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SULPHUR BALANCE IN ANAEROBIC CULTURES OF MICROORGANISMS IN MEDIUM WITH PHOSPHOGYPSUM AND SODIUM LACTATE

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ABSTRACT

Biotransformation of phosphogypsum in two anaerobic cultures of mesophilic and thermophilic bacteria was investigated. In the both cultures an increase of sulphide ions was found, adequate to reduction of ca 50 % of sulphates in phosphogypsum. The mineral fraction of the post-reaction precipitates was equal to 43 and 35 % of the initial mass of phosphogypsum in the meso- and thermophilic cultures, respectively. It comprised, in addition to the residue of phosphogypsum, calcite (in the mesophilic cultures) or calcite and apatite (in the thermophilic cultures). In the both post-reaction precipitates, concentrations of lanthanides increased from 0.56 % in phosphogypsum substrate to 1.28 % in the mesophilic bacteria cultures, and to 1.47 % in the thermophilic ones.

Key words: *sulphur compounds, anaerobic microorganisms, sulphate-reducing bacteria, phosphogypsum, sodium lactate.*

INTRODUCTION

Geochemical circulation of sulphur in the hypergene zone occurs mainly due to the activity of bacteria reducing the oxidized species of sulphur and bacteria oxidizing the reduced species of this element. Sulphur in the oxidized form, e.g. SO_4^{2-}, is being reduced (assimilative reduction) and used by numerous microorganisms as a source of sulphur for synthesis of the sulphur-bearing aminoacids (with HS^- groups). This process, albeit common, does not play any important role in sulphur circulation, because 1 mg sulphur in the form of SO_4^{2-}

is sufficient for the production of 100 to 200 mg of bacterial biomass. Significantly more important for sulphur circulation is the dissimilative reduction of sulphates, because the bacteria involved in this process need 57 times more sulphur in the form of SO_4^{2-} to produce the same amount of biomass. This results from the use of sulphates as electron acceptor in respiratory processes (de Wit 1992, Colleran *et al.* 1995).

According to the stoichiometry of the biologic reduction of sulphates, one mole HS^- is produced from one mole SO_4^{2-} with simultaneous oxidation of 2 moles of organic carbon. The C_2-to-C_4 organic acids, which are the final products of fermentation, are the best acceptable source of carbon for sulphate-reducing bacteria (SRB), as indicated by Postgate (1984). Thus, SRB play an important part in the biodegradation of organic matter in all the anaerobic environments rich in sulphates. The participation of SRB at the final stages of mineralization of organic matter in marine sediments is estimated as in excess of 70% (Jørgensen 1982).

The SO_4^{2-} ions are the most common electron acceptors used by SRB, nevertheless $S_2O_3^{2-}$, SO_3^{2-} and elementary sulphur may replace them, if sulphate(VI) ions are insufficiently abundant. The availability of these ions depends mainly on the solubility of the chemical compounds containing SO_4^{2-}. Thus sodium sulphate as the well-soluble compound is the most common component – source of SO_4^{2-} in the standard culture media for SRB. However, these bacteria are able to use the SO_4^{2-} ions present in relatively poorly soluble compounds like calcium sulphates(VI), *i.e.* gypsum, bassanite and anhydrite, calcium sulphate(IV), *i.e.* hannebachite, hydrous potassium sulphate(VI), *i.e.* jarosite, and even almost insoluble lead sulphate(VI), *i.e.* anglesite or barium sulphate(VI), *i.e.* barite (Ivarson, Hallberg 1976, Przytocka-Jusiak *et al.* 1995, Karnachuk *et al.* 2002).

EXPERIMENTAL AND RESULTS

In the recent years the number of publications devoted to the application of SRB in the biodegradation of the sulphur-rich industrial wastes, both liquid and solid ones, have increased rapidly. Phosphogypsum formed as a waste during the production of the phosphate fertilizers belongs to the latter group (Colleran *et al.* 1995, Deswaef *et al.* 1996). Phosphogypsum precipitates when apatite (or sedimentary phosphate) is treated with sulphuric acid according to the reaction (Burnett *et al.* 1995):

$$\underset{\text{apatite (phosphate) rock}}{Ca_{10}(PO_4)_6F_2} + 10H_2SO_4 + 20H_2O \rightarrow \underset{\text{phosphogypsum}}{10Ca_2SO_4{\cdot}2H_2O} + 6H_3PO_4 + 2HF.$$

In this process, production of 1 tonne of phosphoric acid causes the formation of 4.5 to 5.5 tonnes of phosphogypsum. This waste consists of calcium sulphate(VI) (more than 90% by weight) and residues of the phosphate-bearing rock. The amount and composition of phosphogypsum, that formed in various factories producing phosphate fertilizers, is variable within certain limits and depends mainly on the quality of the raw material being the source of phosphorus (Mays, Mortvedt 1986, Davister 1998).

An attempt of the balance of the process of the phosphogypsum biotransformation in anaerobic mixed cultures is the aim of this paper. Two bacterial assemblages were investigated: the mesophile and thermophile ones. Both assemblages were previously passaged (for above 5 years) under the conditions favouring the selection of SRB. Twelve cultures of each assemblage were set up (to obtain the average results), which were incubated in darkness at the temperatures of 30ºC and 55ºC, respectively. Darkness was necessary because light favours the development of the photosynthesizing bacteria (mainly from the genus *Chlorobium*), which oxidize sulphides to elementary sulphur (Brock, Madigan 1991).

The cultures were maintained in bottles (500 ml) closed with rubber stoppers. The Postgate C medium (Postgate 1984) was used, in which Na_2SO_4 was replaced by phosphogypsum (5 g/l) and the minimum medium containing sodium lactate, NH_4Cl and phosphogypsum in concentrations equivalent to the above defined medium. The pH of the medium was maintained at 7.2. The volume of 50 ml of the culture of anaerobic bacteria assemblage prepared earlier in the same medium was introduced into 450 ml medium. The concentration of HS^- after introduction of the bacteria was equal to ca 80 mg/l in the culture of mesophiles and ca 40 mg/l in the culture of thermophiles. Phosphogypsum, formed during the processing of the Khibiny apatites, was taken from the waste dump at Wizów near Bolesławiec (Kowalski *et al.* 1990). The samples of the cultures were taken with a syringe by piercing the stopper.

Each day of the incubation, samples were taken from two cultures of each series to monitor the changes of the sulphide concentrations and pH of the medium, the remaining cultures were incubated without any interference for 19 and 13 days. The averaged results of the changes of the HS^- concentrations and pH of the medium are given in Fig. 1.

In the cultures of the mesophilic bacteria the increase of the sulphide concentrations was observed till the eleventh day of the incubation. The obtained increase amounted to 556 mg/l, which according to the stoichiometry of the reduction process of sulphates (one mole SO_4^{2-} → one mole HS^-) was equivalent to the reduction of 1568 mg SO_4^{2-}/l. In the cultures of thermophilic bacteria the increases in sulphide ion concentrations ended already on the sixth day and the maximum concentration was 416 mg/l (from reduction of 1173 mg SO_4^{2-}/l). In the cultures of the both assemblages of bacteria a decrease in sulphide concentration was observed in the last days of incubation to ca 100 mg/l. No significant acidification of the medium was observed in any of the cultures, the lowest pH value (~6.5) was found in the thermophilic cultures.

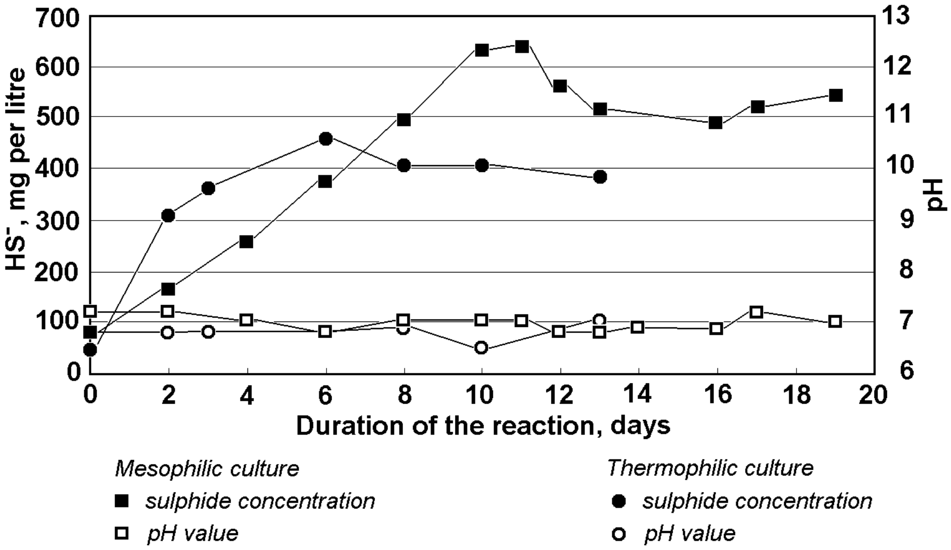

Fig. 1. Changes of the sulphide ion concentrations and pH values in the cultures of the meso- and thermophilic bacteria during the experiments

After completion of incubation, the ten cultures of each series were mixed to obtain material to investigate the effects of the biotransformation of phosphogypsum. After filtration, two five-litres portions of the post-reaction culture liquids and two portions of precipitates were obtained; the precipitates after drying at 40ºC weighed 10.793 g (*i.e.* 2.159 g/l) for the mesophilic series and 8.757 g (*i.e.* 1.751 g/l) for the thermophilic one. The size and morphology of the grains of phosphogypsum and post-reaction precipitates are showed in Figs. 2a–c.

Table 1. Contents of the selected components of the post-reaction precipitates (mg/l)

Component	Phospho-gypsum	Post-reaction precipitate	
		Mesophilic	Thermophilic
Ca	1047	495	396
SO_4^{2-}	2592	824	483
Sr	76	52	41
Ln_2O_3	28.0	27.6	25.8
Precipitate	5000	2159	1751

The concentrations of calcium, sulphate ion, strontium and total lanthanides were determined in phosphogypsum and in the post-reaction precipitates (Table 1). The results of these determinations indicated that the total masses of the both precipitates decreased compared to the mass of phosphogypsum used to the reactions (5 g/l) and amounted to 43% and 35% of the original concentrations.

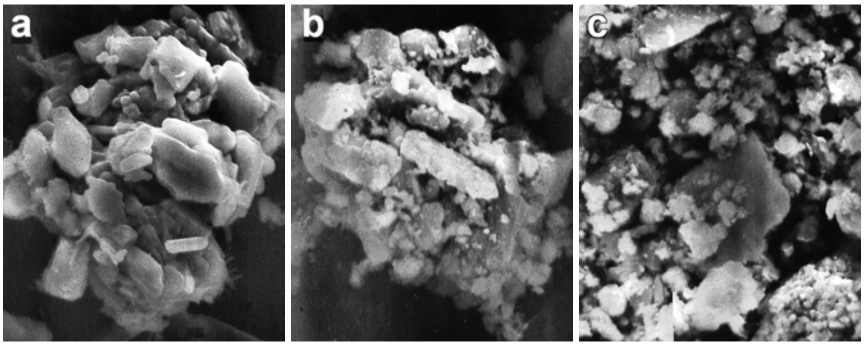

Fig. 2. Morphology of the grains of phosphogypsum (a – note the euhedral habits of gypsum crystals), precipitate from the culture of mesophiles (b) and precipitate from the thermophilic one (c). SEM images, ×2500; scanning microscope Tesla BS-301, pictures taken by Dr. P. Dzierżanowski.

Also, the SO_4^{2-}/Ca ratio changed in both precipitates from 2.48 typical of phosphogypsum to 1.66 and 1.22 in the cultures of meso- and thermophiles, respectively. The compositions of phosphogypsum and the post-reaction precipitates are showed in Fig. 3.

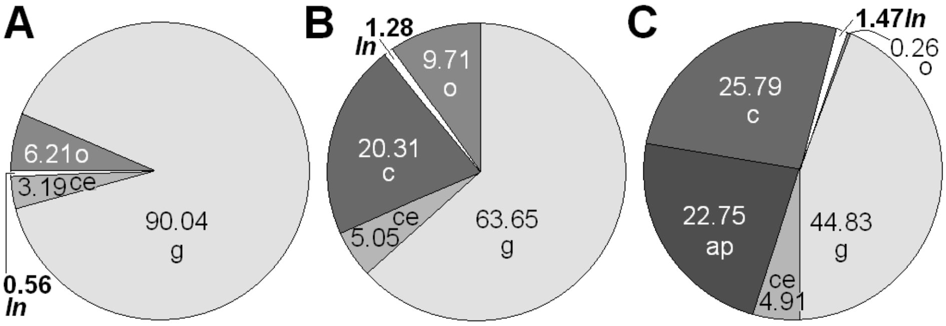

Fig. 3. Chemical composition of phosphogypsum (A) and precipitates formed in the cultures maintained under mesophilic (B) and thermophilic (C) conditions; ap – apatite, c – calcite, ce – celestite, g – gypsum, ***ln*** – lanthanide oxides, o – other components; contents in wt %.

Lanthanide elements concentrated in the post-reaction precipitates from 0.56% in phosphogypsum to 1.28% and 1.47% in the meso- and thermophilic cultures, respectively. In the both post-reaction precipitates, the concentration of gypsum decreased; this component was replaced by calcite and apatite (the latter only in the thermophilic culture due to the addition of 114 mg phosphorus per litre to the Postgate medium). The results of the diffractometric studies of phosphogypsum and post-reaction precipitates are given in Fig. 4. The results of the X-ray analysis correlated well with the data obtained by the chemical methods and confirmed the biotransformation of phosphogypsum. Sulphate ions from phosphogypsum after its dissolution were reduced to the sulphide ions due to the activity of the SRB. In the both post-reaction precipitates the decrease of

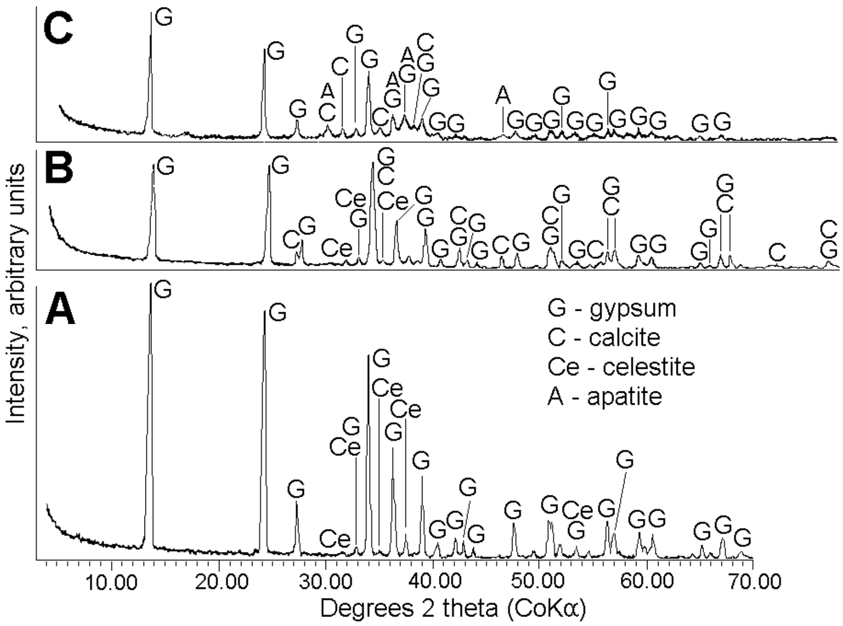

Fig. 4. Diffractogrammes of phosphogypsum (A) and precipitates in the cultures of mesophiles (B) and thermophiles (C). Diffractometer DRON-2, the measurements made by G. Kaproń, M. Sc.

the sulphate sulphur concentration was found, from 864 mg/l in phosphogypsum to 275 mg/l and 356 mg/l in the meso- and thermophilic cultures, respectively (Table 2), what points to the dissolution of 3.4 and 4.1 g phosphogypsum. Similar solubility of phosphogypsum, from 3.5 to 4.0 g/l, was found by Davister (1998), using sea water as the solvent. Nevertheless, a part of the sulphate ions released to the solution was not reduced. In the investigated cultures of the 864 mg sulphates present in phosphogypsum, 46% underwent reduction in the mesophilic cultures and 43% in the thermophilic

Table 2. The balance of sulphur in the meso- and thermophilic cultures of bacteria (mg sulphur per litre)

Sulphur ion	Phosphogypsum	Mesophilic cultures			Thermophilic cultures		
		Precipitate	Solution	Inoculum	Precipitate	Solution	Inoculum
Sulphide	0	0	432.0	75.3	0	389.6	42.4
Sulphate	864	275	232.6	0.0	161	355.7	0.0

ones (Fig. 5); the reduced amounts were equivalent to the biotransformation of 2.3 and 2.1 g phosphogypsum per litre.

Taking into account the error caused by oxidation of sulphides in the period between their maximum concentration and the amount found on the last day of

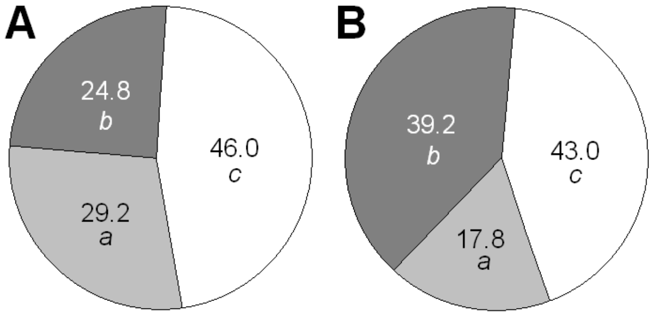

Fig. 5. Balance of sulphur in the mesophilic (A) and thermophilic (B) cultures; *a* – sulphate ions in the precipitate, *b* – sulphate ions in solution, *c* – sulphide ions in solution; contents in wt %.

the incubation (ca 100 mg H_2S), it may be evaluated, that ca 0.5 g more phosphogypsum was transformed into sulphide ions.

CONCLUSIONS

The obtained results unambiguously indicate, that SRB may use the SO_4^{2-} ions present in phosphogypsum as the electron acceptors. The effectiveness of this process did not exceed 140 or 196 mg SO_4^{2-} per litre per 24 hours at different temperatures of the runs and was probably limited by the toxic properties of hydrogen sulphide (Okabe *et al.* 1995). This effectiveness may be enhanced several fold in continuous cultures, in which the toxic influence of hydrogen sulphide may be easier controlled, maintaining its concentrations which are non-toxic for SRB. These conditions may be reached either by the increase of the medium flow velocity or by biological or chemical oxidation of sulphide ions to elementary sulphur.

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