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UTILIZATION OF DIFFERENT CARBON COMPOUNDS BY SULPHATE-REDUCING BACTERIA IN MEDIUMS WITH PHOSPHOGYPSUM

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WYKORZYSTYWANIE RÓŻNYCH ŹRÓDEŁ WĘGLA PRZEZ BAKTERIE REDUKUJĄCE SIARCZANY W PODŁOŻACH Z FOSFOGIPSEM

Badano efektywność biotransformacji fosfogipsu w hodowlach bakterii redukujących siarczany (BRS) namnożonych z gleby terenu stacji benzynowej. Selekcji dokonywano metodami hodowli namnażających i metodą *microcosms* na podłożach: Postgate'a, minimalnym, zawierających różne źródła węgla (fenol, octan, mleczan) i podłożu Emersona. Wszystkie podłoża zawierały fosfogips (5 g/dm^3) jako jedyny akceptor elektronów. Wyizolowane zespoły mikroorganizmów przenoszono na podłoża zawierające różne źródła węgla: etanol, mleczan, fenol, octan, kazeinian lub laktozę w celu sprawdzenia efektywności biotransformacji fosfogipsu w zależności od stosowanego źródła węgla. W hodowlach bakterii na podłożu Postgate'a z mleczanem lub kazeinianem uzyskano najwyższe stężenia siarkowodoru (654 i 540 mg HS^-/dm^3) i największy ubytek masy fosfogipsu odpowiednio 84% i 64%. Wyizolowane zespoły charakteryzowały się szerokim spektrum pokarmowym, wykorzystywały alkohol, cukier, białko, sole kwasów organicznych oraz fenol.

Summary

In this work the effectiveness of the biotransformation of phosphogypsum in the cultures of sulphate-reducing bacteria (SRB) was studied. SRB were isolated from soil contaminated with automobile fuel. The microorganisms were grown by two methods: the enrichment of the cultures and microcosms in: Postgate, minimal medium (with phenol, acetate or lactate) and Emerson medium. All media contained phosphogypsum as electron acceptors. The isolated microorganisms were passage in medium containing different carbon compounds: ethanol, lactate, phenol, acetate casein or lactose to test the effectiveness of biotransformation of phosphogypsum depending on the source of carbon used. In cultures in Postgate medium with lactate or casein there were found the maximal H_2S concentration (654 and 540 mg HS^-/dm^3) and maximal decrease of phosphogypsum 84% and 64%, respectively. The isolated microorganisms utilised alcohol, sugar, protein and phenol.

INTRODUCTION

Phosphogypsum is a form of waste gypsum formed during the production of phosphoric acid from apatites or phosphorites treated with sulphuric acid. On the global scale every year over 22 millions Mg phosphoric acid and about 110 million Mg of phosphogypsum are

formed. The production of 1 Mg phosphoric acid is accompanied by the formation of about 5 Mg of phosphogypsum [23]. Gypsum ($CaSO_4$ x $2H_2O$) and bassanite ($CaSO_4$ x 0.5 H_2O) make up about 90% of its mass, however celestine ($SrSO_4$) and rock residues and other different kinds of contamination [14].

Sulphate reducing bacteria (SRB) represent a group of anaerobic bacteria using sulphates among other oxidized sulphur compounds as a final electron acceptor [7, 17]. Sulphates, which comprise about 50% of the mass of phosphogypsum, can be used by SRB as a final electron acceptor [5].

SRB have been shown to use about 125 organic compounds, including certain hydrocarbons [9]. The preferred carbon sources for these bacteria usually are low molecular weight compounds, such as lactate or ethanol [6]. Certain strains as *Desulfotomaculum antarcticus* can utilize glucose as the sole carbon source, but this phenomenon appears very seldom among SRB [6]. Utilizing hydrocarbons by SRB is common enough [21].

Sulphate reducing bacteria can be met in all anaerobic environments where sulphate and organic compounds are present. But typical environments for these bacteria are marine sediment [22], oil fields and oil reservoirs [17] and environments contaminated with petroleum products [2, 16].

The aim of this study was to isolate anaerobic community of microorganisms from soil contaminated with automobile fuel and to determine the effectiveness of the biotransformation of phosphogypsum in cultures of these microorganisms in different selective media and carbon sources.

EXPERIMENTAL – MATERIALS AND METHODS

Phosphogypsum. The studied phosphogypsum sample was obtained from mounds located in Wizów near Bolesławiec, Lower Silesia.

Media. The following media were used: Postgate medium with phenol (0.5 and 1.5 g/dm^3) or lactate (2.64 and 3.5 g/dm^3), ethanol (3 cm^3/dm^3), acetate (3.8 g/dm^3), caseinate (2.7 g/dm^3), lactose (3 g/dm^3); minimal medium with phenol (0.5 g/dm^3) or lactate (2.64 g/dm^3); Emerson medium in which crude oil was source of carbon (5 cm^3/dm^3). All media contained phosphogypsum (5 g/dm^3) as sole electron acceptor for SRB. To all cultures resazurin in concentration 0.001 g/dm^3 was added as an indicator of redox conditions in the medium.

Inoculum. The inoculum was autochthonous microflora originally isolated from soil contaminated with automobile fuel. The inoculum was multiplied using two methods: *microcrocosms* – soil from the studied cites was placed in 100 cm^3 plastic boxes together with 5 g/dm^3 of phosphogypsum and covered with appropriatly; and enrichment cultures – soil was placed in high glass cylinders. The ratio of soil to water was 1:10.

Three kinds of medium were used: Postgate's with phenol (0.5 g/dm^3) or lactate (2.64 g/dm^3), minimal medium with phenol (0.5 g/dm^3) or lactate (2.64 g/dm^3) and Emerson medium in which the sole source of carbon was fuel oil.

Cultures. Stationary cultures were maintained in 50 or 300 cm^3 glass vessels tightly closed with rubber stoppers through which a needle, topped by a syringe, was inserted. The cultures were incubated at 30°C or 55°C in the dark.

Determinations. Sulphides in the cultures were determined using the iodometric method, sulphates by the hot barium method, COD by the dichromate method, the concentration of

phenol using a colorimetric method involving p-nitroaniline [10]. pH was measured using a pH-meter or with bromothymol indicator and color scale of pH. The reaction of the cultures was corrected with 0.1 N HCl or 0.1 N NaOH.

Determinations involving post-culture sediments and fluids were made using a DRON-2 X-ray diffractometer.

RESULTS AND DISCUSSION

Isolation. 20 cultures were set up altogether: 10 enrichment cultures and 10 *microcosms* (in two groups of 5 incubated at 30 and 55°C. The incubation period was 6 weeks. After the six-week incubation, blackening indicating the presence of sulphides and the characteristic smell of H_2S was observed only in 4 *microcosms* incubated at 30°C. It shows the presence of SRB in studied environment. The obtained community of microorganisms containing SRB is shown in Tab. 1.

Table 1. SRB isolated by the *microcosms* method from soil contaminated with automobile fuel

Symbol of microcosms	Kind of medium	Source of carbon
Ia	Postgate	lactate
Ib	minimal	lactate
Ic	minimal	phenol
Id	Emerson	crude oil

Biotransformation of phosphogypsum in different media. The selected communities of anaerobic microorganisms were used as inoculum for setting up stationary cultures. Culture Ia, isolated in Postgate medium with lactate as sole carbon source was passaged to Postgate medium with lactose, caseinate and lactate. Cultures no Ib and Ic selected in minimal medium with lactate or phenol, were passaged one more time to minimal medium with the earlier mentioned sources of carbon and additionally Ic in Postgate medium with phenol. In Emerson medium, only one culture Id was selected, which was passaged in Postgate with lactate and phenol and additionally in Emerson medium (with oil fuel) and petroleum-refining wastewater. All the media contained phosphogypsum (5 g/dm^3) as electron acceptor for SRB. Altogether 18 cultures were set up. At the time of incubation the concentration of hydrogen sulphides was measured in the cultures rising during biotransformation of phosphogypsum (Fig. 1).

The highest concentrations of HS^- – 597, 410, 597, 435 and 606 mg HS^-/dm^3 were obtained in cultures no 2, 3, 8, 17 and 18 respectively. It corresponded to the reduction 67, 52.6, 67, 49 and 68% the initial mass of phosphogypsum. Only 5 cultures out of 18 were used in further studies (no 2, 3, 8, 17 and 18). No growth was observed in cultures no 5, 6 and 15 (Tab. 2).

These 5 communities (no 2, 3, 8, 17 and 18) were used as inoculum to set up cultures of

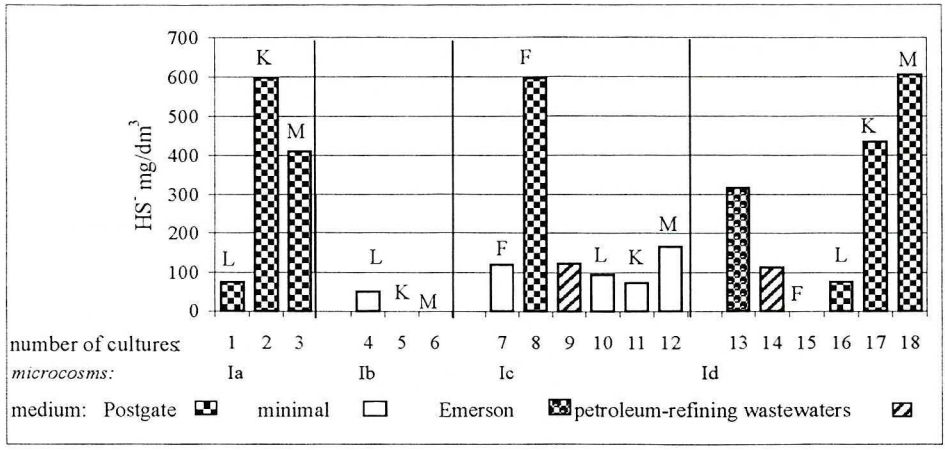

Fig. 1. The maximal concentration of sulphides in cultures of anaerobic microorganisms set up from soil contaminated with automobile fuel (capital letters mean the sources of carbon in medium: L – lactose, K – casein, M – lactate, F – phenol)

Table 2. The cultures of bacteria isolated from soil contaminated with automobile fuel

Number of cultures	2	3	8	17	18
microcosms	Ia	Ia	Ic	Id	Id
used medium	P_k	P_m	P_f	P_k	P_m

Arabic numbers – mean number of cultures as Fig. 1, P – Postgate medium, m – lactate, f – phenol, k – caseinate

sulfidogenic consortium capable of biotransformation of phosphogypsum. Postgate medium with lactate (cultures no 3 and 18), caseinate (cultures no 2 and 17), phenol (culture no 8) were used. All the cultures were passaged five times.

The maximal concentration of HS^- found in the studied cultures ranges from 130 mg HS^-/dm^3 in Postgate'a medium with lactate to 620 mg HS^-/dm^3 in Postgate'a medium with caseinate. The maximal concentration of HS^- in all the cultures in all media was found to be 520 mg HS^-/dm^3 with lactate (culture no 3), 610 mg HS^-/dm^3 with caseinate (culture no 17) and 390 mg HS^-/dm^3 with phenol (culture no 8) which corresponded to the reduction of about 1470, 1750 and 1100 mg SO_4/dm^3, which indicates the biotransformation 59%, 70% and 44% of the initial mass of phosphogypsum respectively (Fig. 2).

Biotransformation of phosphogypsum in Postgate medium with different sources of carbon. In cultures with the highest concentration of HS^- (culture no 3 and 17) after incubation period 84% (4.2 g/dm³) and 64% (3.2 g/dm³) decrease of phosphogypsum was observed respectively. The post cultures sediments were diffractometrically studied. The phase changes of the sediments showing biotransformation were observed only in one sediment (cultures no 3). The post culture sediments were composed of phosphogypsum residues: gypsum and celestine; appatite was observed too (Fig. 3).

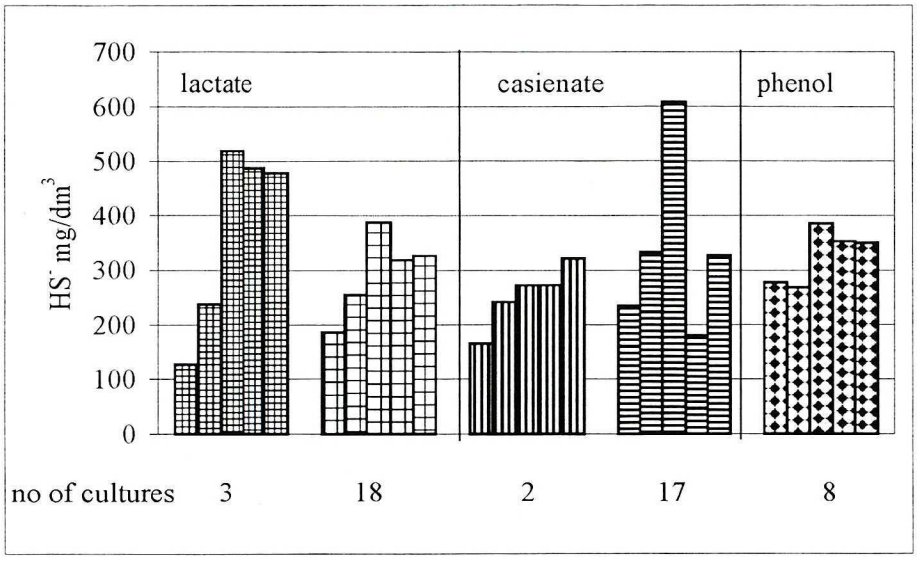

Fig. 2. The maximal concentration of sulphides in consecutive passages of cultures of microflora in different medium

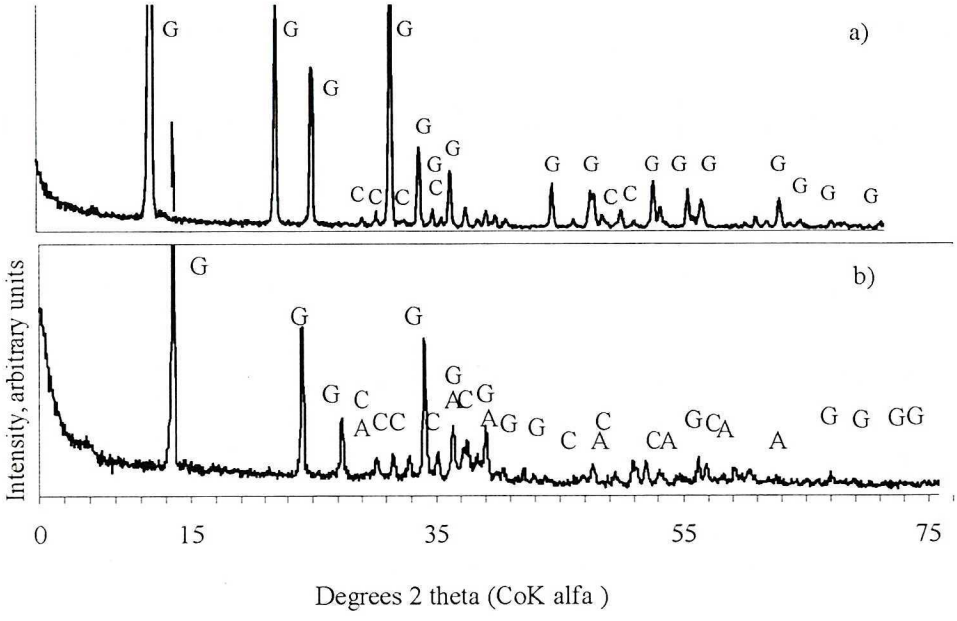

Fig. 3. Diffractogrammes of phosphogypsum (a), and precipitates in the cultures no 3 (symbols mean: G – gypsum, C – celestite, A – apatite)

The efficiency of phosphogypsum biotransformation by isolated microbial communities from cultures no 3 and 17 in mediums with different sources of carbon was tested. 12 cultures were set up in Postgate medium with ethanol, lactate, phenol, acetate, lactose and caseinate. All cultures were passaged twice. Fig. 4 shows the maximum SRB activity (mg HS^-/dm^3) in the studied cultures.

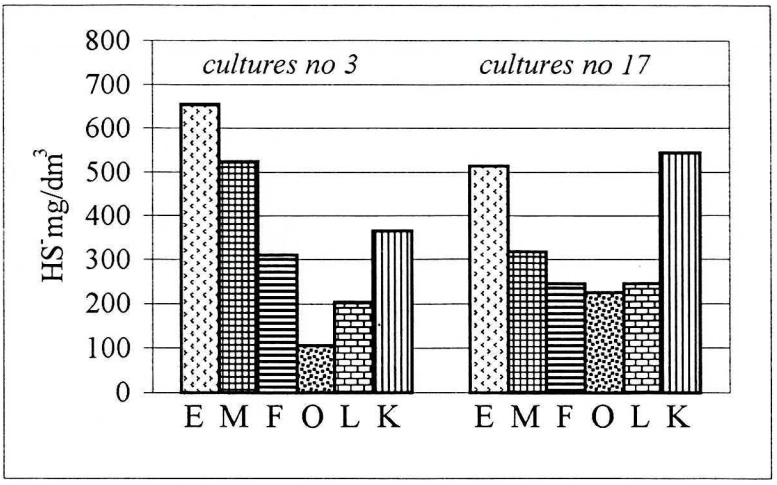

Fig. 4. The maximal concentration of sulphides in Postgate medium with different compounds (capital letters mean the sources of carbon in medium: E – ethanol, M – lactate, F – phenol, O – acetate, L – lactose, K – casein)

The isolated anaerobic sulfidogenic bacterial communities produced the maximal concentration of HS^- in the cultures in medium with ethanol (654 mg/dm^3), caseinate (540 mg/dm^3) and lactate (510 mg/dm^3), which corresponded to the reduction of 1820, 1525 and 1440 mg SO_4/dm^3.

Ethanol presents a good source of carbon for SRB and it is mentioned in the third place after lactate and pirogronate [3]. Some strains of SRB, like *Desulfobacter postgatei*, can oxidise ethanol entirely but others such as *Desulfobulbus propionicus* or *Desulfovibrio baculatus*, oxidize ethanol only to acetate. Utilization of ethanol was observed in over 30 classes and strains belonging to 12 different types of SRB [13, 15]. Lactate, like ethanol, is an easily available source of carbon for SRB and it was regarded till 1984 as an optimal source of carbon for this group of bacteria during isolation [18].

Utilization of caseinate by the studied microbial communities shows that in the cultures there are other bacteria, except SRB, e.g. fermentative. SRB do not contain hydrolytic enzymes, and because of this, in decomposition of polysaccharide or proteins they are included after hydrolyze performed by fermentative bacteria, at the level of volatile fatty acids, amino acids and alcohol [4]. However, single strains of SRB are known which utilize amino acids as a sole source of carbon *Desulfovibrio aminophilus* [1], *Desulfobacterium vacuolatum* [19] and *Desulfovibrio mexicanus* [11]. In medium with acetate and lactose (Fig. 2) the lowest concentration of HS^- was observed which could be caused by low pH of these cultures (pH 4.8). The optimal pH values of medium for SRB range from 5.5–9.0 [12].

So far only one strain of SRB *Desulfobulbus mediterraneus sp. nov.* which utilizes lactose as a sole of carbon source has been isolated and described [20]. The isolated microbial

communities from soil contaminated with automobile fuel contained SRB capable of biotransformation of phosphogypsum. In all the cultures the biotransformation of phosphogypsum was accompanied by COD reduction. The amount of sulphides produced and COD used by the whole bacterial community made it to calculate, the participation in the utilization of organic compounds by SRB based on the stoichiometry of sulphate reduction $COD/SO_4 = 0.67$ [12]. The highest participation of SRB in the utilization of organic compounds was about 40% in the culture no 3 in medium with ethanol. The lowest participation (about 7%) was observed in the medium with acetate. The participation of SRB in the residual cultures ranged from 11 to 36%.

The amount of anaerobic bacteria in soil is about 10% [8] but in the studied community of anaerobic microorganisms higher values were observed. It shows the adaptation and selection processes of the studied microorganisms to created conditions.

In the environmental samples contaminated with petroleum products the presence of SRB which were capable of effective biotransformation of phosphogypsum was observed. All the isolated microbial community contained SRB. Biotransformation of phosphogypsum in the cultures in mediums containing lactate, ethanol, acetate, phenol, lactose and caseinate as a sole carbon sources was observed.

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