The Investigation of Sulphides Composition Created by Bioprecipitation

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Abstract

The objective of this work was to examine the structure and characteristics of biogenic iron sulphides, created by sulphate-reducing bacteria (SRB) cultivation under various conditions.

SRB are anaerobic microorganisms characterized by the ability to perform dissimilatory sulphate reduction with the simultaneous oxidation of the organic substrates. By oxidizing low molecular weight organic compounds (e.g. lactate, acetate) they obtain energy and nutrients. Bacteria reduce sulphate to hydrogen sulphide and this reacts with certain metals dissolved forming insoluble precipitates in process which is called bioprecipitation.

The initial sulphide phase is very often amorphous with poor crystallinity. According to the conditions, solution composition and with longer time more stable and crystalline sulphides can be formed.

The precipitates studied in this paper were synthesized in reagent bottles with SRB culture and modified growth medium at 30°C, in 2 modes, under anaerobic conditions during 10 months. The modification consisted of iron ions addition in form of sulphates and double organic substrate dose. During batch and semi-continuous modes were created 4 biogenic iron sulphides samples. They were examined by EDX, SEM and XRD.

The analyses revealed how variety in bacteria cultivation (nutrient medium compound, cultivation process) reflects in crystallinity, structure, particle size and composition. EDX results confirmed the presence of iron and sulphur as a major part in all samples. XRD showed mainly amorphous or poorly crystalline precipitates (with partial mackinawite and gregite occurrence).

Keywords: iron minerals, bioprecipitation, sulphate-reducing bacteria, cultivation conditions

Introduction

Iron sulphides form in diverse anoxic environments. In freshwater and marine sediments they are the predominant precipitates and their biological origin largely stems from the preceding biological reduction of sulphate (Gramp et al., 2010). They can be formed mainly as a result of the metabolic activity of sulphate-reducing bacteria (SRB). Anaerobic processes based on the SRB use are able to utilize sulphate as an electron acceptor and to form hydrogen sulphide which leads to an increase in the pH of the water and the precipitation of heavy metals, forming insoluble sulphides (Jimenez-Rodriguez et al., 2009).

The initial precipitate is commonly referred to as amorphous iron sulphide. It exhibits poor crystallinity in the form of small crystal sizes and short-range crystal order. Within days, this initial precipitate develops incipient long-range order, and eventually crystallizes to the more stable mackinawite (Herbert et al., 1998). In most sediments they occur as finely dispersed precipitates and as coatings on other minerals, making identification difficult in heterogeneous mixtures. In some cases the initial iron sulphide precipitate was reported to contain greigite alongside mackinawite. It is generally assumed that greigite does not form directly from solution but converts from the pre-existing mackinawite (Csákberényi-Malasics et al., 2012; Watson et al., 2000). Final biogenic sulphides products are strong dependent on solution chemistry. Primarily on redox conditions, the first precipitate may react to form more stable phases such as greigite, marcasite, pyrite and pyrrhotite (Mokone et al., 2010; Larrasoana et al., 2007; Benning et al., 2000). Although many studies were centred on the formation and phase transitions of iron sulphides from the initial precipitate into crystalline form (mackinawite, greigite, etc.), the exact structure, the changes in crystal morphologies, the particle sizes and the physical properties are not satisfactory known (Csákberényi-Malasics et al., 2012). Despite this was found out that iron sulphide minerals can be used in soil or water remediation: they were shown to effectively immobilize heavy metals and toxic ions through sorption mechanisms (Renock et al., 2009; Liu et al., 2008; Mullet et al., 2004; Watson et al., 1995).

The purpose of this work was to better understand the structure of the iron sulphides synthe-

Tab. 1. The conditions for biogenic iron sulphides production

Tab. 1. Warunki biogenicznego powstawania siarczków żelaza

Sample	Medium modification	Cultivation mode
1	$FeSO_4.7H_2O$ addition	Batch
2	$FeSO_4.7H_2O$ and $Fe_2(SO_4)_3.9H_2O$ addition	Batch
3	$FeSO_4.7H_2O$ addition	Semi-continuous
4	$FeSO_4.7H_2O$ and $Fe_2(SO_4)_3.9H_2O$ addition	Semi-continuous

Tab. 2. The particle size characteristics of suspensions

Tab. 2. Skład ziarnowy pyłów zawieszonych

Sample	Mean	Median	Mode	Standard deviation
	[μm]			
1	3.849	4.231	10.18	2.00
2	3.286	3.992	6.617	2.23
3	4.311	4.922	10.53	2.01
4	4.082	4.531	11.53	1.99

Tab. 3. Selected parameters of biogenic iron sulphides

Tab. 3. Wybrane parametry biogenicznych siarczków żelaza

Sample	pH	Dry weight [g]
1	6.50	0.501
2	6.48	0.613
3	6.57	3.084
4	6.28	3.538

sized in cultures of sulphate-reducing bacteria under various laboratory conditions and growth media modifications, which should be used in the next research activities as a heavy metal sorbents.

Materials and methods

Sulphate-reducing bacteria

A mixed culture of sulphate-reducing bacteria (with predominant genus Desulfovibrio) was isolated using nutrient medium Postgate C from mineral water collected at Gajdovka spring (Košice, Slovakia).

It is water with pH 7.5, H_2S odour and with natural content of SRB. Bacteria were maintained at 30°C in glass reaction flasks in anaerobic conditions that had been generated by introducing an inert gas (N_2) and chemically with sodium thioglycollate.

Biogenic iron minerals creation

Iron sulphide precipitates production took under anaerobic conditions at 30°C in modified Postgate C medium 10 months. It is a chemically defined growth medium suitable for SRB cultivation.

The modification consist of an addition of Fe ions in form of sulphates ($FeSO_4.7H_2O$, $Fe_2(SO_4)_3.9H_2O$) and a double dose of sodium lactate as carbon and energy source. The precipitates creation was followed during two modes. The bacteria cultivation parameters during experiments are in Table 1.

The first – "batch mode" was running without fresh nutrient medium addition (sample 1 and 2) all the time. Experiments were performed in duplicate using 1000 ml glass bottles containing 900 ml of growth medium (pH around 7.5) and 100 ml of bacteria inoculum. After inoculation, the bottles were sealed with butyl rubber stoppers and stored in thermostat at 30°C for 10 months. Blank controls (without bacteria) were carried out in the same conditions.

The second mode – "semi-continuous" consisted of several phases. In some predetermined weeks (3, 5, 7, 15, 17 and 19) the fresh medium was supplied to the bottles. The other weeks were identical to batch mode, without a medium exchange until 10 months of experiment duration. The bottles were all the time stored in thermostat at 30°C (sample 3 and 4). Abiotic controls were carried out in the same conditions.

At the end some liquid samples were taken out from each bottle for analyses (pH measurement, hydrogen sulphide presence, particle size distribution determination). The solids were separated from the suspensions by centrifugation at 10000 rpm for 10 minutes, washed once with degassed distilled water and centrifuged again. The samples were consequently freeze-dried for 48–72 hours and stored in a vacuum desiccator. Dry powders were then studied by XRD, EDX, SEM.

Samples characterization

The structures, compositions, particle sizes and morphologies of the precipitated minerals were examined using X-ray powder diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX).

X-ray powder diffraction data were collected over an angular range $10 < 2\Theta < 80°$ with steps 0.05° using a Bruker D8 Advance diffractometer, working with the Cu Kα radiation and equipped

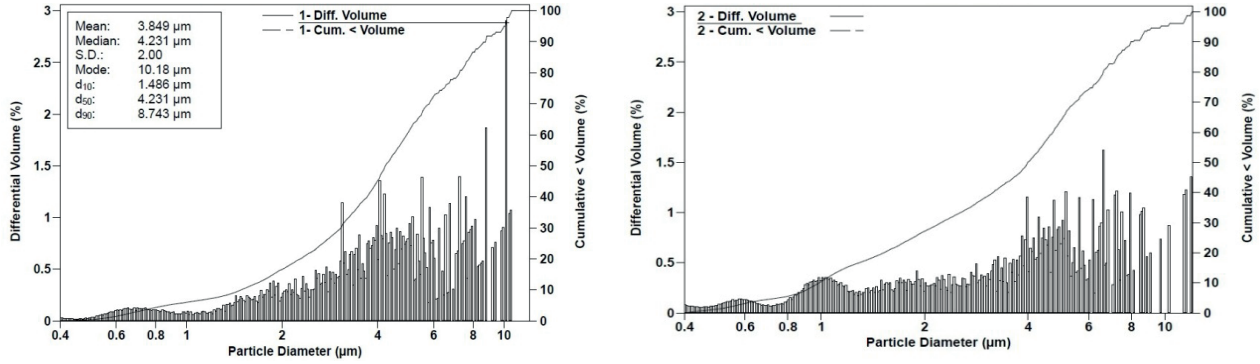

Fig. 1. and 2. Differential and cumulative volume distribution for sample 1 and 2

Rys.1. i 2. Rozkład objętości dla próbek 1 i 2

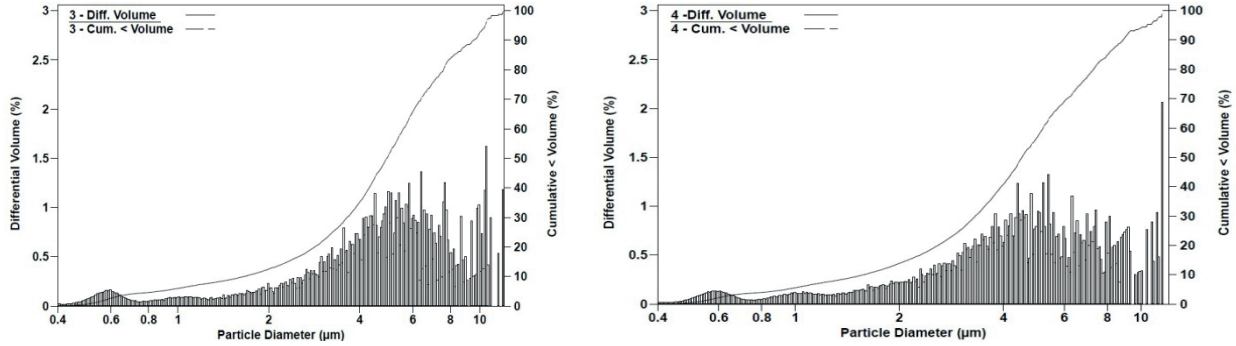

Fig. 3. and 4. Differential and cumulative volume distribution for sample 3 and 4

Rys. 3. i 4. Rozkład objętości dla próbek 3 i 4

with a secondary graphite monochromator. Diffraction patterns were treated with the Diffracplus Basic analysis program.

The SEM studies were performed using a SEM Tescan MIRA 3 FE microscope equipped with energy dispersive X-ray microanalysis system (EDX – Oxford Instruments). The sample was placed on adhesive carbon slice and carbon coated.

The Beckman Coulter Multisizer™ 4 provided particle size distributions of suspensions during bacteria cultivation in number and volume.

Results and discussion

Iron sulphides formation

Regular visual control and hydrogen sulphide examination done during experiments duration confirmed the creation of biogenic iron sulphides in both medium modifications and cultivation modes. The visual controls proved the formation of black precipitates ("FeS") in all nutrient mediums. The SRB occurrence was confirmed by light microscope. The hydrogen sulphate presence was positive.

In blank samples were created grey or grey-green precipitates, hydrogen sulphide presence was negative and no bacteria were attended.

Structure and particle size of precipitates

The suspensions were after 10 months of precipitates creation analysed. Particle size distributions in volume of generated particles were provided by Beckman Coulter Multisizer™ 4 in a sizing range of 0.4 to 12 µm. The statistic data from the measurements are in Table 2. Figures 1–4 show differential and cumulative distribution of each sample.

The pH measurements of liquid phase after bacteria cultivation were realized to find out what a change of pH happened in comparison with initial value 7.5. Consequently, the solids were separated from the suspensions by centrifugation, freeze-dried and dry samples were weighted. Results are in Table 3.

The nutrients addition (during 6 medium exchanges) supported bacterial activity, hydrogen sulphide production and precipitates creation. This all caused an increase in the mass amount of generated precipitates of samples 3 and 4, which were much higher than in sample 1 and 2.

SEM observations of biogenic precipitates revealed particle sizes from low micrometer range to tens of micrometers, but also bigger aggregates were present (Figures 5–8). Very probably, there are small aggregates of biogenic iron sulphide

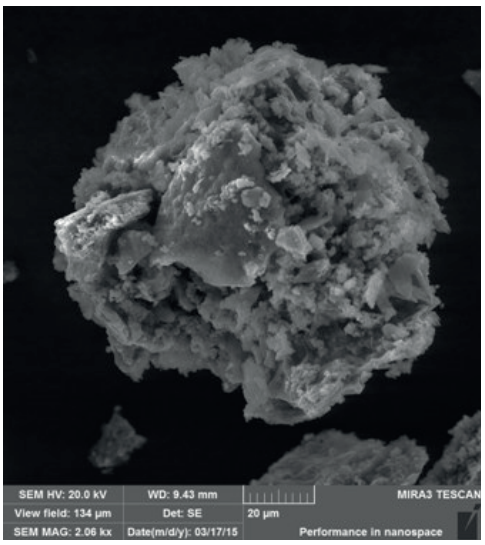

Fig. 5. Biogenic sulphide precipitate – sample 1 (SEM)
Rys. 5. Osad siarczków biogenicznych – próbka 1 (SEM)

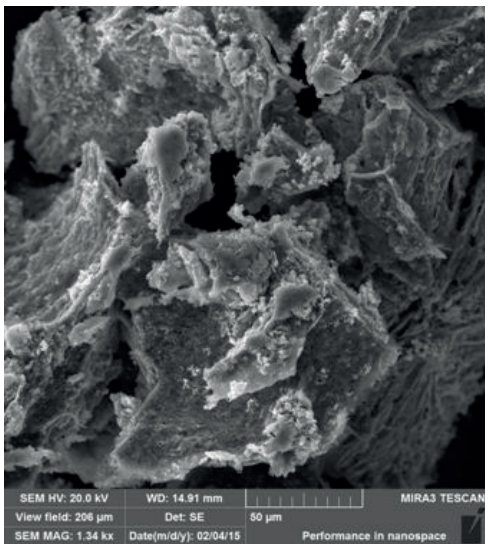

Fig. 6. Biogenic sulphide precipitate – sample 2 (SEM)
Rys. 6. Osad siarczków biogenicznych – próbka 2 (SEM)

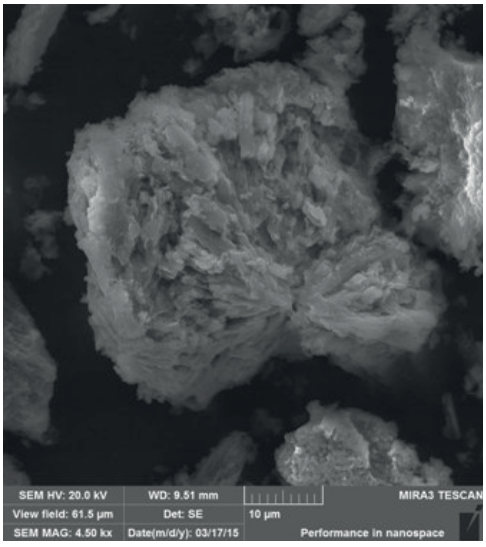

Fig. 7. Biogenic sulphide precipitate – sample 3 (SEM)
Rys. 7. Osad siarczków biogenicznych – próbka 3 (SEM)

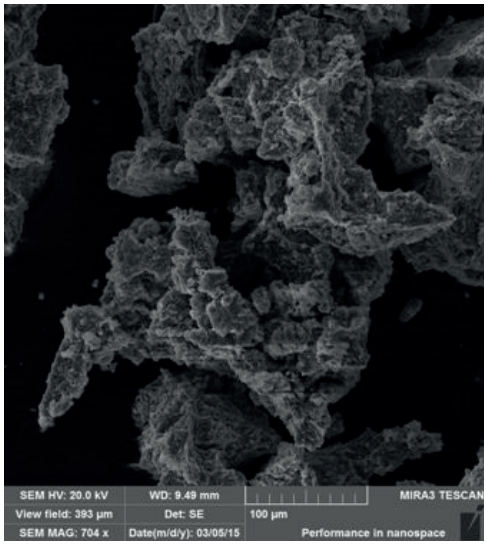

Fig. 8. Biogenic sulphide precipitate – sample 4 (SEM)
Rys. 8. Osad siarczków biogenicznych – próbka 4 (SEM)

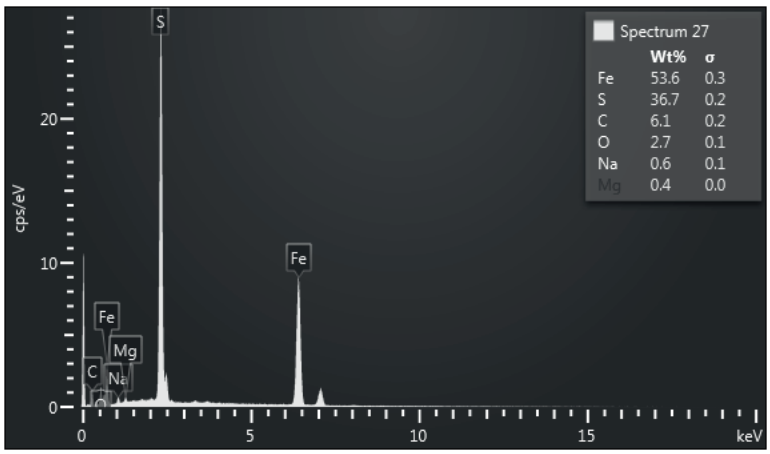

Fig. 9. EDX spectrum of biogenic iron mineral sample
Rys. 9. Spektrum EDX biogenicznych minerałów żelaza

Tab. 4. EDX analyses of biogenic precipitates

Tab.4. Analiza EDX osadów biogenicznych

Element		Sample							
		1		2		3		4	
Fe	[Wt%]	53.6	41.8	53.2	57.0	40.9	51.4	53.2	49.0
S		36.7	33.7	30.3	38.0	34.9	38.4	37.4	34.0
C		6.1	14.2	11.1	1.3	15.6	6.0	4.1	11.5
O		2.7	5.0	2.8	1.2	5.4	2.1	2.6	2.6
N		-	1.9	-	-	-	-	-	0.7
Na		0.6	1.4	1.7	0.9	1.1	1.2	1.4	1.1
Mg		0.4	0.5	0.3	0.3	0.7	0.3	0.4	0.3
K		-	0.4	0.5	0.8	0.6	0.5	0.5	0.6
Ca		-	0.4	0.2	0.3	0.3	0.2	0.3	0.2
Cu		-	0.6	-	-	0.5	-	-	-

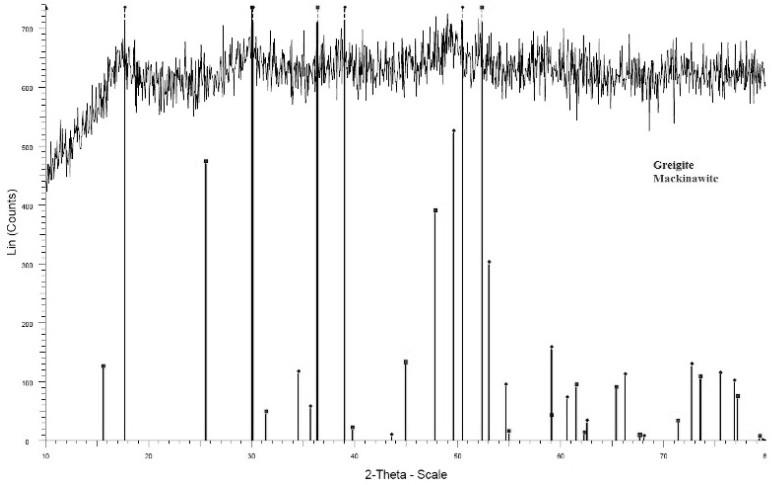

Fig. 10. XRD of precipitates in sample 1

Rys. 10. Analiza XRD osadu w próbce 1

minerals, particles of sulphur, biomass and nutrient medium component remains as noted by the subsequent analysis.

By using a microscope equipped with energy dispersive X-ray microanalysis system we studied the chemical compositions of precipitates (Fig. 9). Several spectra in each sample were monitored. Percentages by weight of elements in analyzed samples are resumed in Table 4. The differences in results can by caused by a certain heterogeneity of precipitates, depending on whether only "FeS" particles were analyzed or there were remains of biomass and elements from the culture medium in the studied spectrum.

Figure 10 illustrates XRD pattern for sample 1. The structure of the biogenic iron precipitates reported in the literature was either as amorphous or as consistent with that of crystalline mackinawite (Herbert et al., 1998). Greigite forms only when conditions are not completely anoxic. It was also noted the material could be extremely fine-grained and aggregated, and therefore difficult to characterize. Diffraction patterns of all our samples confirmed mainly amorphous or poorly crystalline precipitates (with mackinawite and gregite occurrence).

Conclusion

The aim of this paper was oriented on the study of biogenic iron sulphides synthesized with SRB culture under specific cultivation modes and growth media modifications. We attempted to determine particle sizes, chemical composition, structure and crystallinity of created samples by various methods, which revealed that the material is a complex of aggregates consisting of iron sulphide minerals structures with remains of biomass and elements from growth media. XRD analysis confirmed the beginning of mackinawite and greigite creation, with enormous amorphous part in each sample.

Acknowledgements

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Badanie składu siarczków powstałych na wskutek biosedymentacji

Celem niniejszej pracy było przebadanie struktury i właściwości biogennych siarczków żelaza, pochodzących z hodowli bakterii redukującej siarczany (ang. skrót SRB), w różnych warunkach. SRB to beztlenowe mikroorganizmy charakteryzujące się zdolnością do dysymilacyjnej redukcji siarczanów z jednoczesną oksydacją organicznych substratów. Dzięki utlenianiu organicznych związków o niskiej masie cząsteczkowej (np. mleczany, octany) otrzymują energię i wartości odżywcze. Bakterie redukują siarczan do siarczku wodoru, który reaguje z niektórymi rozpuszczonymi metalami tworząc nierozpuszczalny osad w procesie zwanym biosedymentacją. W początkowej fazie siarczek jest często amorficzny i o niskiej krystaliczności. W zależności od warunków, właściwości roztworu i dłuższego czasu można formować stabilniejsze i bardziej krystaliczne siarczki.

Osad badany w niniejszej pracy został syntetyzowany w butlach z odczynnikiem i z kulturą bakterii SRB i zmodyfikowanym środkiem wzrostu w temperaturze 30°C, w dwóch trybach, w warunkach beztlenowych przez okres 10 miesięcy. Modyfikacja polegała na dodaniu jonów żelaza w formie siarczanów oraz podwójnej dawce subtratów organicznych. Podczas trybów półciągłego utworzono 4 biogenne próbki siarczków. Zostały poddane badaniom w EDX, SEM oraz XRD.

Analizy wykazały w jaki sposób hodowla bakterii (związek wartości odżywczych, proces hodowli) odzwierciedla się w procesie krystalizacji, strukturze, wielkości cząsteczki i właściwościach. Badanie EDX potwierdziło obecność żelaza i siarki, jako głównych związków we wszystkich próbkach. Wyniki XRD pokazały głownie amorficzne lub nisko skrystalizowane osady (częściowo z makinawitem i greigitem).

Słowa kluczowe: minerały żelaza, bioosadzanie, bakterie redukujące siarke, warunki wzrostu