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Biotransformation of Phosphogypsum on Distillery Decoctions (preliminary results)

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Abstract

The paper presents the activity of anaerobic bacterial communities isolated from soil polluted by aircraft fuel on distillery decoctions with phosphogypsum. The microorganisms were selected using the microcosms method, and then enriched on Postgate medium with ethanol. The isolated communities became the inoculum to establish a culture on potato and rye distillery decoctions. The obtained results show that a simultaneous removal of two industrial wastes such as phosphogypsum and distillery decoctions is possible. The introduction of a inoculation comprising a selected anaerobic bacterial community into the culture does not influence the increase of the biotransformation process efficiency.

Key words: biotransformation, sulphate reducing bacteria, distillery decoctions

Introduction

Phosphogypsum represents industrial waste originated during the production of phosphoric acid from apatites or phosphorites. Phosphogypsum is composed mainly of gypsum ($CaSO_4 \times 2H_2O$), accompanied by bassanite ($CaSO_4 \times 0.5H_2O$). The mineralogical characteristic of phosphogypsum was presented by Kowalski *et al.* (1990). Phosphogypsum, containing about 50% sulphates, undergoes biotransformation in cultures of sulphate reducing bacteria (Przytocka-Jusiak *et al.*, 1995). Sulphate reducing bacteria (SRB) are heterotrophic and absolute anaerobes, using sulphates as well as other oxidised sulphur compounds (sulphites, tiosulphites, tritronians, tertrationians, elementary sulphur) as the final electron acceptors in respiration processes (Postgate, 1984; Gibson, 1990).

Equally hazardous wastes as phosphogypsum are fluid organic wastes, *e.g.* distillery decoctions. They originate during the production of ethyl alcohol from rye and potatoes. In order to be applied, as the fluid phase to dissolute phosphogypsum, fluid organic wastes should contain organic compounds available for the SRB; nitrogen and a low content of sulphates. The main organic components of decoctions are proteins, pectines, cellulose and fibres (Mayer and Hillebrandt, 1997). They represent a group of organic compounds rather poorly available for the SRB due to the fact that the sulphate reducing bacteria do not produce hydrolytic enzymes. At present the only one species of SRB, *Archeoglobus fulgidus* is known to take part in the hydrolysis process, as it produces amylase (Labes and Schonheit, 2002). The organic components of distillery decoctions after initial fermentation by the accompanying microflora undergo transformations into alcohols or organic acids, being a good source of carbon for SRB (Szewczyk and Pfennig, 1990). The simultaneous biodegradation of two industrial wastes such as phosphogypsum and distillery decoctions would be an interesting solution from the economical point of view. Costs linked with simultaneous biodegradation of two industrial wastes, hazardous to the environment, are always lower than in the case of two separate biodegradation processes.

The main focus of the presented research was obtaining a sulphidogenic microorganisms community containing SRB and testing the possibility of phosphogypsum biotransformation in stationary cultures with simultaneous treatment of distillery decoctions.

Experimental

Materials and Methods

Phosphogypsum. The studied sample of phosphogypsum was collected from a waste dump from Wizów near Bolesławiec in Lower Silesia (Table IA).

Microorganisms. Community of anaerobic bacteria isolated from soil polluted by aircraft fuel and autochthonic microflora from distillery decoctions.

Media. (a) Modified Postgate medium (Postgate, 1984), in which Na_2SO_4 (4.5 g/dm^3) was replaced by phosphogypsum (5.0 g/dm^3). The source of carbon in the medium was ethanol (3 cm^3/dm^3). (b) Potato and rye distillery decoctions. The standard composition of distillery decoctions is presented in Table IB. Resasurine in the concentration 0.001 g/dm^3 was added to all cultures.

Table I
Chemical composition of phosphogypsum (A) and distillery decoctions (B)

A. Phosphogypsum

Component (% weight)	Kowalski *et al.* (1990) – Wizów
SO_3	42.18
CaO	29.61
P_2O_5	2.24
SrO	1.62
SiO_2	0.65
F	0.5
Al_2O_3	0.24
Na_2O	0.37
Fe_2O_3	0.14
K_2O	0.10
BaO	0.03
MgO	0.05
H_2O cryst.	20.18

B. Distillery decoctions

Component (% weight)	potato	rye
dry mass	5.6	8
ash	0.7	0.3–0.5
starch	–	–
other polysaccharides	2.9–3.2	4.0–4.5
fibres	0.4–0.7	0.5–1.1
proteins	1.2–1.5	1.7–3.5
fats	0.04	0.5

Culture conditions. The cultures were enriched in 100 or 300 cm^3 volume bottles, tightly sealed with rubber plugs, with permanently attached needles with syringes. The relation of the inoculum to the medium was 1:10. The cultures were incubated in temperature of 30° or/and 55°C.

Enrichment of microorganisms. The microorganisms were enriched from soil polluted by aircraft fuel using the microcosm method. The soil suplemented with phosphogypsum (5 g/dm^3) was placed in 100 cm^3 boxes, and next the Postgate medium with ethanol was added as the only source of carbon. The boxes were tightly sealed and incubated for 6 weeks in temperature of 30° and 55°C in order to select anaerobic sulphidogenic consortions able to biotransform phosphogypsum.

Measurements. The following parameters were determined in the cultures: sulphides using the iodometric method, sulphates using the bar method and emission spectrometry method (induction excitation in an ICP medium), COD using the bi-chromate method. The reaction (pH) of the culture was corrected using 0.1N HCl or 0.1N NaOH.

The post-culture deposits were analysed with the radiography method using a DRON-2 diffractometer.

Results and Discussion

Isolation and enrichment of SRB from soil polluted by aircraft fuel. The most characteristic environments in which SRB occur are marine sediments (Boopathy *et al.*, 1998; Kuever e*t al.*, 1999), where the concentration of sulphates typically reaches an average of 28 mM (de Wit, 1992), oil fields and oil reservoirs (Voordouw *et al.*, 1996; Telang *et al.*, 1997; Jenneman and Gevertz, 1999; Magot *et al.*, 2000; Gieg and Suflita, 2002), as well as environments polluted by oil-derived products (Wolicka and Kowalski, 2005). The analyses were preceeded by the enrichment of an active community of anaerobic microorganisms in conditions favouring the selection of SRB.

The microorganisms were isolated from soil polluted by aircraft fuel. After 6-week incubation, the enrichment of selected anaerobic communities of microorganisms began on a Postgate medium with ethanol and phosphogypsum. The obtained sulphidogenic microorganisms community (ethanol community) was passaged 5 times. Each passage lasted for 14 days. The maximal content of sulphides, 720 mg HS^-/dm^3,

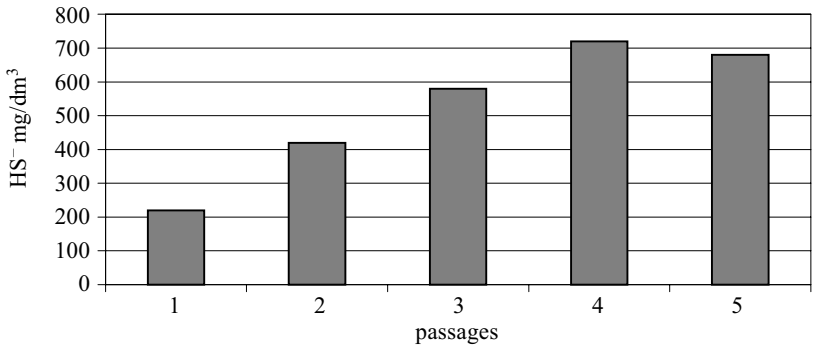

Fig. 1. Maximal concentration of sulphides obtained in the following passages of the ethanol culture

was observed in the 4th passage of the culture, what corresponded to the reduction of 2030 mg SO_4/dm^3, *i.e.* 46% of phosphogypsum introduced into the medium (Fig. 1).

Enrichment of bacterial communities on distillery decoctions. The isolated ethanol community was used as the inoculum to establish cultures on potato and rye distillery decoctions. Ten cultures were established: 4 on potato distillery decoctions (P) and 4 on rye distillery decoctions (R). Control cultures (C), cultures no 5 and 10, were cultured on a Postgate medium with ethanol inoculated with the ethanol community. They were managed in two variants: with and without pH regulation in the medium. All cultures were passaged 6 times in order to adapt the microorganisms to the distillery decoctions environment, and also in order to select a bacterial community able to actively biotransform phosphogypsum. The obtained results are presented in Table II.

As shown in Table II, the activity of the selected microorganisms in the enriched cultures depends on three factors: temperature, pH and type of decoctions. Higher concentrations of HS^- were observed in cultures where the pH during the incubation was optimal for the SRB and reached 6.6–7 (cultures no 1 and 3). In these cultures the content of HS^- reached over 380 mg HS^-/dm^3. In cultures no 2 and 4, in which the pH values were not corrected (pH 4.8–5.2) was observed lower activity of the SRB. Only some SRB can be active in media with such pH; they include bacteria growing in acidic mine waters (Elliot *et al*., 1998).

Analysing the data from Table II it can be concluded that higher values of HS^- were obtained in mesophilic cultures. In cultures no 1 and 3 a similar concentration of HS^- was obtained – 612 and 610 mg/dm^3, what corresponds to the reduction of 1726 and 1720 mg SO_4/dm^3 and 34.5% phosphogypsum/dm^3 in relation to 5 g/dm^3 introduced into the medium. In cultures of thermophilic communities of microorganisms, regardless the type of applied decoctions and the pH value in the medium, high concentrations of HS^- were not observed. The maximal content of HS^- – 280 mg/dm^3 was observed in cultures no 6 and 8, what

Table II
Maximal concentration of HS^- (mg/dm^3) in the following passages of the cultures on distillery decoctions

Type of medium		No of culture	pH	Incubation temp. (°C)	No of passage					
					1	2	3	4	5	6
					HS^- (mg/dm^3)					
Decoctions	P	1	reg.	30	448	460	495	612	568	497
		2	n. reg.	30	320	280	280	220	200	220
	R	3	reg.	30	380	470	520	610	580	540
		4	n. reg.	30	472	442	408	529	612	529
	C	5	n. reg.	30	380	420	470	628	640	620
	P	6	reg	55	220	180	240	280	220	180
		7	n. reg.	55	220	180	120	120	160	180
	R	8	reg	55	180	200	190	280	220	200
		9	n. reg.	55	180	180	160	120	100	120
	C	10	n. reg.	55	180	220	200	200	180	160

C – control cultures; reg. – pH regulation of the medium; n.reg. – without pH regulation of the medium

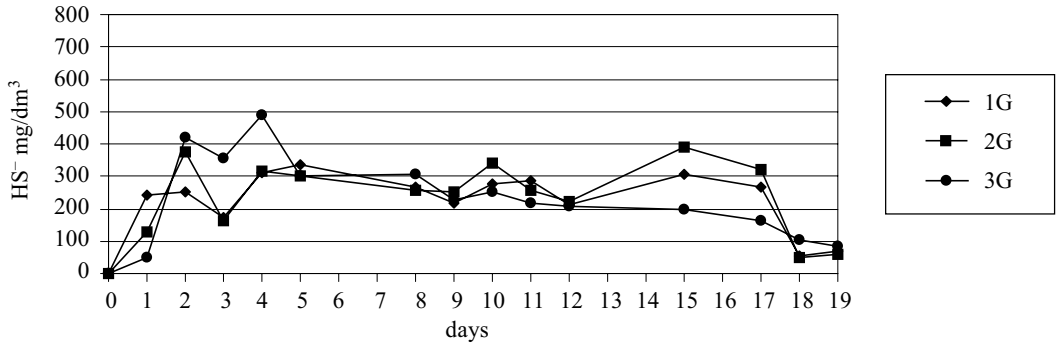

Fig. 2. The content of HS^- obtained in cultures of bacterial communities on distillery decoctions

corresponded to the reduction of 790 mg SO_4/dm^3 and 15.8% phosphogypsum/dm^3 in relation to 5 g/dm^3 introduced into the medium.

Regardless the applied decoction (potato or rye), the activity of the isolated bacteria was comparable.

Influence of the inoculum on the activity of phosphogypsum biotransformation in distillery decoctions. The next stage of the study was focused on testing the influence of the applied inoculum on the effectiveness of the phosphogypsum biotransformation process in distillery decoctions. In this case the most active microorganism community no 1 was used as the inoculum to establish a culture on distillery decoctions. Three cultures were established: 1G – on barren potato decoctions inoculated with the inoculum; 2G – on non-barren decoctions inoculated with the ethanol inoculum. Culture 3G comprised non-barren non-inoculated potato decoctions (control culture). All cultures contained phosphogypsum as the only electron acceptor. The production of sulphides obtained in these cultures is shown in Fig. 2.

The increase of concentration of hydrogen sulphide was observed in all cultures. The highest concentration of HS^- – 488 mg/dm^3 was noted in culture 3G, on non-barren non-inoculated decoctions. In 1G and 2G similar values of 336 and 343 mg/dm^3 of HS^- were noted. This corresponded to the reduction of about 55%, 38% and 39%, respectively, of phosphogypsum in relation to 5 g/dm^3 introduced into the medium.

The costs linked with simultaneous biodegradation of two industrial wastes, particularly hazardous to the environment, are always lower than for each of these wastes treated separately. Therefore, the removal of organic wastes from decoctions is equally important as the biotransformation of phosphogypsum. The COD of distillery decoctions was high and reached 2.2 g O_2/dm^3, whereas the content of sulphates was rather low – 50 mg/dm^3. Addition of phosphogypsum to distillery decoctions makes them high-sulphur wastes as in the case of wastes from the production of citric acid from sugar cane (2.9 g SO_4/dm^3). The calculated ratio COD/SO_4 reached 8.8. If this ratio is higher, then more organic compounds are decomposed during the formation of methane. At COD/SO_4 >10 sulphides are not produced (Li and Humphre, 1989; Oude Elferinck *et al.*, 1995). It is assumed that the co-existence of the two groups of microorganisms is possible when COD/SO_4 ratio is between 1.7 and 2.7; below 1.7 the SRB dominate (Clancy *et al.*, 1992). The reduction of COD and biotransformation of phosphogypsum in the particular cultures are presented in Fig. 3.

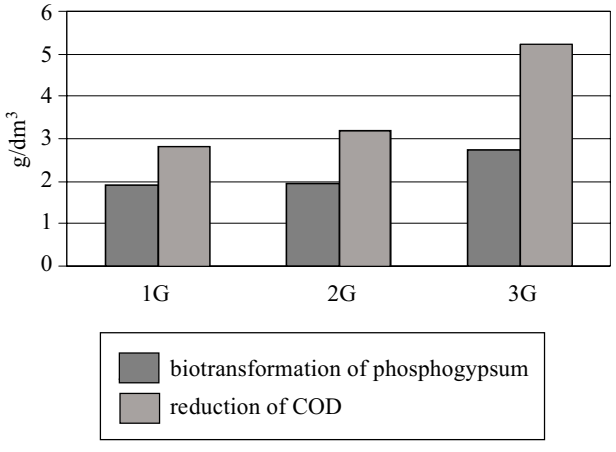

Fig. 3. Reduction of COD and biotransformation of phosphogypsum in cultures on distillery decoctions

It can be assumed that addition of a selected anaerobic inoculum comprising a community of microorganisms to the decoctions did not increase the effectiveness of phosphogypsum transformation. Community 3G comprising non-barren non-inoculated distillery decoctions most effectively took part in the biotransformation process (55% reduction of phosphogypsum) with simultaneous purification of the distillery decoctions (24% reduced COD). Calculations showed similar results for cultures 1G and 2G: 38% reduction of phosphogypsum and about 13.5% reduction of COD. The activity of SRB in COD reduction was 22.7% in culture 1G, 22.2% in 2G and 17.7% in 3G. The obtained results of a *ca.* 20% activity of SRB in the utilisation of organic compounds are similar to those obtained by Gupta *et al.* (1994) in bioreactors purifying sewage.

After incubation the post-culture deposits were separated. The observed reduction of the phosphogypsum mass was: 15% for culture 1G (0.9 g/dm^3), 2G – 20% (1 g/dm^3) and 3G – 55% (2.75 g/dm^3). Diffractometric analyses did not show any substantial phase changes in the sediment in comparison to phosphogypsum.

In conclusion the community of bacteria isolated from soil contaminated by air fuel contained SRB capable of the biotransformation of phosphogypsum. It can be also stated that the biotransformation of phosphogypsum in cultures in distillery decoctions is possible but is not very effective and its optimization requires further research.

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