



The Application of Biogenically Created Sorbent for Metal Ions Elimination

Jana JENČÁROVÁ¹⁾, Alena LUPTÁKOVÁ²⁾

¹⁾ Ing., Ph.D.; Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 040 01 Košice, Slovakia; email: jencarova@saske.sk

²⁾ Ing., Ph.D.; Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 040 01 Košice, Slovakia; email: luptakal@saske.sk

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Abstract

The aim of this work was to study the sorption ability of biogenic iron sulphides created by sulphate-reducing bacteria (SRB). They were isolated using growth media Postgate C, at 30°C, under anaerobic conditions from mineral spring Gajdovka (Košice). Biogenic sorbent precipitates were synthesized in sulphides form in reagent bottles with SRB culture and modified nutrient medium during 10 months in semicontinuous cultivation mode. The mode consisted of several phases, when fresh medium was added to the bottles in order to support the bacterial growth and precipitates creation. Abiotic controls of samples were performed in same conditions, but without bacteria.

At the end of cultivation, the generated samples were separated from suspensions by centrifugation, dried, analysed (EDX, SEM, XRD) and used for sorption experiments. EDX results confirmed the presence of iron and sulphur as a major part of biogenic precipitates. Abiotic (non-biogenic) samples consisted of iron phosphate. SEM analysis revealed non-smooth particles and aggregates up to tens of micrometers. XRD showed mainly amorphous or poorly crystalline precipitates. Non-biogenic precipitates were identified as mineral vivianite.

Sorption of zinc and cadmium from model solutions was realized by prepared sorbent samples in 100 ml Erlenmeyer flasks, with sorbent dose 1g/l, during 24 hours. The concentrations of metal ions were determined by AAS. Sorption process was most remarkable during initial 30 minutes. The metals cations from model solutions with low initial concentration (10 and 20 mg/l) were adsorbed well and the equilibrium was attained very quickly. Sorbent showed better affinity for cadmium than for zinc.

Keywords: biogenic sorbent, sulphate-reducing bacteria, cadmium, zinc

Introduction

Wastewaters originating from mining and metallurgical industry are often acidic and typically characterized by a significant content of soluble metals, such as zinc, copper, cadmium, lead, iron, nickel. The presence of metals in final industrial effluents is undesired, as they may accumulate to toxic levels and cause ecological damage under certain environmental conditions. They are not biodegradable and thus tend to accumulate in living organisms, causing various diseases and disorders (Motsi et al., 2009).

Many techniques have been developed for the treatment of heavy metal polluted waters, which can include chemical precipitation, adsorption, ion exchange, ultra-filtration, membrane and electrochemical technologies. The choice of method is based jointly on the concentration of heavy metals in the solution and the cost of treatment.

Several studies revealed that biogenic sulphides produced by sulphate-reducing bacteria (SRB) are useful as adsorbents and can decrease concentrations of common wastewater and acid mine drainage cations to low levels (Renock et al., 2009; Marius et al., 2005; Mullet et al., 2004; Watson et al., 1995). It was found out that iron sulphide material an excellent adsorbent for different metal ions such as Pb, Zn, Ni, Cu, As, Cd

and has a very high specific uptake capacity from solution (Jong and Parry, 2004; Neal et al., 2001).

SRB are anaerobic heterotrophs characterized by the ability to perform dissimilatory sulphate reduction with the simultaneous oxidation of the organic substrates (Postgate, 1984). They utilize a range of substrates, e.g. lactate, acetate, alcohol, hydrocarbons (Liamleam and Annachhatre, 2007) and sulphates as terminal electron acceptors that are reduced to hydrogen sulphide (Odom and Singleton, 1993). This reacts with certain metals dissolved, such as copper, iron and zinc, forming insoluble precipitates (Azabou et al., 2007).

The requirements for SRB growth and biogenic sorbent creation are: a near-neutral pH, appropriate temperature, a source of organic carbon, nutrients, a reducing environment, a source of sulphate, a solid support for microbial attachment and development, and a way to physically retain metal sulphide precipitates (Garcia et al., 2001).

The initial precipitate in iron-rich environment is commonly referred to as amorphous iron sulphide with poor crystallinity. Within days, it crystallizes to the more stable mackinawite (Herbert et al., 1998) or may react to form phases such as greigite, marcasite, pyrite and pyrrhotite (Larrasoana et al., 2007; Benning et al., 2000). Final sulphides are strong dependent on solution

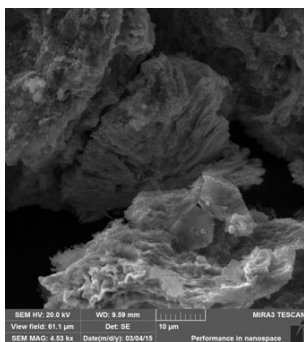


Fig. 1 Biogenic precipitate (SEM)

Rys. 1 Osad biogeniczny (SEM)

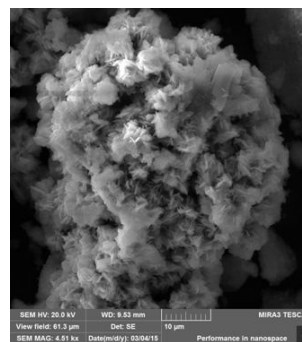


Fig. 2 Non-biogenic sample (SEM)

Rys. 2 Osad niebiogeniczny (SEM)

Tab. 1 EDX analysis of precipitates

Tab. 1 Analiza EDX osadu

Element	Biogenic sorbent	Non-biogenic sample
	[Wt%]	
Fe	53.1	49.6
S	34.9	-
P	-	13.8
C	7.1	-
O	2.0	36.2
N	0.9	-
Na	0.8	-
Mg	0.3	-
K	0.5	-
Ca	0.3	0.4

chemistry (Lewis, 2010). In many cases the precipitates identification in heterogeneous mixtures is quite difficult; the exact structure, the changes in crystal morphologies, the particle sizes and the physical properties are not satisfactory known (Csákberényi-Malasics et al., 2012).

The objective of this work was to prepare biogenic sorbent samples by sulphate-reducing bacteria cultivation, to characterize the synthesized precipitates and to realize the experiments in order to verify their sorption ability to eliminate metal ions (cadmium, zinc) from water.

Materials and methods

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). Bacteria were maintained and stored in glass reaction flasks in anaerobic conditions at 30°C.

Biogenic sorbent

The precipitates in form of biogenic iron sulphides were created by SRB activity in a modified Postgate C medium. The modification consist of an ad-

dition of Fe ions in form of sulphates ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$). The growth medium contains double dose of sodium lactate as carbon and energy source.

The sorbent production was performed in semi-continuous cultivation mode under anaerobic conditions 10 months. Experiments were performed in duplicate using 1000 ml glass bottles containing 900 ml of growth medium and 100 ml of bacteria inoculum at the beginning. The pH of nutrient medium was adjusted to 7.5. After inoculation, the bottles were sealed with butyl rubber stoppers and stored in thermostat at 30°C.

Semi-continuous cultivation mode consisted of several phases. In some predetermined weeks (3, 5, 7, 15, 17 and 19) the fresh medium (900 ml) was supplied to the bottles. The other weeks were running in "batch" mode, without a medium exchange. The bottles were all the time maintained at 30°C. Abiotic controls (non-biogenic) – without SRB were performed in the same conditions.

At the end of cultivation the liquid samples were taken out from each bottle for analyses (pH measurement, hydrogen sulphide presence proof) and 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

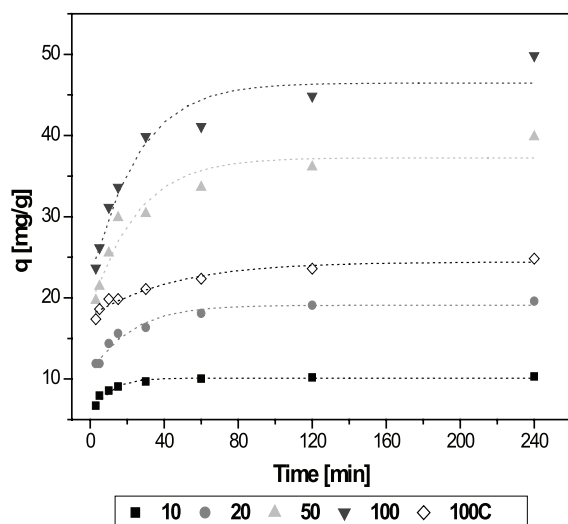


Fig. 3 Cadmium ions adsorption from model solutions
Rys. 3 Adsorpcja jonów kadmu z roztworu modelowego

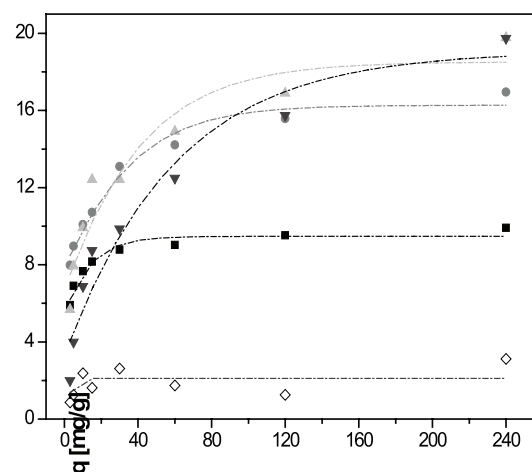


Fig. 4 Zinc ions adsorption from model solutions
Rys. 4 Adsorpcja jonów cynku z roztworu modelowego

and stored in a vacuum desiccator. Dry powders were then studied by EDX, SEM and XRD analyses.

Samples characterization

The structure, composition, particle sizes and morphology of the precipitated samples were examined using scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX) and X-ray powder diffraction (XRD).

X-ray powder diffraction data were collected over an angular range $10 < 2\theta < 80^\circ$ with steps 0.05° using a Bruker D8 Advance diffractometer. SEM studies were performed using a SEM Tescan MIRA 3 FE microscope equipped with energy dispersive X-ray microanalysis system (EDX – Oxford Instruments).

Model solutions

Stock solutions with metal ions (Me^{2+}) concentration 1 g/l were prepared by dissolving the $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ (p.a. grade) in distilled water. Model solutions for sorption experiments with various concentrations of metal ions (10–100 mg/l) were made from prepared stock solutions.

Sorption experiments

Sorption experiments were conducted by mixing 0.1 g of biogenic sorbent sample with 100 ml of model solution containing the desired concentration of metal ions (10, 20, 50, and 100 mg/l). One test was carried out by using 0.1 g of non-biogenic sample from abiotic control with 100 ml of model solution with initial metal concentration (Cd^{2+} , Zn^{2+}) 100 mg/l. The pH of solutions before sorption experiments was adjusted at $\text{pH } 5.8 \pm 0.1$ with 0.01M NaOH and 0.01M HCl. Experiments were realized at room temperature.

The mixtures were continuously stirred in plastic Erlenmeyer flasks using mechanical laboratory shaker for 24 hours. In each test the sorption kinetics was followed. 2 ml of liquid phase were taken out in the following intervals: after 3, 5, 10, 15, 30, 60, 120, 240 minutes and at the end, after 24 hours. The concentration of cadmium and zinc ions in the extracts was determined by atomic absorption spectrometry (AAS).

Results and discussion

Precipitates formation

Regular visual controls done during 10 months confirmed the creation of solid phase in solutions. The formation of black precipitates was proved in biogenic samples. The hydrogen sulphate presence was positive and the SRB occurrence was confirmed by light microscope. In blank samples grey-green precipitates were created and no bacteria were confirmed. The hydrogen sulphide examination was negative.

Samples characterization

At the end of cultivation the created precipitates (after separation from suspension and drying) were followed by scanning electron microscope. SEM observations revealed particle sizes from low micrometer range to tens of micrometers, but also bigger aggregates were present.

Figure 1 shows the structure of biogenic sorbent. It is evident that there is a marked grooving, surface is not smooth. Very probably, there are small aggregates of iron sulphide minerals, particles of sulphur, biomass and nutrient medium component remains. On Figure 2 is a SEM photo of sample from abiotic control. The structure is visibly different to sorbent, seems to have an acicular character in.

Tab. 2 Maximum sorption capacity (after 24 hours)
Tab. 2 Maksymalna pojemność sorpcyjna (po 24 godzinach)

Initial metal concentration [mg/l]	Sample for sorption experiment	q _{max} [mg/g]	
		Cadmium	Zinc
10	Biogenic sorbent	10.3	9.9
20	Biogenic sorbent	19.7	17.9
50	Biogenic sorbent	44.8	22.1
100	Biogenic sorbent	62.3	21.9
100	Non-biogenic sample	31.0	3.99

The chemical composition of precipitates was studied by using a microscope equipped with energy dispersive X-ray microanalysis system. Table 1 resumes percentages by weight of elements in analyzed samples. The result of sorbent sample confirms the presence of iron sulphates “FeS” and little amounts of elements from nutrient medium. Non-biogenic sample consists very probably of iron phosphate.

Results of X-ray analysis show that the biogenic sorbent sample consists mainly of amorphous or poorly crystalline precipitates. But there were some peaks identified as mackinawite, greigite, and sulphur alpha. The last one is caused very probably by incomplete sulphate reduction or sulphides oxidation. XRD of non-biogenic precipitates do not reveal any iron sulphide minerals. Only one compound was identified as vivianite $\text{Fe}_3(\text{PO}_4)_2 \cdot 8(\text{H}_2\text{O})$ (Jenčárová et al., 2014).

Sorption experiments

The created precipitates were after the characterization and structure study used for sorption experiments. Biogenic iron sulphides and non-biogenic vivianit were applied as sorbents. Their dose was 1 g/l. Model solutions contained concentration of cadmium and zinc ions 10, 20, 50 and 100 mg/l. Sorption test with vivianit (marked 100C) was carried out for the highest metal concentration only.

Figures 3 and 4 illustrate quantity of adsorbed metals from model solutions calculated from sorption kinetics study within 4 hours of sorption experiment duration. We can see that sorption processes were most remarkable during initial 30 minutes. The metals cations from model solutions with low initial concentration (10 and 20 mg/l) were adsorbed well and the equilibrium was attained very quickly. Cadmium

removal was recorder either by non-biogenic vivianit. Zinc sorption from solution with higher initial concentration (50 and 100 mg/l) was significantly slower and after 240 minutes did not reach the values of cadmium, not half. From this finding we can state that our sorbent samples showed better affinity for cadmium than for zinc. Zinc sorption by vivianit was negligible.

Table 2 summarizes maximum sorption capacities of sorbent samples which were achieved after 24 hours of sorption experiments. It confirms the previous statement that cadmium sorption by biogenic iron sulphides from solutions with higher initial metal concentration was twice bigger than zinc sorption.

Conclusion

This paper was oriented on the study and characterization of biogenic sample synthesized with SRB culture and non-biogenic sample created chemically in abiotic conditions. The SEM observations, EDX and XRD analyses revealed that biogenic precipitates consisted predominantly of iron sulphides, non-biogenic material was vivianit. Afterwards, the samples were used as sorbents for cadmium and zinc sorption from model solutions with various concentrations of metal ions (10–100 mg/l). The results showed that biogenic iron sulphides prepared by sulphate-reducing bacteria cultivation are utilizable for the metal elimination from water. Higher specific uptake capacity from solutions was achieved for cadmium. Partial removal of cadmium was noticed also by abiotic sample.

Acknowledgements

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Literatura – References

1. AZABOU, S. et al. 2007. Zinc precipitation by heavy-metal tolerant sulfate-reducing bacteria enriched on phosphogypsum as a sulfate source. *Minerals Engineering* 20 (2): 173–178.
2. BENNING, L.G. et al. 2000. Reaction pathways in the Fe-S system below 100°C. *Chemical Geology* 167 (1): 25-51.
3. CSÁKBERÉNYI-MALASICS, D. et al. 2012. Structural properties and transformations of precipitated FeS. *Chemical Geology* 294-295: 249-258.
4. GARCIA, C. et al. 2001. Bioremediation of an industrial acid mine water by metal-tolerant sulphate-reducing bacteria. *Minerals Engineering* 14 (9): 1001-1008.
5. HERBERT, B.R. et al. 1998. Surface chemistry and morphology of poorly crystalline iron sulfides precipitated in media containing sulfate-reducing bacteria. *Chemical Geology* 144 (1): 87-97.
6. JENČÁROVÁ, J. et al. The examination of biogenic and non-biogenic iron precipitates created by hydrogen sulphide. 2014. *Inżynieria Mineralna - Journal of the Polish Mineral Engineering Society* 15 (2): 281-286.
7. JONG, T., PARRY, D.L. 2004. Adsorption of Pb(II), Cu(II), Cd(II), Zn(II), Ni(II), Fe(II), and As(V) on bacterially produced metal sulfides. *Journal of Colloid and Interface Science* 275 (1): 61-71.
8. LARRASOANA, J.C. et al. 2007. Diagenetic formation of greigite and pyrrhotite in gas hydrate marine sedimentary systems. *Earth and Planetary Science Letters* 261 (3): 350-366.
9. LIAMLEAM, W., ANNACHHATRE, A.P. 2007. Electron donors for biological sulfate reduction. *Biotechnology Advances* 25 (5): 452–463.
10. LEWIS, A.E. Review of metal sulphide precipitation. 2010. *Hydrometallurgy* 104 (2): 222-234.
11. MARIUS, M.S. et al. 2005. Influence of iron valency on the magnetic susceptibility of a microbially produced iron sulphide. *Journal of Physics: Conference Series* 17 (1): 65–69.
12. MOTSI, T. et al. 2009. Adsorption of heavy metals from acid mine drainage by natural zeolite. *International Journal of Mineral Processing* 92 (1): 42-48.
13. MULLET, M. et al. 2004. Removal of hexavalent chromium from solutions by mackinawite, tetragonal FeS. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 244 (1-3): 77-85.
14. NEAL, A.L. et al. 2001. Iron sulfides and sulfur species produced at hematite surfaces in the presence of sulfate-reducing bacteria. *Geochimica et Cosmochimica Acta* 65 (2): 223-235.
15. ODOM, J.M., RIVERS SINGLETON, J.R. 1993. *The Sulfate-reducing Bacteria: Contemporary Perspectives*. New York: Springer-Verlag, New York. 249 p. ISBN 387978658.
16. POSTGATE, J.R. 1984. *The sulphate-reducing bacteria*. 2nd edition. Cambridge: Cambridge University Press. 208 p.
17. RENOCK, D. et al. 2009. Chemical and structural characterization of As immobilization by nanoparticles of mackinawite (FeSm). *Chemical Geology* 268 (2): 116-125.
18. WATSON, J.H.P. et al. 1995. Heavy metal adsorption on bacterially produced FeS. *Minerals Engineering* 8 (10): 1097-1108.

Zastosowanie biosorbentów do usuwania jonów metali

Celem pracy było zbadanie zdolności sorpcyjnej biogennych siarczków żelaza tworzonych przez bakterie redukujące siarczany (SRB). Bakterie zostały wyizolowane ze źródła wody mineralnej Gajdovka (Košice) na pożywce Postgate C, w temperaturze 30°C, w warunkach beztlenowych. Biogenne osady sorbentu zostały zsyntetyzowane do siarczków w butelkach odczynników z pożywką SRB w ciągu 10 miesięcy w trybie uprawy półciągłej. Metoda składa się z kilku etapów, w których dodaje się świeżą pożywkę, aby wspomagać wzrost bakterii i wytrącanie osadu. Abiotyczną kontrolę próbek przeprowadzono w tych samych warunkach, lecz bez bakterii.

Pod koniec hodowli, powstałe osady oddzielono od zawiesiny przez odwirowanie, następnie wysuszono i przeprowadzono analizowano (EDX, SEM, XRD) a także wykonano eksperymenty sorpcyjne. Wyniki EDX potwierdziły obecność żelaza i siarki, w większej części osadów biogennych. Abiotyczne (nie biogenne) próbki składają się z fosforanu żelaza. Analiza SEM wykazała występowanie cząsteczek o niegładkiej powierzchni i wielkości poniżej kilkudziesięciu mikrometrów. Analiza XRD wykazała głównie występowanie osadów bezpostaciowych. Nie biogenne osady zidentyfikowany jako minerał vivianit.

Sorpcja cynku i kadmu z modelowych roztworów była analizowana w przygotowanych próbkach sorbenta w kolbkach Erlenmeyera o pojemności 100 ml, z dawką sorbentu 1 g/l, w ciągu 24 godzin. Stężenie jonów metalu oznaczono metodą AAS. Proces sorpcji był najszybszy podczas początkowych 30 minut. Do kationów metali z roztworów wzorcowych o niskim stężeniu początkowym (10 i 20 mg/l) równowaga była osiągana bardzo szybko. Sorbent wykazał lepsze powinowactwo do kadmu niż cynku.

Słowa kluczowe: sorbent biogeniczny, bakterie redukujące siarczany, kadm, cynk