally contaminated with microbiota. The dilutions contained brown soil dried and were rested at room temperature. The following proportions were used 1: 2<sup>n</sup>, i.e. 1:1, 1:16, 1:32, 1:64, 1:128. Four concentrations of brown soil (10 wt.%, 20 wt.%, 30 wt.% and 35 wt.%) were tested for only in sample No. P-O.

After mixing with the drilling mud sample and hydration, the natural soil microbiota created the microbial inoculum and this was irrespective of the natural contamination of drilling fluids during the transport, disposal and in contact with microorganisms. All results are related to 1 g dry mass of drilling fluids or mixtures with soil.

The respiratory quotient (RQ) was calculated from the ratio:

$$\text{RQ} = \text{CO}_2 \text{ produced} / \text{O}_2 \text{ consumed} \quad (1)$$

where: the  $\text{CO}_2$  and  $\text{O}_2$  were to be given in the same units.

The *Penicillium sp.* was determined based on conidiophore branching patterns observed under microscope in *Penicillium* according to Visagie et al. (Visagie et al. 2014).

## Results

### FTIR absorption

The FTIR absorption and analysis of the obtained spectrograms showed that the following components dominated in most of the analyzed mud samples: clayey minerals of layered structure – smectite (montmorillonite, illite or mixed package smectite/illite type), and also chlorite, kaolinite (the strongest absorption bands between 420–525  $\text{cm}^{-1}$  and 900–1,200  $\text{cm}^{-1}$ ), quartz (visible bands in 780, 798, 1,085 and 1,099  $\text{cm}^{-1}$ ). Additional absorption maxima result from the presence of carbonate mineral (calcite) at 875  $\text{cm}^{-1}$  and about 1,430  $\text{cm}^{-1}$ . In the case of samples No. 4 and 6, barite was a major mineral component with the absorption bands 609, 637, 1,085 and 1,175  $\text{cm}^{-1}$ , while quartz (780 and 798  $\text{cm}^{-1}$ ) and calcite (712 and 875  $\text{cm}^{-1}$ ) were minor minerals.

The FTIR spectra also corroborate a high content of a polymeric substance, which has been inferred from many bands, e.g. by the presence of wide and intensive bands in the range of 3,000–3,700  $\text{cm}^{-1}$  with a maximum at 3,418–3,419  $\text{cm}^{-1}$  attributable to the valance  $\text{NH}_2$  vibrations within the amide groups, valance  $\nu$  vibrations of the carboxyl groups and  $\nu$  vibrations of the OH molecules of  $\text{H}_2\text{O}$ . The valance vibrations of the  $\text{CH}_2$  and  $\text{CH}_3$  groups were responsible for the multiple band in the 2,800–3,000  $\text{cm}^{-1}$  range. In a lower range of wave numbers there was, among others, a weak maximum of about 1,741  $\text{cm}^{-1}$  of the valance  $\text{C}=\text{O}$  vibrations of the carboxyl groups, a medium intensity maximum about 1,607–1,620  $\text{cm}^{-1}$  of asymmetric stretching vibrations of the carboxylate  $\text{COO}^-$  anions and, probably, deformation vibrations of  $\text{NH}_2$  within the amide groups. Finally, it may be concluded that the polymeric compounds in this spent drilling mud represent a mixture of PHPA and, also of a biopolymer of the *XCD Polymer* type. An exemplary FTIR spectrogram of a WBM sample was presented in Fig. 1. and a SBM sample was presented in Fig. 2.

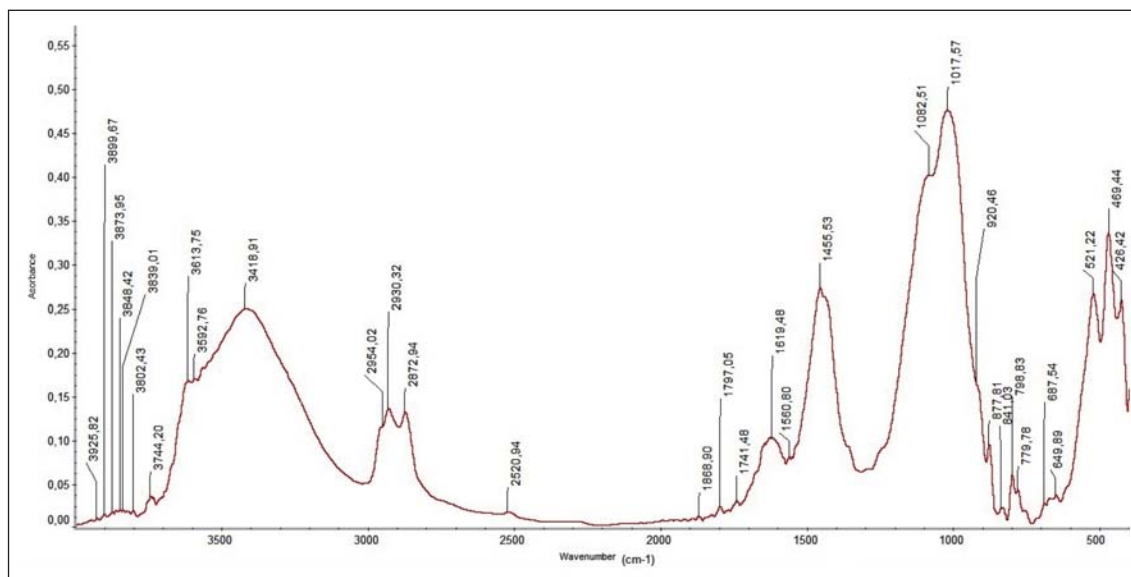


Fig. 1. FTIR infrared spectrum of the sample P-1/K-P (3)

The spectrogram of the SBM sample presented in Fig. 2 indicates that barite ( $\text{BaSO}_4$ ) is the dominating phase. This is caused by the presence of a number of diagnostic bands, e.g. the peak at  $609\text{ cm}^{-1}$  (from deformation vibrations  $\nu_4$  of  $\text{SO}_4^{2-}$ ) and the strongest maximum  $1,086\text{ cm}^{-1}$  (from asymmetrical stretching vibrations  $\nu_3$ ). The subordinate components include: calcite (diagnostic maxima from vibrations  $\nu_3$  of  $\text{CO}_3^{2-}$  at  $1,433\text{ cm}^{-1}$  and  $\nu_2$  at  $877\text{ cm}^{-1}$ ), quartz (diagnostic maxima from symmetrical stretching vibrations at  $\sim 800\text{ cm}^{-1}$ , and also asymmetrical stretching vibrations Si-O-Si at  $950\text{--}1,200\text{ cm}^{-1}$ , partly coinciding with vibrations  $\nu_3$  of  $\text{SO}_4^{2-}$  in the structure of barite). The presence of clayey minerals of smectite/illite type was also documented (diagnostic bands: bending of the spectral line  $\sim 1,040\text{ cm}^{-1}$  from stretching vibrations Si-O, and maxima  $467$  and  $519\text{ cm}^{-1}$  from bending vibrations, partly coinciding with quartz bands. Moreover, small maxima from valence vibrations O-H of hydroxyl groups at  $3,620\text{ cm}^{-1}$  were also observed.

An intense, broad absorption band from  $3,000$  to  $3,650\text{ cm}^{-1}$ , with a maximum at  $3,422\text{ cm}^{-1}$ , comes from stretching vibrations O-H in  $\text{H}_2\text{O}$  particles, which are present in the sample.

A weaker band of  $2,800\text{--}3,000\text{ cm}^{-1}$  should be ascribed to the stretching vibrations in  $\text{CH}_2$  and  $\text{CH}_3$  groups, which comes from aliphatic hydrocarbons, and which are part of the base oils.

### Measurement of microbial respiration rate

Metabolic activity measurements of drilling fluids are a key indicator of decomposition and/or resistance for decomposition of organic compounds and the rate of these processes. Metabolism requires energy for vital processes and for synthesizing new organic material. According to the biochemical definition, respiration refers to a metabolic process in which the organism obtains energy by oxidizing nutrients and releasing waste products. In aerobic conditions, oxygen is used in the process of oxidation of an organic substrate and  $\text{CO}_2$  is produced as waste. Both  $\text{O}_2$  and  $\text{CO}_2$  can easily be measured. Generally, the higher the consumption of oxygen, the faster the rate of decomposition, and the conditions are not toxic for

microorganisms. The consumed  $\text{O}_2$  to the released  $\text{CO}_2$  ratio informs us about the quality of the decomposed substrate. If in a substrate molecule the C:O ratio is 1:1 (as in glucose), the  $\text{CO}_2/\text{O}_2$  is 1. If the  $\text{O}_2$  content is higher or lower than that of C, the respiratory quotient RQ is higher or lower than 1. In this sense, the RQ parameter informs us about oxidation of the organic substrate.

The metabolic rate of the drilling mud samples is presented in Fig. 3. Measurements of respiration gave pairs of data (for  $\text{O}_2$  and  $\text{CO}_2$ ) for each investigated spent mud/soil mix. During 4 days of surveying metabolic rates were almost constant. The cumulative hourly measurements were arranged linearly (Fig. 3). The cumulative oxygen consumption and  $\text{CO}_2$  evolution during 96 hours was usually below  $35\text{ }\mu\text{g}\cdot\text{g}^{-1}$  of dry mass soil/spent mud mixture. The bigger the admixture of SBM, the slower the respiration rate.

Almost all raw WBM was biologically inactive. The high salt concentration and high pH in WBM are the main reason for poor microbiota growth in spent drilling mud not diluted with soil. Dilution with soil in the proportion of 1:1, excluding bentonite sample K-1/B, was insufficient for microbiota growth, Fig. 4 A, B.

Bigger dilutions, usually 1:16, promote aerobic growth of bacteria and fungi. Dense colonies of *Penicillium* sp. were observed on the surface of some mud samples (Fig. 5), even on the stem of undiluted samples.

The chloride-polymer inhibited (W-1/Cl-P) sample, the biological activity of which was comparable with the brown soil only at a higher dilution than 1:64, was the most toxic.

The remaining samples, i.e. W-2 (polymer inhibited), L2-K (saline-barite), Kram-1P (potassium), K1/P-Cl (chloride-polymer) had comparable toxicity. The dilution 1:16 or 1:32 created sufficient conditions for almost normal microbial activity and life processes. Buffer capacity of brown soil reduced alkalic pH and Cl-concentration in spent mud sample No. W-2, especially in higher solutions.

Soil enriched 10 wt% SBM had nearly a twice as high respiration rate as the reference brown soil. Higher admixtures

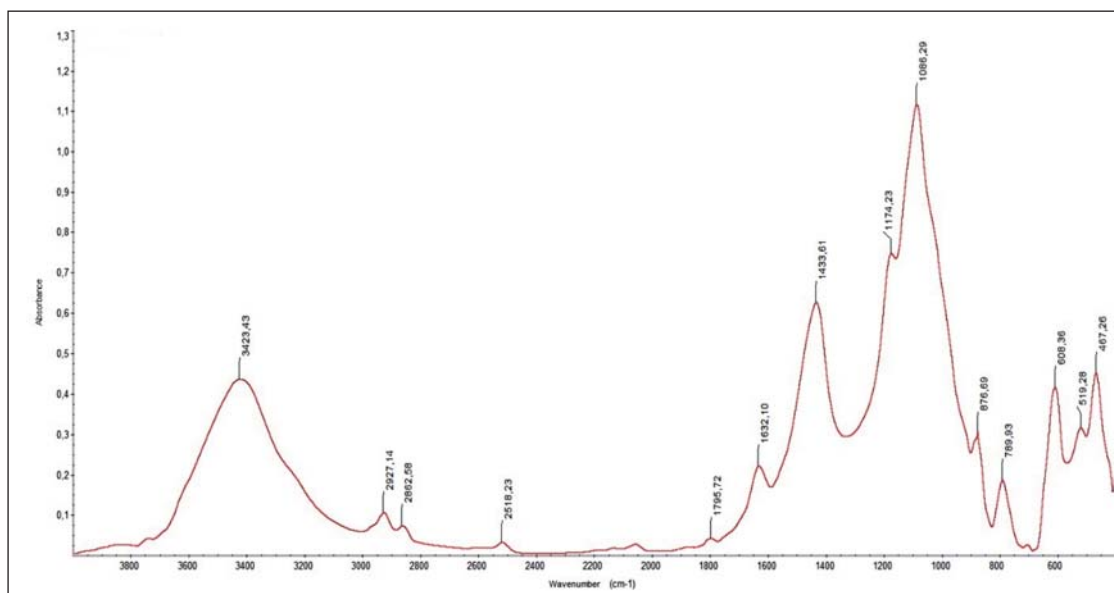


Fig. 2. Absorption spectrum of sample SBM in FTIR infrared

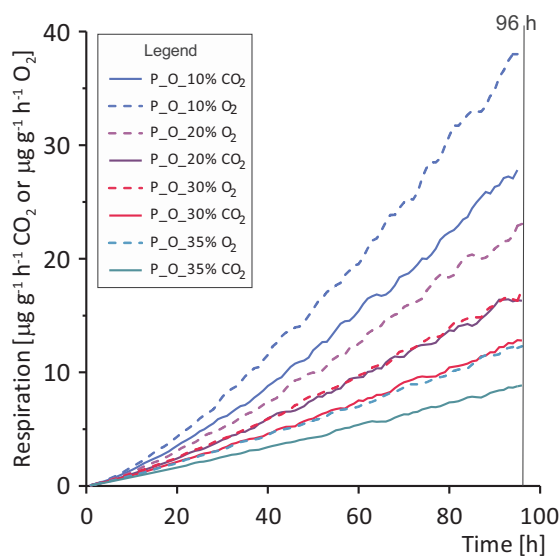


Fig. 3. Family of curves obtained for the measurement respiration of mixture SBM mud with brown soil

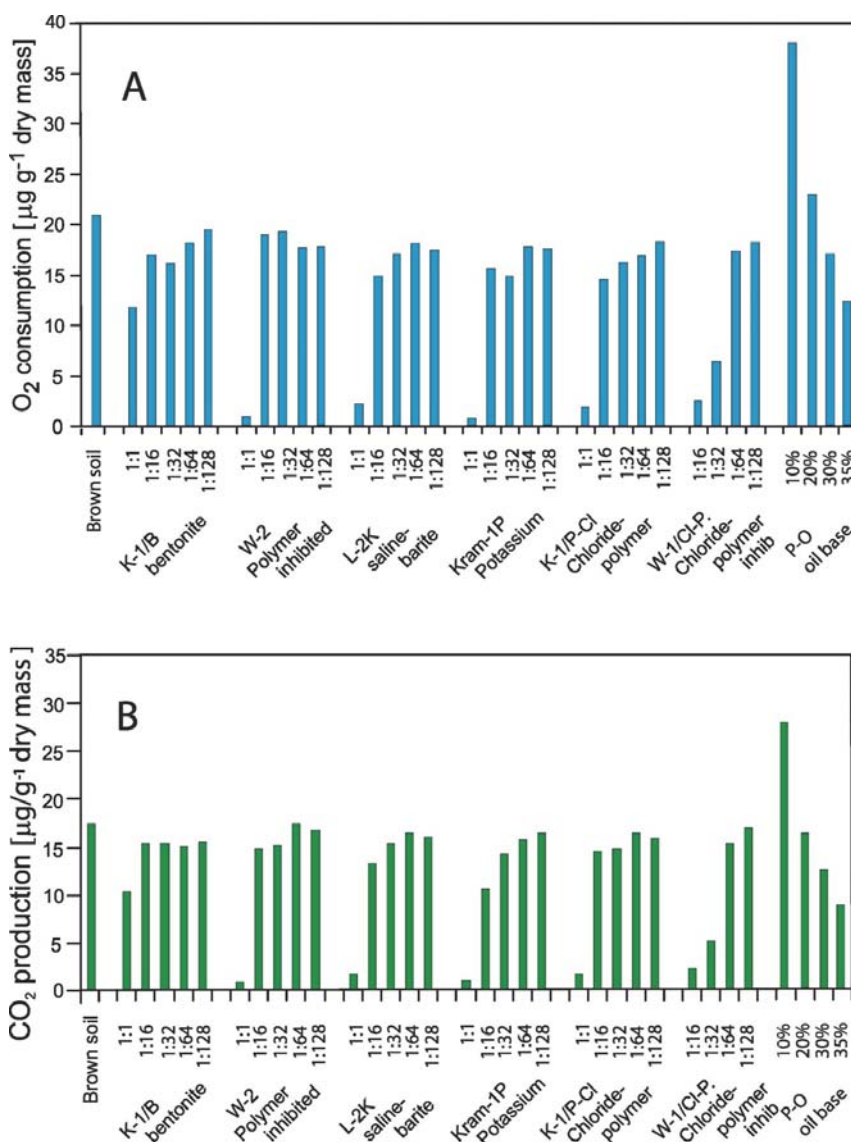


Fig. 4. Oxygen consumption and carbon dioxide production after 96 h in spent drilling mud during respiration after 96 h

(20wt%) inhibit respiration, whereas a 35wt% additive diminished respiration by circa 50% below the reference level (Fig. 4A).

The respirometric tests were supported by toxicological test carried out on a minute branchiopod crustacean of the order Cladocera *Simocephalus vetulus*, green algae and higher plant – *Lepidium sativum* and received similar results (Żurek et al. 2017).

### Respiratory quotient RQ

The respiration quotient value varied from slightly above 1 in single dilutions of K1-B, (1.07), W2 and KRAM-1P to the lowest value for sample KRAM-1P in dilutions 1:1 and 1:16. In a majority of tested samples and dilutions, RQ was between 0.8 and 1, Fig. 6.

The RQ for the reference arable soil was 0.82. In oil contaminated soil RQ was slightly below 0.8, regardless the concentration, though the metabolism was inhibited (Fig. 6). The remaining muds had different patterns of the RQ changes depending on the dilution. One drilling mud had an RQ independent of dilution, despite the inhibited microbial activity (e.g. L-2K, K1/P-Cl), another, had a low RQ at low dilution and had a RQ high in the low dilution, such as KRAM-1P. Still

another, K-1/B, had an RQ similar to those at low dilutions and decreasing at high dilutions (1:64 and 1:128). The standard error of RQ for all samples and dilutions varied from 0.017 to 0.056.

### Discussion and conclusions

The dominating components of the analyzed used drilling muds are mechanically degraded clayey minerals (smectite, smectite/illite, chlorite and quartz). Considerable amounts of halite (NaCl), sylvine (KCl), barium sulfate ( $\text{BaSO}_4$ ) and calcite ( $\text{CaCO}_3$ ) were also identified in the mud. Spent drilling muds are made from multiphase, polymeric aggregates and agglomerates of submicron crystallites (mainly clayey and carbonate minerals), in the formation of which organic polymeric substances actively participate (Jamrozik et al. 2017).

Drilling fluids are constantly exposed to large numbers and different types of micro-organisms, starting from the preparation, and ending on the storage stage. The contamination with microorganisms from the well in the process of drilling can hardly be prevented, if possible at all. Drilling fluids can be contaminated with bacteria living deep in the rock mass. Zhang et al. (2005, 2007) demonstrated the presence of many anaerobic, thermophilic bacterial species in the rocks



Fig. 5. *Penicillium* sp. is able to colonize drilling fluids after diluting them with soil in proportion 1:1

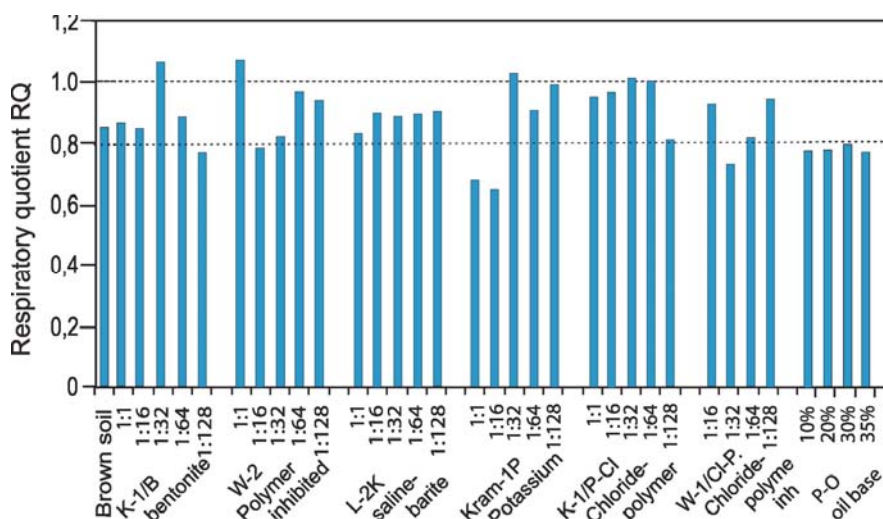


Fig. 6. Respiration quotient after 96 hours of experiment

at 529 depths of 2,026 m and at 95°C. Then, at the stage of drilling, the fluids were endogenously contaminated.

Drilling fluids contain different components which could be carbon and sulfate sources of microorganisms. The FTIR analyses showed the presence of polymeric and macromolecular organic compounds in the analyzed used oil samples. These are mainly PHPA of XCD Polymer type, carboxymethyl cellulose (CMC) as well as macromolecular organic colloids (lignosulphonate or humic substances).

CMC, lignosulphonates, tannins, lignins, synthetic polymers such as polyacrylamides, high-molecular-weight polymers and many other compounds, which are added to muds, are all found to be susceptible to biodegradation (Bland et al. 1993). This biodegradation is not desired during drilling. Microorganisms are accused of biogenic sulphide production, and corrosion is observed at natural gas wells. Those microorganisms in drilling muds are responsible for the deleterious effects (Struchtemeyer et al. 2011, Turkiewicz 2011).

On the other hand, from the point of view of utilization of spent drilling muds, the microbiological activity and toxicity are very important. Unfortunately they still create problems with the management of spent fluid in bioremediation processes (Gao et al. 2019).

The respiration of spent drilling mud, naturally contaminated with microbiota was rarely of interest to researchers. Manufacturers of drilling fluids are aware of the biological degradation of some components of this mud and block the development of microbiota with various chemicals (Aslanoglu 2019). Over time, spent drilling fluids are attacked by bacteria and fungi that use organic compounds (Steliga 2011). In nature, it is usually the mud surface which is eluted by rains and thus less toxic or the mud is generally of low toxicity. A soil admixture additionally brings the microbiota as inoculum which can use the organic components of drilling fluid into the soil.

The purpose of the work was to clarify some of the biological aspects of drilling waste utilization. For the needs of the experiments, the spent drilling mud was diluted with brown soil. This soil had a typical carbon-to-nitrogen ratio. For most arable fields the C/N ratio varied between 8:1 and 9.5:1. Microbial respiration depends on this ratio and is positively correlated with the litter C:N ratio (Spohn 2015).

The organic matter was decomposed by microorganisms from naturally contaminated mud and soil. The analysis of Fig. 4A shows that the consumption of oxygen in a sample where the higher drilling mud content was lower. A higher soil content (bigger dilution) decreased the toxic effect. Similar results were obtained in the toxicological assessment carried out on *Simocephalus vetulus*, green algae and higher plant – *Lepidium sativum* (Żurek et al. 2017).

We tried to compare our results with similar measurements of the respiration of drilling mud enriched with P, N and litter to get an optimal proportion of the general formula of organic matter  $C_{106}H_{263}O_{110}N_{16}P_1$  in a 600 h experiment (Żurek et al. 2005). After recalculating their data for 96 h and after changing units from wet mass to dry mass (as in our experiment), their 96 h  $O_2$  consumption was in the range 71.4 to 195.8  $\mu g\ g^{-1}$  dry mass. This means that the metabolic activity was 3 to about 7 times bigger. Taking the optimized nutrient proportion in their

experiment into account, the relatively low metabolic activity in our mud/soil mix seems to be justified. The measurement of the effect of soil temperature and water content on soil respiration showed that the highest metabolic activity was obtained for water content in the range 210,000 to 370,000  $mg\ O_2\ kg^{-1}$  of dry mass (Wang et al. 2003). The moisture content of our drilling mud was therefore within these optimal limits, Table 1.

Respiratory quotient in bentonite, polymer inhibited, saline-barite, and chloride-polymer inhibited mud was slightly above 0.8. This value is comparable with the basal metabolism of soils not enriched with glucose, as in Dilly's measurements (Dilly 2001, 2003). After enriching the soil with glucose the soil's RQ was approximately 1.3 or higher, and significantly varied, depending on the land use. In our tested samples similar results were observed in oil-based mud for low admixtures (10 to 20%) and in chloride-polymer mud. Values higher than 1 were possible when alternative electron acceptors, such as  $NO_3^-$ ,  $SO_4^{2-}$  or organic acids, were significantly involved in the current degradation of organic substances. When oil concentration in the sample was higher than 30–35wt.%, the fungi-bacterial consortium activity diminished but the value of RQ remained constant and stable on a level slightly below 0.8. We assigned this low value to the decomposition of hydrocarbons. A simple example of decane explains why,  $2C_{10}H_{22} + 31O_2 \rightarrow 20CO_2 + 22H_2O$ , then the theoretical RQ is  $20:31=0.645$ . The high oxygen demand was derived from this equation. And indeed, this oil-contaminated sample consumed much more oxygen than the reference soil, Fig 2A. This result shows the biological degradation of drilling oil in the aerobic process after dilution. Nweke and Okpokwasili (2003) also stated that *Staphylococcus* sp. isolated from oil-contaminated soil was grown in 1% of drilling fluid base synthetic oil, HDF-2000 in suspension in sea water, as a sole source of carbon and energy.

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## Aktywność mikrobiologiczna odpadów wiertniczych

**Streszczenie.** Podczas prac wiertniczych generowane są znaczne ilości odpadów wiertniczych. Skład chemiczny odpadów wiertniczych oraz ich toksyczność zależy przede wszystkim od warunków geologiczno-technologicznych wiercenia, rodzaju przewiercanych utworów skalnych oraz od rodzaju i składu stosowanej płuczki wiertniczej. Rozkład polimerów organicznych przez mikroorganizmy chroniony jest w płuczce poprzez dodatek biocydów. Jednakże podczas prac wiertniczych, płuczki wiertnicze, są w stałym kontakcie z mikroorganizmami które je infekują i namnażają się tylko te gatunki mikroorganizmów, które są zdolne do przetrwania i korzystania z zasobów tego konkretnego środowiska. Z tego powodu w pracy zbadano możliwość przeżycia mikroflory w środowisku zużytych płuczek wiertniczych domieszkowanych różnymi stężeniami gleby brunatnej. Próbkę płuczek pochodziły



z wierceń wykonanych w różnych rejonach Polski, między innymi w rejonie Podkarpacia, Niżu Polskiego oraz rejonu Pomorza. Próbkę zużytych płuczek pobrano z różnych otworów i głębokości. Zużycie tlenu po 96 godzinach oscylowało w granicach około  $20 \mu\text{g} \cdot \text{g}^{-1}$  masy suchej gleby lub mieszanki gleby z płuczkami na bazie wodnej. Mieszanki glebowe domieszkowane 10% wag. płuczkami na bazie oleju syntetycznego (SBM) charakteryzowały się wyższym zużyciem tlenu wynoszącym  $38 \mu\text{g} \cdot \text{g}^{-1}$  suchej masy. Zużycie tlenu gwałtownie spada wraz ze wzrostem zawartości SBM. Wyższe stężenie SBM (35% wag.) zmniejszyło metabolizm tlenowy o ponad 50%. Wysokie stężenie zredukowanego węgla (węglowodory alifatyczne) obniżyło wartość współczynnika oddychania (RQ) do 0,7. Wszystkie badane odpady wiertnicze wykazują aktywność mikrobiologiczną. Natomiast w próbkach odpadów nie domieszkowanych glebą brunatną skład chemiczny tych odpadów (zasolenie, inhibitory, biocydy itp.) silnie hamuje rozwój mikroflory, a w konsekwencji blokuje oddychanie tlenowe.