The Examination of Biogenic and Non-Biogenic Iron Precipitates Created by Hydrogen Sulphide

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Summary

Metabolism of sulphate-reducing bacteria (SRB) generally consists of organic substrates or molecular H2 oxidation and sulphates reduction under anaerobic conditions. By oxidizing low molecular weight organic compounds (e.g. lactate, acetate) they obtain energy and nutrients. Biological-chemical process based on the ability and activity of these bacteria to reduce sulphates results in hydrogen sulphide creation which binds with metal cations in solutions to insoluble precipitate forms.

In anoxic sedimentary environment with iron redundancy the amorphous iron sulphide is often the initial sulphide phase to be formed and exhibits poor crystallinity in the form of small crystal sizes and short-range crystal order. Although many studies have focused on the mineralogy and surface chemistry of the more crystalline and more thermodynamically stable sulphides such as pyrite and pyrrhotite, an understanding of the crystal habit and surface chemistry of the more poorly crystalline Fe sulphides is critical in assessing their reactivity and the potential for transformation to more stable phases.

The aim of this work was to study the properties and composition of biogenic precipitated materials synthesized in reagent bottles with SRB culture under specific laboratory conditions and modified growth media. The modification consists of iron ions addition in form of sulphates and organic substrate dose. In two cultivation modes were by bacterially produced hydrogen sulphide precipitated 4 biogenic samples. The realisation of abiotic control experiments without SRB results in 4 non-biogenic samples production. All created materials were examined by EDX, SEM and XRD. The composition of samples varies according to cultivation and growth media composition. The biogenic precipitates contain greigite, mackinawite and sulphur, non-biogenic samples consist of vivianite.

Keywords: biogenic precipitates, sulphate-reducing bacteria, hydrogen sulphide

Introduction

Sulphate-reducing bacteria (SRB) are anaerobic heterotrophs that utilize a range of organic substrates and sulphates as terminal electron acceptors that are reduced to hydrogen sulphide during bacterial sulphate reduction (Odom and Singleton, 1993). The hydrogen sulphide produced from sulphate reduction plays a major role in metal sulphides immobilization in sediments but has also been applied to bioremediation of metals in waters and leachates (Cao et al., 2009; Skousen et al., 1998).

In freshwater and marine sediments the predominant precipitates are iron sulphides and their biological origin largely stems from the preceding biological reduction of sulphate (Gramp et al., 2010).

Often the initial iron sulphide phase to form in anoxic sedimentary environments is 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 the amorphous iron sulphide and mackinawite occur as finely dispersed precipitates and as coatings on other minerals. Heterogeneous mixtures make mineral identification difficult (Fečko et al., 2009).

Previous studies on biogenic iron sulphide formation in sedimentary pyrite formation have identified poorly crystalline mackinawite and greigite as major solid phases (Schoonen, 2004). Also many intermediate iron sulphides may exist between disordered mackinawite and well crystallized pyrite in anaerobic environments, including greigite, marcasite, smythite and pyrrhotite (Donald and Southam, 1999; Gramp et al., 2010). Final biogenic sulphides products are strong dependent on solution chemistry (Benning et al., 2000; Mokone et al., 2010).

The purpose of this work was to identify iron sulphides synthesized in cultures of sulphate-reducing bacteria under various laboratory conditions and growth media modifications. X-ray diffraction (XRD) was used as the primary technique to identify the crystallized synthesis products. Other analyses of samples were provided by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX).

Materials and methods

Bacteria

A mixed culture of sulphate-reducing bacteria was isolated from mineral water collected at Gajdovka spring (Košice, Slovakia). The SRB were grown in a Postgate C medium. The predominant genus in mixed cultures of SRB is usually Desulfovibrio. Bacteria were maintained at 30°C in anaerobic conditions that had been generated by introducing an inert gas (N_2) and chemically with sodium thioglycollate.

Biogenic precipitates

The precipitates in form of iron sulphides were created in a chemically defined growth medium for SRB cultivation - Postgate C. In order to prepare and to compare different biogenic samples the medium was modified. The modification consists of an addition of Fe ions in form of sulphates ($FeSO_4.7H_2O$, $Fe_2(SO_4)_3.9H_2O$). The growth medium contains double dose of sodium lactate as carbon and energy source.

The production was performed in two modes (batch and semi-continuous) under anaerobic conditions.

The first mode – "batch" was running without addition of fresh nutrient medium (samples A and B).

All experiments were performed in duplicate using 1000 ml glass bottles containing 900 ml of growth medium and 100 ml of bacteria inoculum with pH around 7.5. After inoculation, the bottles were sealed with butyl rubber stoppers and stored in thermostat at 30°C for 3 months. After cultivation precipitates were filtered through 0.85 μm membrane filter, dried and stored in anaerobic conditions. Dry samples were used for further analyses and experiments. In the same conditions but without SRB (samples ABIO A and ABIO B) were abiotic controls performed. Solid phase from abiotic control was filtered through 0.85 μm membrane filter, dried and stored in anaerobic conditions, too.

During the second mode – "semi-continuous" the fresh medium was supplied to the bottles every 7 days during the first month of experiment duration (samples C and D). Next 2 months were identical to batch mode. The bottles were stored in thermostat at 30°C. After cultivation precipitates were filtered through 0.85 μm membrane filter, dried and stored in anaerobic conditions. Dry samples were used for further analyses and experiments. Abiotic control was performed in the same conditions without SRB (samples ABIO C and ABIO D). Solid phase from abiotic control was filtered through 0.85 μm membrane filter, dried and stored in anaerobic conditions.

The modifications of the medium composition and other cultivation parameters are described below in Table 1.

Tab. 1 The conditions for biogenic precipitates creation

Tab. 1 Warunki tworzenia biogennych wytrąceń osadów

Sample name	Bacteria	Medium modification	Cultivation mode
A	SRB	$FeSO_4.7H_2O$ addition	Batch
ABIO A	without SRB	$FeSO_4.7H_2O$ addition	Batch
B	SRB	$FeSO_4.7H_2O$ and $Fe_2(SO_4)_3.9H_2O$ addition	Batch
ABIO B	without SRB	$FeSO_4.7H_2O$ and $Fe_2(SO_4)_3.9H_2O$ addition	Batch
C	SRB	$FeSO_4.7H_2O$ addition	Semi-continuous
ABIO C	without SRB	$FeSO_4.7H_2O$ addition	Semi-continuous
D	SRB	$FeSO_4.7H_2O$ and $Fe_2(SO_4)_3.9H_2O$ addition	Semi-continuous
ABIO D	without SRB	$FeSO_4.7H_2O$ and $Fe_2(SO_4)_3.9H_2O$ addition	Semi-continuous

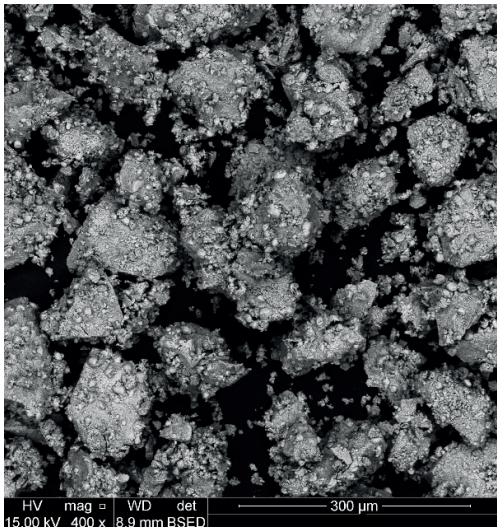

Fig. 1 SEM photo of precipitates created in media containing sulphate-reducing bacteria

Rys. 1 Zdjęcie SEM wytrąceń stworzonych w roztworze bakterii redukujących siarkę

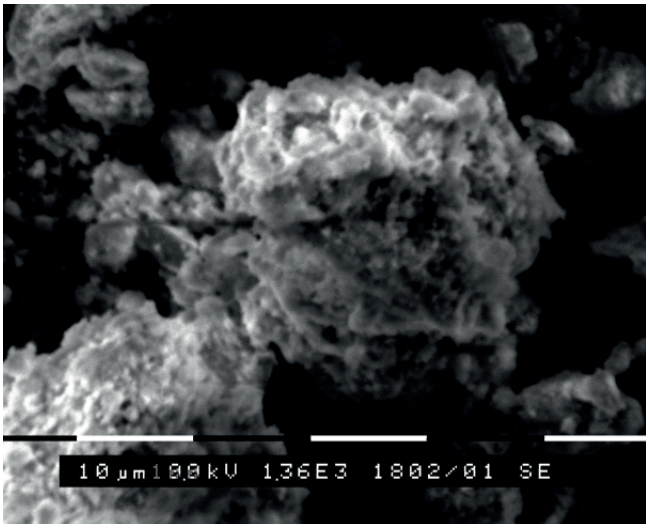

Fig. 2 Biogenic sulphide precipitate (SEM)

Rys. 2 Osad biogenny siarczku (SEM)

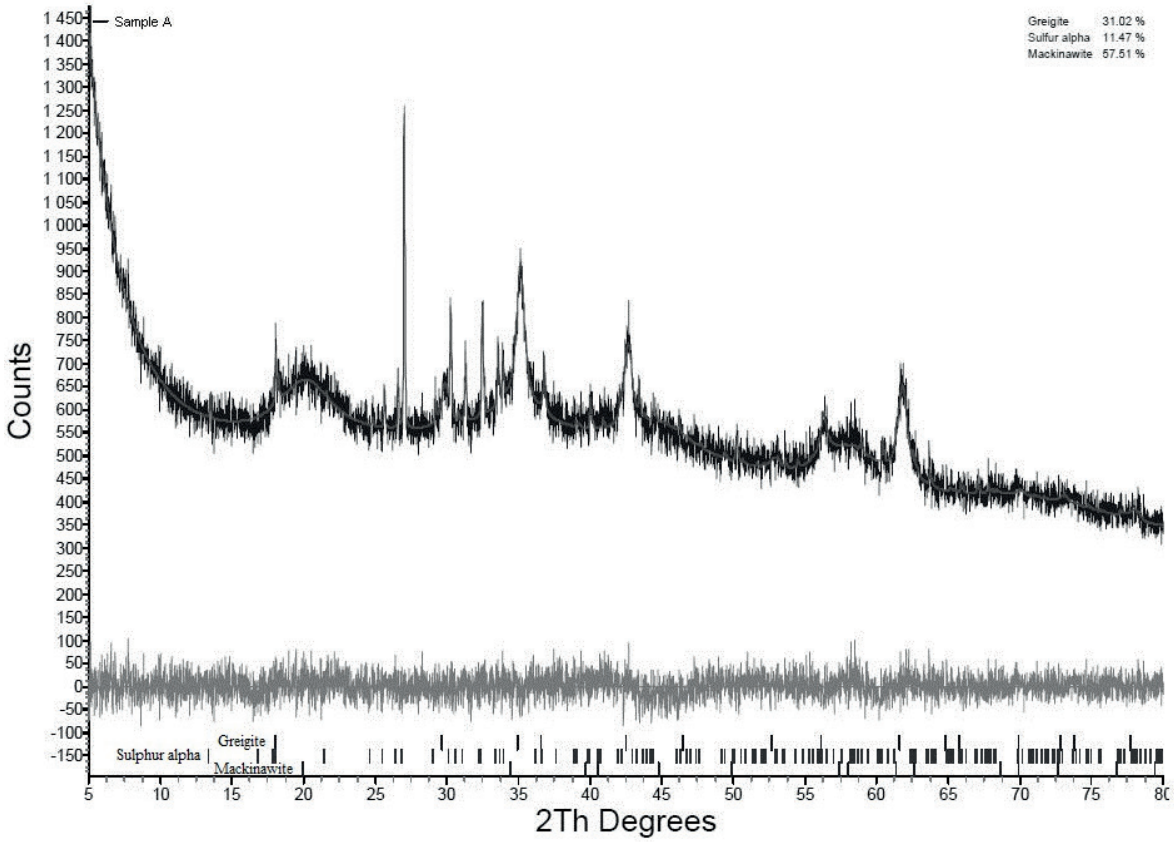

Fig. 3 XRD of produced precipitates in sample A

Rys. 3 XRD wytworzonych wytrąceń w próbce A

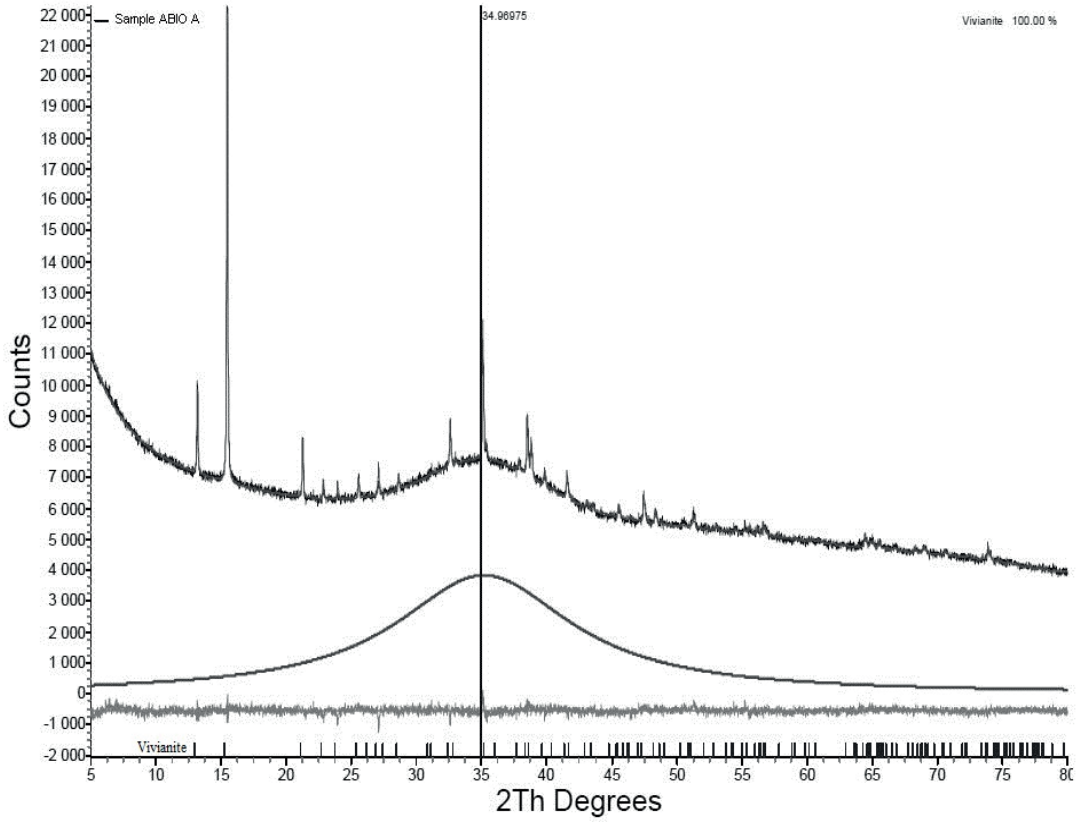

Fig. 4 XRD analysis of precipitates in sample ABIO A

Rys. 4 Analiza XRD wytrąceń w próbce ABIO A

Tab. 2 The composition of precipitates created in media containing SRB

Tab. 2 Skład wytrąceń wytworzonych w roztworze zawierającym BRS

Sample	Compounds		
A	Greigite 31.02%	Sulphur alpha 11.47%	Mackinawite 57.51%
B	Greigite 37.32%	Sulphur alpha 11.53%	Mackinawite 51.15%
C	Greigite 20.68%	Sulphur alpha 14.77%	Mackinawite 64.55%
D	Greigite 20.97%	Sulphur alpha 10.44 %	Mackinawite 68.59%

Analyses

The samples composition was evaluated by using X-ray powder diffraction measurement on Bruker-AXS D8 Advance device and related Bruker software (Conditions for measurements: 2Θ/Θ goniometer, radiation CoKα/Fe, position sensitive detector LynxEye).

SEM observations and analyses were done using the microscope Quanta 650 FEG equipped with EDX, WDA, EBSD and CL detectors.

Results and discussion

The production of biogenic iron sulphides was successful in both medium modifications, as well as in batch and in semi-continuous conditions. During 3 months of experiment duration were provided regular visual control indicated sulphide precipitates creation in bottles and hydrogen sulphide presence examination. The visual control indicated black precipitates in all nutrient mediums. The hydrogen sulphate presence was positive. In all cases the SRB occurrence was confirmed by light microscope. So this way, we prepared 4 different biogenic samples – named A, B, C and D.

Abiotic controls performed in same conditions without bacteria (ABIO A, ABIO B, ABIO C and ABIO D) were exposed to identical analyses in the same time. In all samples during whole experiment were not any bacteria confirmed by light microscope, hydrogen sulphide examinations were negative. Visual control indicated creation of grey precipitates.

At the end of cultivation the samples were filtrated, dried and prepared for analyses. At first, SEM observations of biogenic "iron sulphides" were realized (Fig. 1, Fig. 2), which revealed particle sizes in the range of ten micrometers to very small particles. Very probably, the bigger particles are particles of sulphur and the small particles are aggregates of biogenic iron sulphide minerals, as noted by the subsequent X-ray analysis (software estimation of crystallite sizes).

Results of X-ray analysis show which kinds of minerals were created by biogenic method induced by bacteria. Figure 3 illustrates XRD pattern for sample A. The major peaks have been identified as mackinawite, greigite, and then sulphur alpha. These compounds were confirmed in all samples (A, B, C, D).

According to the nutrient medium composition and cultivation mode there were some variations in precipitates composition. They are resumed in Table 2.

Table 2 declares that predominant compound in all samples is mackinawite. Batch cultivation mode without fresh nutrient medium supplying supports more the creation of greigite phase than in semi-continuous mode. Also medium modification reflects a bit in greigite and mackinawite ratio in generated precipitates. Solution chemistry in suspensions during semi-continuous mode of SRB cultivation produces such conditions that mackinawite is created most markedly. In all samples was presence of sulphur alpha recorded, caused probably by incomplete sulphate reduction or sulphides oxidation.

The mass amounts (in grams) of generated precipitates for samples C and D (per 1 liter of medium) were twice higher than for samples A and B. It confirms the assumption that fresh medium addition supports the SRB activity, incite the hydrogen sulphide production and consequently the precipitates creation.

Figure 4 of non-biogenic sample ABIO A demonstrates that precipitates created in bottles with medium Postgate C without SRB do not contain any iron sulphide minerals. Only one compound was identified - vivianite $Fe_3(PO_{4)2}.8(H_2O)$. In all abiotic samples (ABIO A, ABIO B, ABIO C and ABIO D) we obtained the same result. This indicates that iron sulphide precipitation is dependent on bacteria presence and we can conclude that vivianit creation is a chemical process dependent probably only on solution composition and some factors as pH value, temperature, etc. and iron sulphides are result of processes biological-chemical (hydrogen sulphide generation by metabolism of SRB and sulphide precipitation).

Conclusion

The aim of this paper was oriented on composition study of biogenic and non-biogenic precipitated materials synthesized in reagent bottles with SRB and without SRB culture under specific laboratory conditions (cultivation modes) and growth media modifications. XRD analysis of the precipitates demonstrated the presence of mackinawite and greigite as the main iron sulphides produced under biotic conditions and vivianit creation under abiotic conditions. Medium modification reflects only a bit in a compounds ratio. Cultivation conditions influenced the composition of generated precipitates more.

Results obtained in this work will help for next experimental work oriented on the application of biogenic sulphide formation through bacterial sulphate reduction in bioremediation of metal containing waste streams such as mine waters.

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Badanie biogennych i nie-biogennych wytrąceń żelaznych z siarkowodoru

Metabolizm bakterii redukujących siarczany (SRB) składa się generalnie z utleniania organicznych substratów lub molekularnego H2 oraz redukcji siarczanów w warunkach beztlenowych. Poprzez utlenianie molekularnych składników organicznych (np. mleczan, octan) zyskują one energię oraz pożywki. Proces biologiczno-chemiczny oparty na zdolności i aktywności tych bakterii do redukcji siarczanów daje efekt w postaci utworzenia siarczku wodoru, który wiąże się z kationami metalu w roztworach do nierozpuszczalnych form wytrąceniowych.

W sedymentacyjnym środowisku beztlenowym z redukcją żelaza, amorficzny siarczek żelaza często wstępną fazą siarczkową, która zostaje utworzona i wykazuje słabą krystaliczność o małych rozmiarach cząstek i słabym uporządkowaniu. Chociaż wiele badań było poświęconych mineralogii i chemii powierzchni bardziej ukrystalizowanych i bardziej stabilnych termodynamicznie siarczkom, takim jak piryt czy pirotyn, zrozumienie zachowań kryształu i chemii powierzchni słabiej ukrystalizowanych siarczków żelaza jest bardzo małe, zwłaszcza w kwestii oceny ich reaktywności i potencjału do transformacji w bardziej stabilne fazy.

Celem tej pracy było zbadanie właściwości i składu biogenicznego wytrącanych materiałów wiązanych następnie w butelkach reakcyjnych z kulturą SRB w specyficznych warunkach laboratoryjnych i przy modyfikowanych warunkach rozwoju. Modyfikacja polegała na dodawaniu jonów żelaza w formie siarczków oraz dawki substratu organicznego. W dwóch trybach kultywacji 4 próbki biogeniczne były wytrącane dla siarczku wodoru utworzonego przy udziale bakterii. Realizacja kontroli abiotycznej eksperymentów bez udziału SRB dało efekt w postaci czterech nie-biogenicznych próbek. Cały utworzony materiał był następnie badany przy użyciu metod EDX, SEM oraz XRD. Skład próbek różnił się w zależności od kultywacji i składu ośrodka rozwoju. Wytrącenia biogeniczne zawierały greigit, makinawit oraz siarkę, nie-biogeniczne próbki zawierały wiwianit.

Słowa kluczowe: wytrącenia biogeniczne, bakterie redukujące siarkę, siarkowodór