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MICROBIAL TRANSFORMATIONS OF FOSSIL ORGANIC MATTER OF KUPFERSCHIEFER BLACK SHALE – ELEMENTS MOBILIZATION FROM METALLOORGANIC COMPOUNDS AND METALLOPORPHYRINS BY A COMMUNITY OF INDIGENOUS MICROORGANISMS

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Abstract: The presented article describes the role of community of indigenous microorganisms occurring in the Kupferschiefer black shale in elements mobilization from fossil organic matter of the deposit. The short description of metalloorganic compounds and metalloporphyrins extracted from black shale was presented as well as the characterization of indigenous microorganisms. The processes of fossil organic matter colonization, biofilm development and elements mobilization accompanying bacterial growth was summarized.

Keywords: *black shale, fossil organic matter, metalloporphyrins, metalloorganic compounds, biotransformation, microbial community*

Metalloorganic compounds and metalloporphyrins occurring in Kupferschiefer black shale

The ~256-million-year-old polymetallic Kupferschiefer black shale located in Fore-Sudetic Monocline is a sedimentary rock containing large accumulation of organic carbon. The concentration of organic carbon is between 7–16% and it is typically of marine origin. It represents kerogen type II and contains a mixture of three maceral groups: liptinite, vitrinite and inertinite, as well as other organic components such as amorphous sapropelic mass and solid bitumens (Oszczepalski, 1999). The organic carbon of the Kupferschiefer is mainly comprised of long-chain saturated and unsaturated aliphatic hydrocarbons and polycyclic aromatic hydrocarbons (Sklodowska et al., 2005).

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A major part of metals present in black shale occur in organic compounds (Sawlowicz, 1985; 1989; 1991; Szubert et al., 2006). Two types of organic compounds containing metals were identified in black shale – metalloporphyrins and metalloorganic compounds. The first group is represented among other by: tetraphenyl porphyrin, tetramethoxyphenyl porphyrin, tetramethyl porphyrin and metalloporphyrins derivatives such as copro-porphyrin permethyl ester and azoporphynates (Table 1). Among metalloorganic compounds determined in black shale organic matter were: sandwich type compounds, carbonyl and carbamate compounds (Table 1).

Table 1. The list of organic compounds containing metals detected in Kupferschiefer shale

Chemical compounds	Chemical formula
Metallorphyrines and metalloporphyrins derivatives	
α,β,γ,δ-tetraphenylporphyrin (nickel, lead cobalt copper)	$C_{44}H_{28}N_4Ni$ (*or Pb, Co, Cu)*
Magnesium 5, 10, 15, 20 – tetrakis chlorophenyl porphyrin	$C_{44}H_{24}Cl_4N_4Mg$
[Tetramethyl 3,8,13,18-tetramethyl-21H,23H-porphine-2,7,12,17-tetrapropnoato[2-]-N21,N22,N23,N24]-(zinc, copper)	$C_{40}H_{44}N_4O_8Zn$ (*or Cu)*
Tetra[1,2-(4-t- butyl)-benzo]-tetra-azaporhinato-oxyvanadium	$C_{48}H_{48}N_8OV$
Tetra[1,2-(4-t- butyl)-benzo]-tetra-azaporhinato-chloroaluminium	$C_{48}H_{48}N_8AlCl$
Chloro-(5,10,15,20) -tetramesitylporphinato chromium (III)	$C_{56}H_{52}N_4ClCr$
Metalloorganic compounds	
Methyltriisopropyltine	$C_{10}H_{24}Sn$
Ditelluride, bis(2,4,6trimethylphenyl	$C_{18}H_{22}Te_2$
Tungsten,(2,3- eta)-bicyclo[2.2.1]hepta-2,5-diene]pentacarbonyl	$C_{12}H_8O_5W$
Platinum, dichloro[(1,1',2,2'-eta.)-3,3'-oxybis[propene]]	$C_6H_{10}Cl_2OPt$
Tungsten, phenyltricarbonyl-pi-cyclopentadienyl	$C_{14}\ H_{10}O_3W$
Zirconium,bis(1,2,3,4,5-eta)-1-(1,1-dimethyl)-2,4-cyclopentadien-1-yl	$C_{23}H_{34}Zr$

Indigenous community of microorganisms isolated from Kupferschiefer black shale

A community containing eight bacterial strains were isolated form black shale in the Lubin copper mine using cultivation based methods (Matlakowska and Sklodowska, 2009). Phylogenetic analysis based on 16S rRNA gene homology showed that five strains belonged to the γ-Proteobacteria (*Pseudomonas* sp., *Acinetobacter* sp.), one to the Firmicutes (*Bacillus* sp.) and two to the Actinobacteria (*Microbacterium* sp.).

Detailed analysis of these isolates revealed the adaptation of indigenous bacteria to geochemical conditions of the examined environment. They exhibited such properties as:

- ability to transform black shale ore and use it as a carbon and energy source,
- extremely high-level resistance to metals and metalloids (As, Co, Cu, Ni and Zn),

- proficiency in assimilation of organic acids (capric, adipic malic acids, pyruvic acid, galacturonic acid, itaconic acid),
- the activity of esterase, lipase and dioxygenase,
- the ability to degrade some aliphatic and aromatic hydrocarbons,
- the production of siderophores and organic acids which showed the ability to complex elements from black shale,
- the transformation of synthetic metalloporphyrins.

Organic matter colonization and biofilm development

It was demonstrated that an enriched community of indigenous heterotrophic microorganisms isolated from black shale grown under aerobic conditions could utilize shale organic matter as the sole carbon and energy source.

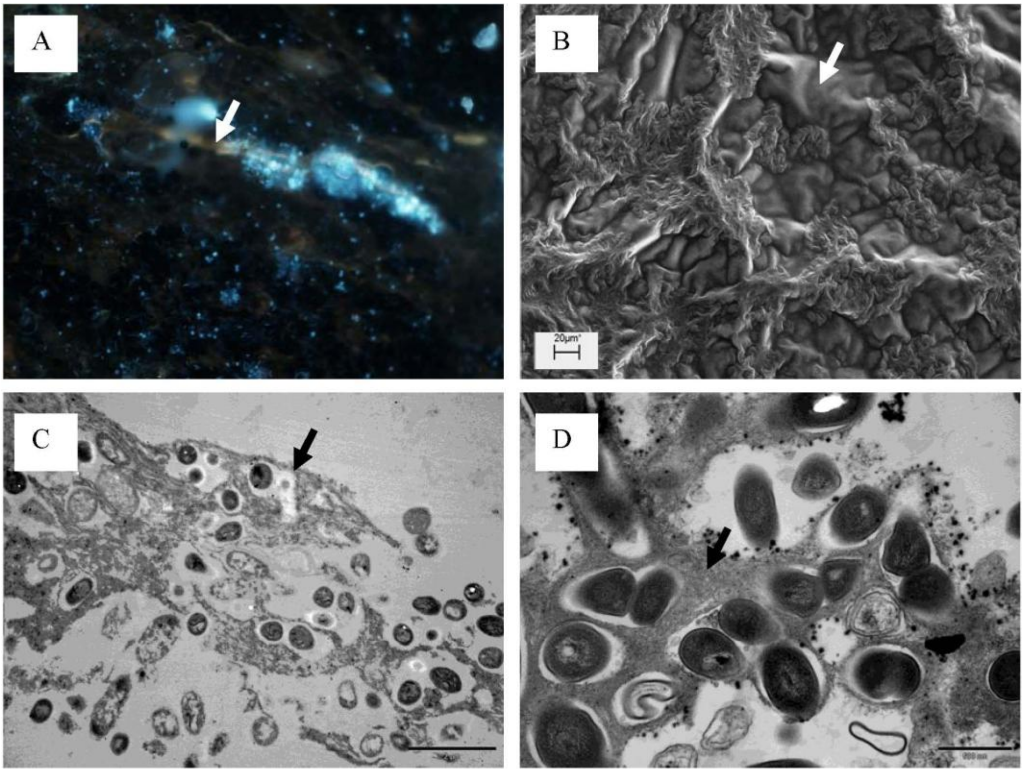

Fig. 1. (A) Epifluorescence micrograph of black shale sections showing the distribution of DAPI-stained microorganisms on the organic-rich laminae on the surface of black shale; (B) scanning electron microscopy (SEM) photomicrographs of bacterial colony-like form grown on a solid mineral medium covered with organic matter extracted from black shale as sole energy and carbon source. (C, D) Ultrathin section TEM photomicrographs of bacterial cells cultivated on mineral medium with organic matter extracted from black shale. The arrows indicate organic matter

Indigenous bacteria directly interact with black shale organic matter to produce a widespread biofilm on the Kupferschiefer shale surface (Matlakowska et al., 2012). They exhibit a specific affinity to fine laminae within the black shale that are rich in organic matter (Fig. 1A). The selective affinity was demonstrated by the localization of the biofilm along these strips oriented parallel to the lamination of the black shale.

Bacteria were able to grow in aerobic conditions on liquid mineral medium supplemented with organic matter extracted from black shale as the sole carbon and energy source. Figure 1B shows photomicrograph of a colony-like form from a mixed culture of bacteria growing on solid mineral medium overlaid with organic matter as the sole carbon and energy source. Colonization of shale extract is apparent and it is a direct evidence of capability of indigenous bacteria to use organic matter as energy and carbon source (Matlakowska and Sklodowska, 2011). Ultrathin cross and longitudinal sections of bacteria grown on solid mineral medium supplemented with organic matter were examined using transmission electron microscopy (TEM) (Figs 1C, D). A direct attachment of cells to organic matter is visible. Intracellular reserve material was also detected in bacterial cells. This material was identified as phosphorus by X-ray microprobe analysis (Matlakowska and Sklodowska, 2011).

Fossil organic matter biodegradation and elements mobilization

The study of biodegradation of black shale organic matter was based on the simultaneous analysis of the concentration of released organic carbon and the analysis of metal concentration mobilized to aqueous phase of cultures and chloroform extracts of this phase. The chemical analysis of biodegradation products was also performed.

Degradation of the black shale organic matter was clearly demonstrated by the release of organic carbon (DOC) into the aqueous phase of the cultures (Fig. 2). The level of DOC in the liquid phase reached a maximal concentration of 16 mg/dm^3 after 25 days of cultivation.

It was confirmed that about 70% of organic matter was degraded after 30 days of experiment. The main biodegradation intermediates and products were identified as phosphonic acid dioctadecyl ester, cyclopropene butanoic acid, phthalic acid bis(methyl nony) ester, hexadecanoic acid, octadecanoic acid, hexadecanol and chloropropionic acid. At the end of the cultivation a part of these compounds completely disappeared indicating that they were metabolized and isoindole-1,3 was detected which is a well-known metabolite of i.e. pyrene, fluoranthene and phenanthrene produced by bacterial degradation (Matlakowska and Skłodowska, 2011).

Simultaneous analysis of metals in aqueous phase and chloroform extracts allowed comparing the total concentration of metals released from organic matter and their content in organic compounds. The concentration of cobalt, copper and nickel directly

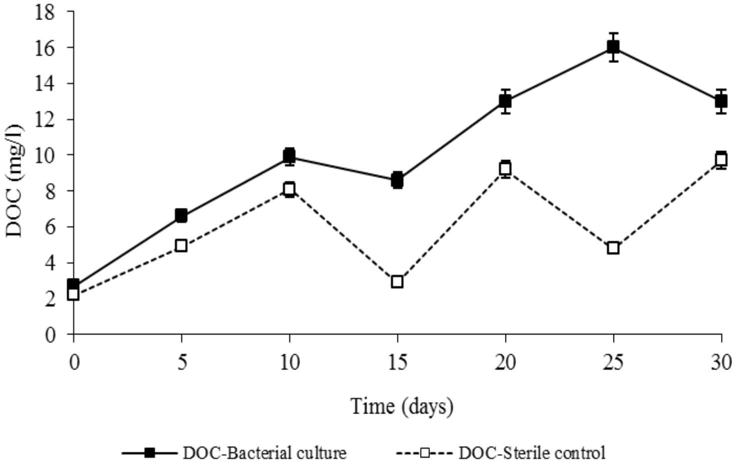

Fig. 2. Organic carbon mobilization from shale organic matter – the changes of the dissolved organic carbon (DOC) concentration in the aqueous phase of bacterial cultures during growth of microorganisms.

released from organic matter extracted from black shale to the aqueous phase of cultures and control as well as chloroform extract of this phase and are presented on Figs 3A, B, C. It was clearly showed that the biodegradation of organic matter cause the release of metals into the culture solution.

The concentration of cobalt, copper and nickel in organic fraction (chloroform extract) reached the highest value in bacterial cultures between 20 and 25 days what is in correlation with the concentration of organic carbon in the aqueous phase. At the end of experiment the concentration of these elements in chloroform extracts decreased. Simultaneously the increase of the concentration of cobalt and copper in the aqueous phase was observed (Figs 3 A, B).

Obtained results support the hypothesis that the biodegradation of organic compound leads to the release of chemical elements to the aqueous phase.

The most interesting results were obtained for copper. About 74% of copper present in black shale substrate was released to aqueous phase of bacterial culture after 30 days of cultivation. In the case of control copper extraction was about 10%. Interestingly, the percent of copper occurring in the form of organic compounds decreased from 19% to 0.15% in bacterial culture in the course of experiment, what confirmed the biodegradation of organic compounds containing this metal. In the case of control, the concentration of copper in chloroform extract was stable and represented the dissolved compounds. The concentration of cobalt and nickel release to aqueous phase of culture from black shale organic matter was 1.4% and 8.9% respectively, while in control 3.4% and 13.8%. The concentration of these metals in bacterial culture was lower than in control and indicated specific fluctuations during experiment. It was calculated that approximately 10% of cobalt and 20 % of nickel were accumulated by bacterial cells during experiment.

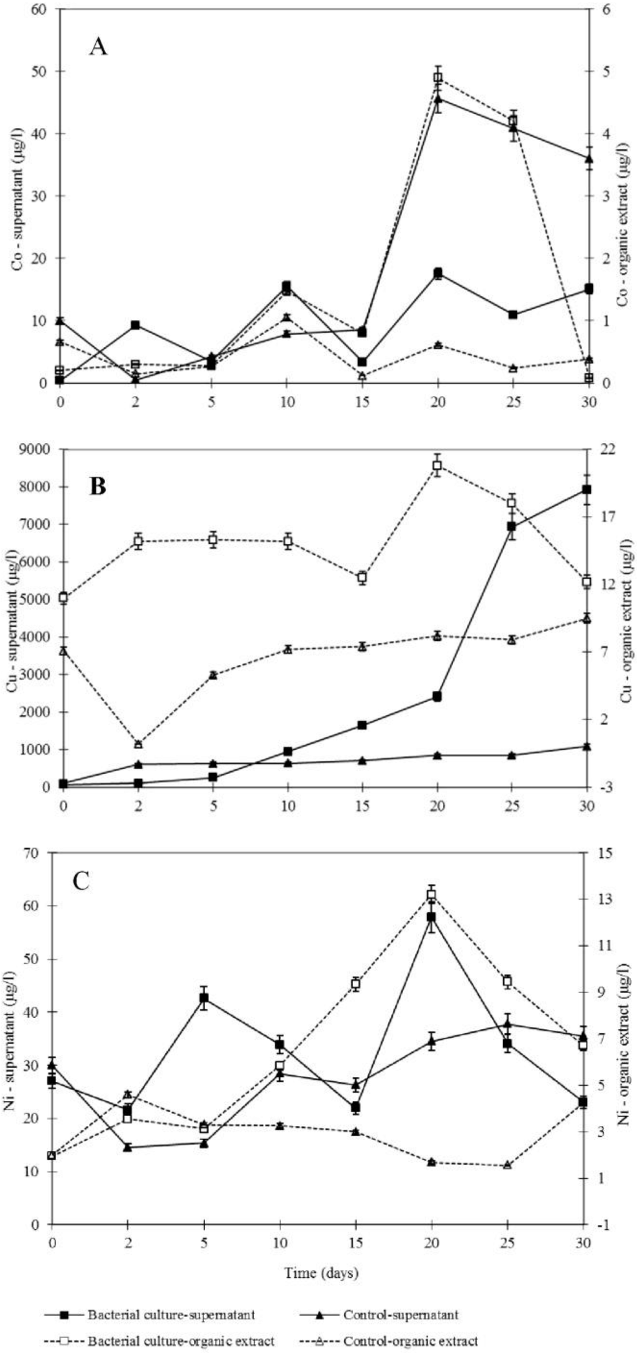

Fig. 3. Concentration of cobalt (A), copper (B) and nickel (C) in the aqueous phase and chloroform extracts of bacterial cultures and sterile controls over the 30 days of cultivation

The biodegradation of organic compounds containing metals were also confirmed by the analysis of UV-Vis spectra of chloroform extracts of aqueous phase. It revealed the significant differences between the bacterial cultures and the sterile control cultures as well as between the bacterial cultures at different days of cultivation (Fig. 4).

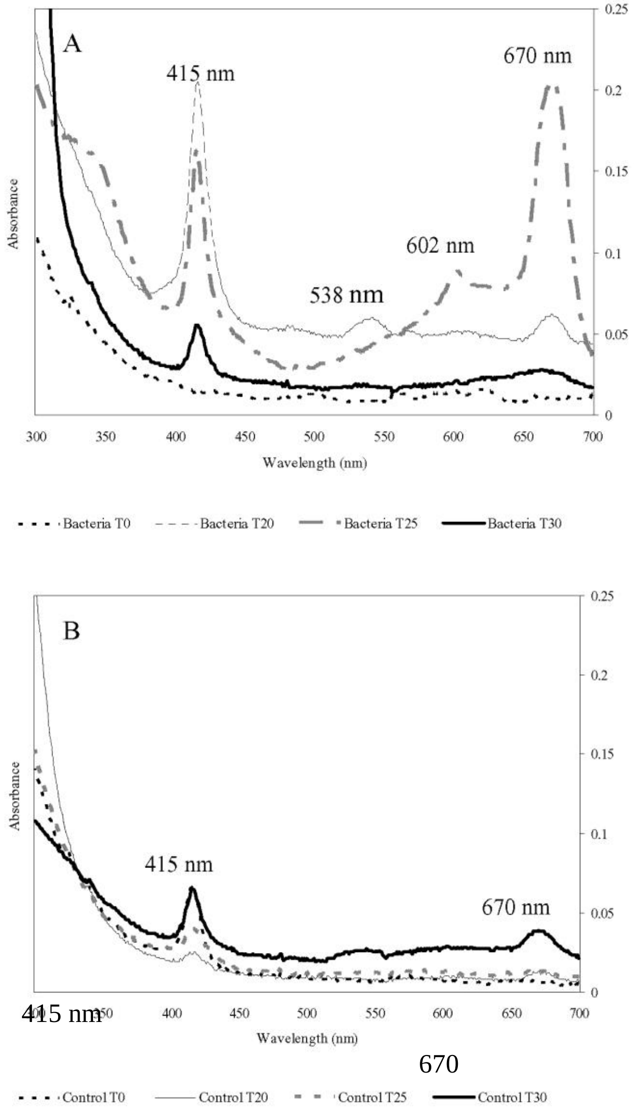

Fig. 4. UV-Vis spectra of chloroform extracts of the aqueous phase of bacterial cultures (A) and sterile control (B) containing organic matter extracted from black shale at the beginning of the experiment (T0), and after 25 (T25) and 30 days (T30) of cultivation

The spectrum of the sample after 20 and 25 days of bacterial treatment exhibited absorption bands typical for metalloporphyrins which is a Soret band (390–425 nm) and Q band (480–700 nm) (Kadish et al., 2000). An intense Soret band with maximum absorption at 415 nm and Q bands with maximum absorption peaks at 602 and 670 nm were apparent and they were considerably stronger than in the control samples. All of these peaks had decreased after 30 days of bacterial cultivation, indicating the degraation of these compounds. Similar results were obtained in earlier experiment in which the biodegradation of whole black shale and synthetic metalloporphyrins were studied (Matlakowska and Sklodowska, 2010, 2010a). The first step in synthetic metalloporphyrin biotransformation was identified as the highly efficient uptake of these compounds by bacterial cells and further degradation of them in bacterial cells (Matlakowska and Sklodowska, 2010a). The changes of UV-VIS spectra observed in this study also reflected the metalloporphyrins uptake and biodegradation.

Conclusions

- The results of this study confirm that indigenous bacteria play a role in the biotransformation of black shale and can influence the geochemical cycles of ancient organic carbon in the deep terrestrial subsurface.
- The biodegradation of organic matter was accompanied by the metal mobilization and release of organic compounds.
- Biodegradation of resistant compounds such as geoporphyrins was confirmed.
- The presence of phosphonic acid dioctadecyl ester in bacterial culture and intracellular reserve material containing phosphorus confirm the role of indigenous microorganisms in phosphorus mobilization from organic matter and simultaneous immobilization in cells.
- The capability of indigenous bacteria to degrade black shale organic matter may strongly influence carbon mobilization from deposit as well as shale fraction of tailings. This phenomenon may also cause the mobilization of potentially toxic compounds to the soil and groundwater.

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