BIOTECHNOLOGICAL REMOVAL OF HEAVY METALS FROM MINING WASTEWATERS BY DISSIMILATIVE SULPHATE REDUCTION

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Abstract: *Acid drainage wastewaters contaminated with Cu – 200 mg/l, Fe – 20 mg/l, Zn – 20 mg/l, Ni – 10 mg/l, Cd – 10 mg/l and Co – 10 mg/l were remediated by hydrogen sulphide precipitation reaction in laboratory-scale installation. The installation design includes anaerobic bioreactor, chemical reactor and settler, connecting in series. The recirculation of treated wastewater from settler to anaerobic bioreactor was performed by peristaltic pumps. Sulphate-reducing bacterial consortium is adhered in biofilm, which is immobilized on zeolite particles in the anaerobic bioreactor. The bacteria were cultivated on a medium containing lactate as a source of carbon and energy. Heavy metals removal was achieved in a chemical reactor by biogenic produced H_2S and bicarbonate ions. In addition, X-ray diffraction analyses proved that the precipitated above heavy metals are mainly in forms of relevant insoluble sulfides and carbonates. In batch conditions was investigated also the resistance of sulfate-reducing bacteria to various heavy metals. The reported treating method allows to removes heavy metals from wastewaters below the permeable level for water intended for use in the agriculture and/or industry.*

Keywords: *Acid mine drainage, sulphate-reduction, bioreactor, heavy metals, metal sulphides.*

1. Introduction

Acid mine drainage is a major environmental hazard that affects the aquatic ecosystems around mines. Wastewater generated from mining industry is often acidic and typically characterized by a significant content of sulphates and soluble metals, such as Zn, Fe, Cu, Ni, Pb and Cd. Sulphate rich wastewater is derived also by many industrial processes that use sulphuric acid. Conventionally, hydroxide precipitation is the most commonly applied method for the treatment of metal containing waters. There are some serious limitations in terms of application and effectiveness in this treatment. The production of high quantities of sludge is the main disadvantage of the method. Also, sulfate removal is only possible when Ca^{2+} containing chemicals, such as lime, are used for neutralization. In recent years, the use of sulphate reducing bacteria (SRB) to reduce sulphate and precipitate metals has been proposed as an alternative to hydroxide precipitation (Kaksonen and Puhakka, 2007; Liamleam and Annachhatre, 2007; Hoa et al., 2007; Costa et al., 2007].

Sulphate-reducing bacteria oxidaze simple organic compounds (such as lactate, acetate, butirate and other products of fermentations) with sulphate under anaerobic conditions:

$$2CH_3CHOHCOOH + SO_4^{2-} \rightarrow 2CH_3COOH + H_2S + 2HCO_3^- , \quad (1)$$

$$CH_3COOH + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-, \quad (2)$$

The HCO_3^- ions increase pH and alkalinity of the water. The soluble sulfide reacts with the metals in wastewater to form insoluble metal sulfides:

$$M^{2+} + H_2S \rightarrow MS\downarrow + 2H^+ \quad (3)$$

where M includes metals such as Fe, Cu, Zn, Ni, Cd.

Numerous reactor designs for microbial sulphate reduction have been reported (Kaksonen et al. 2004; Bayrakdar et al. 2009; Nagpal et al. 2000; Steed et al. 2000; Nevatalo L. et al. 2010). Biological sulfate reducing reactors used for metal precipitation can have either one or more stages, i.e. the sulfate reduction and metal precipitation can occur simultaneously, or in separate process units. Single-stage processes are low-cost to operate, but it may not be viable if the treated wastewater is high acidic or contains high concentrations of heavy metals (Hao, 2000). Metals can be pre-precipitated to the biological step by reaction with sulphide- or H_2S- containing solution. The

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separation of microbial sulphate reduction and metal precipitation alleviates toxicity on SRB.

The stoichiometry of sulfide addition to the metal solution should be carefully controlled, because the unreacted sulfide remains in the solution and needs to be removed (Veeken et al. 2003). Cadmium, copper, iron, lead, mercury, nickel, and zinc are some of the metals that precipitate as metal sulphides. Arsenic, antimony, and molybdenum form more complex sulphide minerals. Metals such as manganese, iron, nickel, copper, zinc, cadmium, mercury, and lead may also be removed to some extent by co-precipitation with other metal sulphides.

The present work study the treatment of acid wastewaters containing Cu, Fe, Zn, Ni – 10, Cd and Co by means of laboratory-scale installation. The two processes – microbial sulphate-reduction and formation of insoluble metal sulphides are divided into separate reactors. The composition of the metal precipitates in vertical-flow settler was determined.

2. Materials and methods

Mixed SRB culture was obtained from a laboratory anaerobic cell with mixed solid organic matter (cow manure, spent mushroom compost and sawdust) for treatment of model acid mine water solutions. Modified Postgate medium was used for the cultivation of SRB. It contained per liter of distilled water: K_2HPO_4 - 0.5g, NH_4Cl - 1.0g, Na_2SO_4 - 2.0g, $CaCl_2$ - 0.1g, $MgSO_4.7H_2O$ - 4.0g, $FeSO_4.7H_2O$ – 0,5g sodium lactate - 6.0g, yeast extract - 0.25g. The initial pH is adjusted to 6.5. The formation of ferrous sulphide was detected as a black precipitate. It indicates that bacterial growth had taken place.

2.1 The effect of Fe, Cu, Zn, Ni, Co and Cd on dissimilatory sulphate reduction

Experiments with heavy metals were carried out using the modified nutrient medium, which does not contains Fe(II). Batch experiments were carried out in 20 ml glasses bottles heavy metals and nutrient solution. Iron ($FeSO_4.7H_2O$), copper ($CuSO_4.5H_2O$), zinc ($ZnSO_4.7H_2O$), nickel ($NiSO_4.7H_2O$), cobalt ($CoCl_2$) and cadmium ($CdCl_2.5H_2O$) were added separately to the bottles reach to final concentration in the range of 0.01 to 2 g/l for the different experiments. The initial pH was adjusted to 6.0.

2.2 Treatment of waters polluted with heavy metals by the laboratory installation

The laboratory installation for heavy metals removal from waters is given in fig.1. The geometric volume of an anaerobic fixed bed reactor (2) is 1.2 dm^3 and it is filled with 1.13 kg zeolite and 0.67 dm^3 modified Postgate medium. The anaerobic bioreactor is inoculated with 40 ml enriched microbial culture of sulfate-reducing bacteria. After formation of active biofilm of SRB, the reactor was kept with medium feeding and continuous cultivation. Nutrient medium (1) is fed with adjustable flow rate into the bioreactor through a peristaltic pump (9). Homogenization in the reactor is due to upward flow performed by a recirculating pump (11). A sand filter (7) is provided in the scheme to precipitate the insoluble particles. The microbial produced H_2S is in contact with the solution of heavy metals in a chemical reactor (4). The solution of heavy metals is fed into the chemical reactor through a peristaltic pump (10) from tank (3). The geometric volume of the reactor is 0.5 dm^3. The insoluble sulfides precipitated in a vertical-flow settler with a volume of 0.85 dm^3. The greatest part of the effluents of settler was pumped into mix tank by a recirculating pump (13). The other part of effluents accumulates into collector tank (8) with volume of 6 dm^3. Adjustable flow peristaltic pumps (10 and 11) support the necessary volume loading rates with sulfates in the bioreactor and the maintenance the total organic carbon content to final electron acceptor ratio of 0.67.

The concentrated medium with lactate was used for the cultivation of SRB. It contained per liter of distilled water: K_2HPO_4 - 1.0 g, NH_4Cl - 2.0 g, $CaCl_2$ - 0.5 g, sodium lactate - 16.0 g, yeast extract - 1.0 g. The initial pH is adjusted to 7.0.

The heavy metals Cu – 200 mg/l, Fe – 20 mg/l, Zn – 20 mg/l, Ni – 10 mg/l, Cd – 10 mg/l and Co – 10 mg/l are involved in the synthetic solution in the forms of $CuSO_4.5H_2O$, $FeSO_4.7H_2O$, $NiSO_4.7H_2O$, $ZnSO_4.7H_2O$, $CdCl_2.2.5H_2O$ and $CoCl_2$. The other ingredient of synthetic solution of heavy metals is $MgSO_4.7H_2O$ - 2.5 g/l. The pH of the solution is adjusted in the range of 2.5 – 2.7 with 1 N H_2SO_4. The experiment is performed at temperatures ranging from 21^oC to 22 ^oC.

2.3 Analytical methods

In some certain sampling points of the installation are determined the parameters pH and Eh, mV. In the same points are conducted spectrophotometrical measurments of the concentrations of sulfates using $BaCl_2$ reagent at a wavelength of 420 nm and of hydrogen sulphide - using a Nanocolor test 1-88/05.09 at a wavelength of 620 nm. The concentration of heavy metals was measured by ICP. X-ray diffraction (XRD) patterns were obtained with a DRON-1 diffractometer (CuKα radiation, Ni filter, I = 20 mA, U = 30 kV) and a 57.3 mm Debye-Scherrer TUR–M–60 camera.

Numbers of sulphate-reducing, anaerobic heterotrophic and fermenting sugars bacteria with gas production in the liquid phase of anaerobic bioreactor are counted through standard microbiological methods, including those of most probable number and colony-forming unit on plate with nutrient agar.

3. Results and discussion

3.1 The effect of Fe, Cu, Zn, Ni, Co and Cd on dissimilatory sulphate reduction

The effect of heavy metals on microbial sulphate reduction was estimate after 14 days of incubation. The data about established growth of sulphate reducing bacteria in different concentration of heavy metals are sowed in table 1.

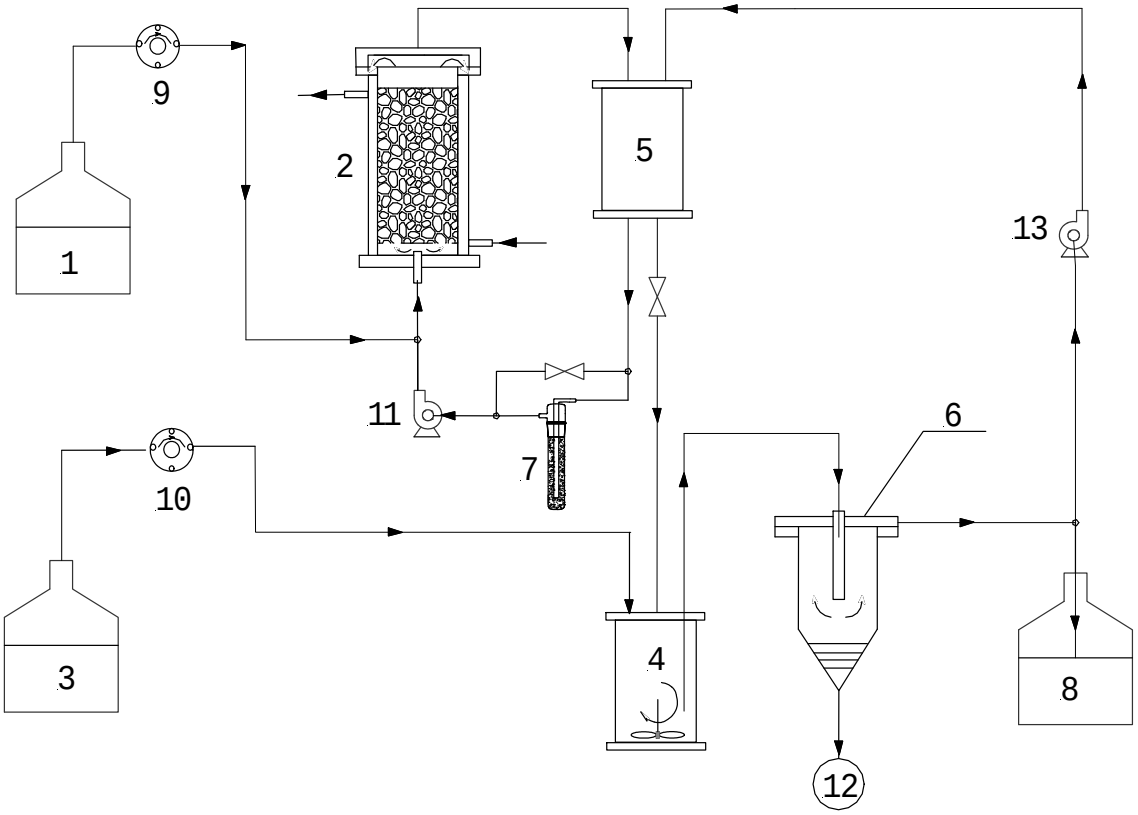

Fig. 1 *Schematic diagram of the laboratory installation for active treatment of wastewaters polluted with heavy metals: 1 – Nutrient medium tank, 2 - anaerobic fixed-bed biofilm reactor, 3 - Heavy metals solution tank 4 – chemical reactor, 5 - mix tank, 6 – settler, 7- sand filter, 8 - collector tank, 9 and 10 – peristaltic (roller) pump, 11 and 13 – recirculating pump, 12 – sludge*

During incubation, black precipitate was detected on the bottom of all glasses bottles at the concentrations of ferrous ions in range 0.01 to 2.0 g/l. However, it was found that bacterial growth is strong affected by the presence of the other studied metal ions. Reduction of sulphates was carried out at maximum concentration of Co and Zn respectively 0.6 and 0.2 g/l. Copper and zinc have strong inhibitory effect on enrichment mixed culture of sulphate-reducing bacteria at concentrations above 0.06 g/l. Bacterial growth was found only at 0.01 g/l cadmium. Experiments with heavy metals showed that cadmium toxicity to this mixed SRB culture is highest.

The reported toxic concentrations of heavy metals to sulphate-reducing bacteria in different studies range from a few mg/l to as much as 100 mg/ (Utgikar l.et al. 2002). Cabrera et al. (2006) reported that the relative order for inhibitory metal concentration was Cu >Ni >Mn > Cr > Zn for two cultures of SRB (Desulfovibrio vulgaris and Desulfovibrio sp.), as an increase in the metal concentration in solution led to a decrease in the sulphate reduction rate and this effect was accompanied by a low level of metal precipitation. In other study (Medircio et al. 2007) were assessed the effects of cadmium and manganese at concentrations 20 mg/l on the SRB growth rates and the batch experiments showed 90% manganese and 85% cadmium precipitation.

3.2 Treatment of waters polluted with heavy metals by the laboratory installation

The adherence of active biofilm of SRB onto the natural occurred zeolite is carried out for a period of three months. For this purpose the

Postgate medium is inoculated with obtained mixed SRB culture. For a period of some months progressively is decreased the residence time and respectively increases volume loading rate of the bioreactor with sulphates. The data about Influence of SO_4^{2-} volume loading rates (SO_4^{2-}g/l.h) to the rates of microbial sulphate-reduction are estimated in previous studies (Bratkova et al. 2011). During this study with the same fixed bed bioreactor was established a maximum rate of sulphate-reduction - 273 mg SO_4^{2-}/l.h at initial concentration of sulphates – 3 g/l and the residence time is 10.5 h.

Table 1. *Growth of SRB in different concentration of heavy metals*

	Concentration of heavy metals, g/l											
	0.01	0.02	0.04	0.06	0.08	0.1	0.2	0.6	0.8	1.0	1.5	2.0
Fe	+	+	+	+	+	+	+	+	+	+	+	+
Cu	+	+	+	+								
Zn	+	+	+	+	+	+	+					
Ni	+	+	+	+								
Co	+	+	+	+	+	+	+	+				
Cd	+											

The other important parameter is total organic carbon/SO_4^{2-} ratio (Vossoughi et al. 2003). Velasco et al. 2008 reported that the feed COD/SO_4^{2-} ratio can be an useful parameter to control hydrogen sulfide production in the metal precipitation process when ethanol is used as sole electron donor and carbon source. Kaksonen et al. 2004 showed that the stoichiometric COD/SO_4^{2-} ratio of 0.67 was adequate to attain around 60% of sulfate reduction with an initial sulfate concentration of 2000mg/L in a fluidized-bed reactor inoculated with SRB capable to completely oxidize ethanol to CO_2. Cao et al. (2009) reported that when lactate is used as sole electron donor, while COD concentration increased from 963 mg/L to 17330 mg/L with the same concentration of SO_4^{2-}, sulfate removal rate increased and strong sulfate-reducing activity was achieved under the initial COD/SO_4^{2-} ratio of 3.0.

In this study the stoichiometric total organic carbon content to final electron acceptor ratio is 0.67. The concentration of sulphates in the solution of heavy metals is in the range 1.10 - 1.15 g/l. Both adjustable flow peristaltic pumps (for polluted acid water and nutrients) support the maintenance of volume loading rates with sulfates of the fixed-bed sulphate-reducing reactor in the range of 190 - 200 mg/l.h. In the course of this volume loading rate and COD/SO_4^{2-} ratio 2.7, the rate of microbial sulphate-reduction is in the range of 180 - 185 mg/l.h. It was found the removal of heavy metals from polluted waters in the range 98.9 - 99.9% (Table 2).

Table 2 *General parameters measured in sample points at the outlets of main facilities of the laboratory installation*

Parameter	**Solution of heavy metals**	**Outlet of the anaerobic bioreactor**	**Outlet of the settler**
pH	2.50 – 2.73	7.56- 7.80	7.46 – 7.69
Eh, mV	-	- 231 - -258	-209 - -234
SO_4^{2-},g/l	1.09 – 1.23	0.08 – 0.10	0.11 – 0.12
H_2S, mg/l	-	24 – 37	16 – 34
Cu, mg/l	194.8 – 207.3	0.01 – 0.08	0.001 – 0.07
Fe, mg/l	18.9 – 20.86	0.42 – 1.43	0.63 – 0.98
Zn, mg/l	17.6 – 21.44	0.21 – 2.76	0.03 – 0.76
Ni, mg/l	8.81 – 9.72	0.08 - 0.21	0.05 – 0.10
Cd, mg/l	9.35 – 10.29	0.006 – 0.01	0.006 – 0.01
Co, mg/l	9.32 – 10.17	0.026 – 0.04	0.031 – 0.05
Removal of heavy metals ratio, %			98.9 - 99.9

The effluents contain low concentration of sulphates – 0.11 – 0.12 g/l. Also the excess amounts of H_2S are determined in the sample points after chemical reactor and settler. This result shows that it is necessary to perform precise dosing of flow rates into the chemical reactor and maintaining the optimal ratio between the concentrations of heavy metals and that of microbial produced sulphide or after the settler to be provided the necessary oxidation step to remove the excess of H_2S as elemental sulphur.

In Table 3 are presented the data about analyzed microbial content of the liquid phase of anaerobic bioreactor. The dominants in a formed mixed culture are sulphate-reducing bacteria. They are using lactate as a carbon and energy source. The concentrations of all investigated groups of

microorganisms are higher with orders in the microbial biofilm.

The precipitation of metal ions as metal sulphides from solution is dependent on the availability of HS^- ions in solution, which in turn is pH-dependent. Due to microbial generated bicarbonate ions, the pH of the treated wastewater remains relatively unchanged between 7.46 and 7.69. Under these conditions precipitation of metal ions as metal sulphides is enhanced. The results obtained for composition of metal sulphide sludge are shown in Table 4.

Table 3 *Number of main physiological groups of microorganisms in effluent from fixed bed anaerobic bioreactor*

Physiological group	Number, cells/ml
Anaerobic heterotrophic bacteria	$2{,}5.10^6$
Fermenting sugars bacteria with gas production	$5{,}0.10^5$
Sulphate-reducing bacteria, using lactate	$1{,}3.10^7$

Table 4 *The composition of the sludge of the settler*

Element	Weight, %
Cu	41.00
Fe	2.24
Zn	4.09
Ni	2.30
Cd	2.14
Co	0.50
S	26.50

Based on theoretical stoichiometry of relative reactions between heavy metals and HS^- ions, the metal precipitates in sludge are metal sulphides. The metal precipitates were analysed as XRD diagrams in order to verify their composition (Figure 2). XRD diagrams of metal precipitates revealed that the precipitates were predominantly CuS, FeS, ZnS, NiS, CoS_2 CdS, and $CuFeS_2$. Steed et al. (2000) reported that in addition to sulphides, metals precipitate as hydroxides or carbonates. XRD analysis of the precipitates (figure 2) demonstrated also the presence of insignificant amounts of carbonate phases ($CuCO_3$, $FeCO_3$, $ZnCO_3$ and $CdCO_3$) Hydroxide and carbonate precipitation is of concern especially when the pH of the wastewater was 6.2–7.5, wastewater was treated with filters containing alkaline materials or when the pH of the treatment process was adjusted with e.g. NaOH, NH_4OH or Na_2CO_3 containing buffers (Kaksonen et al. 2003). The conditions in the settler (pH between 7.46 and 7.69) allowed the precipitation of insignificant amounts of metal ions as metal carbonates.

The amount of fine particles leave the setter due to the high recycle ratio and it was detected in fixed bed bioreactor and effluent. The metal sulfides with low solubility products tend to initially form very small particles and later on small crystals in the solution. Remoundaki et al. (2008) reported the formation of mainly amorphous Zn and Fe sulfides in a sulphate reducing fixed-bed reactor, which are not suitable for the recovery of metals. Other authors (Kaksonen et al. 2003, Bijmans et al. 2009) reported the formation of crystalline sphalerite, pyrite and wurtzite for fluidized-bed reactors and gas-lift reactors.

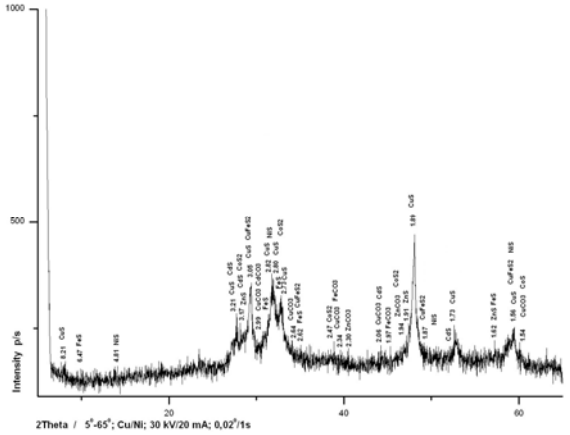

Fig. 2 *X-ray diffractogram of metal sulphide sludge*

4. Conclusions

The following conclusions can be drawn from this study:

The mixed culture of sulphate reducing bacteria showed high resistance to ionic forms of Fe, Co, Zn, Cu. and Ni. Cadmium has highest inhibitory effect on sulphate reducing bacteria. A high removal rate of heavy metals Cu – 200 mg/l, Fe – 20 mg/l, Zn – 20 mg/l, Ni – 10 mg/l, Cd – 10 mg/l and Co – 10 mg/l was achieved by fixed bed anaerobic bioreactor when lactate was used as sole source of carbon and energy and the initial COD/SO_4^{2-} ratio was around 2.7. The produced alkalinity neutralized the wastewater from pH 2.50 – 2.73, resulting in effluent pH of 7.46 – 7.69.

References

1. **Bayrakdar A., Sahinkaya E. , Gungor M., Uyanik S., Atasoy A**
Performance of sulfidogenic anaerobic baffled reactor (ABR) treating acidic and zinc-containing wastewater, Bioresource Technology 100 4354–4360., 2009.

2. **Bratkova S., Angelov A., Nikolova K., Babanova E.** *Removal of Cu(II) ions from waters using biogenic hydrogen sulphide,* Proceedings of the XI-th national conference with international participation of the open and underwater mining of minerals, Varna, Bulgaria, 19-23 june 2011.

3. **Buisman C., Huisman J., Dijkman H, Bijmans M.**
Trends in application of industrial sulphate reduction for sulphur end metal recycling. Proceedings of European Metallurgical Conference, 11-14 June 2001, Düsseldorf, Germany vol 2:383-387, 2007.

4. **Cabrera G., Perez R., Gomez J., Abalos A., Cantero D.**
Toxic effects of dissolved heavy metals on Desulfovibrio vulgaris and Desulfovibrio sp. Strains, Journal of Hazardous Materials A135 (2006) 40–46, 2006.

5. **Cao J., Zhang G., Mao Z., Fang Z., Yang C.**
Precipitation of valuable metals from bioleaching solution by biogenic sulfides, Minerals Engineering 22 (2009) 289–295

6. **Costa,M.C., Martins,M., Jesus, C., Duarte, J.C.**
Treatment of acidmine drainage by sulphate-reducing bacteria using low cost matrices. Water Air Soil Poll. 189,149–162, 2007

7. **Hao, O.J.**
Metal effects on sulfur cycle bacteria and metal removal by sulfate reducing bacteria. In: Lens, P.N.L. and Hulshoff Pol, L. (eds.) Environmental technologies to treat sulfur pollution, Principles and engineering. IWA Puplishing, London. Pp.393-414, 2000.

8. **Hoa, T.T.H., Liamleam,W., Annachhatre, A.P.**
Lead removal through biological sulfate reduction process. Bioresour. Technol. 98, 2538–2548, 2007.

9. **Kaksonen, H., Franzmann, P.D., Puhakka, J.A..**
Effects of hydraulic retention time and sulfide toxicity on ethanol and acetate oxidation in sulfate-reducing metal-precipitating fluidized-bed reactor. Biotechnol. Bioeng. 86, 332–343, 2004

10. **Kaksonen, A.H., Puhakka, J.A.**
Sulfate reduction based bioprocesses for the treatment of acid mine drainage and the recovery of metals. Eng. Life Sci. 7, 541–564, 2007.

11. **Kaksonen, A.H., Riekkola-Vanhanen, M.L., Puhakka, J.A**
Optimization of metal sulphide precipitation in fluidized-bed treatment of acidic wastewater. Water Res. 37, 255–266, 2003.

12. **Liamleam, W., Annachhatre, A.P.**
Electron donors for biological sulfate reduction. Biotechnol. Adv. 25, 452–463, 2007.

13. **Medircio S., Leao V., Teixeira M.**
Specific growth rate of sulfate reducing bacteria in the presence of manganese and cadmium. Journal of Hazardous Materials 143 593–596, 2007.

14. **Nagpal, S., Chuichulcherm, S., Peeva, L., Livingston, A.**
Microbial sulfate-reduction in a liquid-solid fluidized bed reactor. Biotechnol. Bioeng. 70, 370–380, 2000.

15. **Nevatalo L., Mäkinen A., Kaksonen A., Puhakka J.**
Biological hydrogen sulfide production in an ethanol–lactate fed fluidized-bed bioreactor, Bioresource Technology 101 276–284, 2000.

16. **Remoundaki E., Kousi P.,Joulian C., Battaglia-Brunet F., Hatzikioseyian A.,Tsezos M.**
Characterization, morphology and composition of biofilm and precipitates from a sulphate-reducing fixed-bed reactor Journal of Hazardous Materials 153, 514–524, 2008.

17. **Steed, V., Suidan, M., Gupta, M., Miyahara, T., Acheson, C., Sayles, G.**
Development of a sulfate-reducing biological process to remove heavy metals from acid mine drainage. Water Environment Research 72: 530-535, 2000

18. **Utgikar, V.P., Harmon, S.M., Chaudhary, N., Tabak, H.H., Govind, R., Haines, J.R.**
Inhibition of sulfate-reducing bacteria by metal sufide formation in bioremediation of acid mine drainage. Environ. Toxicol. 17, 40–48, 2002

19. **Veeken, A.H.M., Akoto, L., Hulshoff Pol, L.W., Weijma, J.**
Control of the sulphide concentration for optimal zinc removal by sulphide precipitation in a continuously stirred tank reactor. Water Res. 37, 3709–3717, 2003

20. **Velasco A., Ramırez M., Volke-Sepulveda T., Gonzalez-Sanchez A., Revah S.**
Evaluation of feed COD/sulfate ratio as a control criterion for the biological hydrogen sulfide production and lead precipitation, Journal of Hazardous Materials 151, 407–413, 2008

21. **Vossoughi, M., Shaketi, M., Alemzadeh, I.**
Performance of anaerobic baffled reactor treating synthetic wastewater influenced by decreasing COD/SO_4^{2-} ratios. Chem. Eng. Process 42, 811–816, 2003