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REMOVAL OF Cu, Fe, Ni AND Zn IONS FROM WATERS WITH MICROBIAL PRODUCED HYDROGEN SULFIDE

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ABSTRACT. Acid waters with pH in the range at about 1,8 – 1,9 and content of Cu, Fe, Ni and Zn in equimolar concentrations (each of 15 mM) were treated by means of a laboratory installation. The removal of the heavy metals was realized in a chemical reactor due to biogenic H_2S. The pollutants precipitated in settler in forms of relevant insoluble sulphides. The hydrogen sulfide was obtained through the biofilm of sulphate-reducing bacteria, adhered to solid substrate – zeolite in anaerobic bioreactor. Bacteria were cultivated on a medium containing lactate as a carbon and energy source in concentration 12,0 g/l and sulphate - 6,0 g/l. The concentration of investigated ions of heavy metals decreased below the permeable level for water intended for use in the agriculture and/or industry.
Key words: *Anaerobic bioreactor, sulphate-reduction, heavy metals, acid waters*

ОТСТРАНЯВАНЕ НА Cu, Fe, Ni **и** Zn **ЙОНИ ОТ ВОДИ, ПОСРЕДСТВОМ МИКРОБНО ПРОДУЦИРАН СЕРОВОДОРОД**

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РЕЗЮМЕ. Кисели води с pH в интервала 1,8 – 1,9 и съдържащи Cu, Fe, Ni и Zn в еквимоларни концентрации (всеки по 15 mM) са третирани посредством лабораторна инсталация. Отстраняването на тежките метали се реализира в химичен реактор чрез биогенен H_2S. Замърсителите преципитират в утаител под формата на съответните неразтворими сулфиди. Сероводородът се получава от биофилм сулфат-редуциращи бактерии, имобилизирани върху носител – зеолит в анаеробен биореактор. Бактериите са култивирани на хранителна среда, съдържаща като източник на въглерод и енергия лактат – 12,0 g/l и сулфати 6,0 g/l. Концентрациите на изследваните йони на тежки метали спадат под пределно допустимите концентрации за води, използвани за селскостопански и/или индустриални нужди.
Ключови думи: Анаеробен биореактор, сулфат-редукция, тежки метали, кисели води

Introduction

Wastewater generated from mining and metallurgical industries 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 or sulphate rich feed stocks (e.g. fermentation or sea food processing industry). Also the use of reduced sulphur compounds in industrial processes, i.e. sulphide (tanneries, Kraft pulping), sulphite (sulphite pulping), thiosulphate (fixing of photographs) or dithionite (pulp bleaching) contaminates wastewater with sulphate.

Conventional treatments of these wastewaters use lime to precipitate metals as carbonates and hydroxides. These treatments present some serious limitations in terms of application and effectiveness. They usually result in production of unstable metal hydroxides which also lead to a greater disposal expense. 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 (*Gaikwad et al*., 2008). Soluble metal- and sulphate-bearing wastewater treatment schemes are usually based on sulphate-reducing reactors recently developed in both pilot and full scale (*Dvorak et al., 1992; Foucher et al., 1992; Kaksonen et al., 2003, 2004).*

Sulphate-reducing bacteria *at* oxidize simple organic compounds (such as lactate, acetate, butirate and another products of fermentations) with sulphate under anaerobic conditions, and thereby generate hydrogen sulphide and bicarbonate ions(*Widdel et al., 1991; Martinsat al,2009)*.

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- , \qquad (1)$$

where - CH_2O represent the organic matter.

The produced hydrogen sulphide reacts with dissolved metals to form insoluble metal sulphides that subsequently precipitate according to the reaction:

$$M^{2+} + H_2S \rightarrow MS\downarrow + 2H^+ , \qquad (2)$$

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

Sulphate-reducing bacteria are found as dominant representatives of the microflora in anaerobic bioreactors for wastewater treatment from heavy metals, mainly from genera Desulfomicrobium, Desulfobacter, Desulfobulbus and Desulfovibrio [10]. One of the problems when using a biomass for acid wastewater treatment in the same reactor system, in which the metals are settled down, is that the precipitates have to be subsequently recovered from the biomass containing sludge (*Alvarez at al, 2007*). Therefore it is necessary to separate biological stage from the precipitation stage for the treatment of waters. Another important issue is the separation of heavy metal sulphides to their further utilization. There are studies for the separate precipitation of Cu and Zn using sulfide produced in anaerobic baffled reactor (ABR), fed with ethanol and sulphate (*Sahinkaya at al, 2009)*, as the two metals precipitated separately at low pH using sulfide transported from ABR effluent via N_2.

The aims of this of this study are:

1. Through the applying of an adequate technological scheme the two processes – microbial dissimilative sulphate-reduction (MDSR) and formation of insoluble metal sulphides to be divided into separate reactors;
2. To establish parameters under which there is a complete removal of the investigated heavy metals from synthetic solution and;
3. To explore the possibility of selective precipitation of ions of heavy metals through different technological regimes.

Materials and methods

The laboratory installation for heavy metals removal from waters is given in fig.1. The geometric volume of the anaerobic reactor (4) is 1.2 dm^3 and it is filled with 1.13 kg natural zeolite and 0.67 dm^3 Postgate C medium. The surface of zeolite is completely covered. The anaerobic bioreactor is inoculated with 40 ml enriched microbial culture of sulfate-reducing bacteria. After the formation of active biofilm of SRB continuous cultivation and feeding with medium begin. Nutrient medium (6) 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 (10). A sand filter (7) is provided in the scheme to precipitate the insoluble particles. The microbial produced H_2S contacts with the solution of heavy metals in a chemical reactor (2). The geometric volume of the reactor is 0.5 dm^3. The solution of heavy metals is fed into the chemical reactor through a peristaltic pump (8) from tank (1).

Adjustable flow peristaltic pumps (8 and 9) support the necessary volume loading rates with sulfates in the bioreactor, feeding the chemical reactor with a heavy metal solution in with different flow rates and provide the necessary residence time in all facilities of the laboratory installation. The insoluble sulfides precipitated in a vertical-flow settler which volume is 0.85 dm^3. Effluents accumulate into collector tank (12) with volume of 6 dm^3. The experiment is performed at temperatures ranging from 21 ^oC to 22 ^oC.

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 - 4.0g, $CaCl_2$ - 0.1g, $MgSO_4.7H_2O$ - 8.0g, sodium lactate - 12.0g, yeast extract - 0.25g. The initial pH is adjusted to 6.5. The concentration of sulfates in the medium is 6 g/l as the organic carbon content to final electron acceptor ratio equals to 0.67. The biofilm containing SRB and other metabolic related bacteria is adhered to a natural occurred zeolite, clinoptilolite of 2.5 - 5.0 mm size fraction, taken from Beli Plast deposit, Eastern Rhodopes, Bulgaria. Zeolite used in this study is with the following composition: SiO_2 - 67.96, Al_2O_3 - 11.23, Fe_2O_3 - 0.83, K_2O - 2.85, Na_2O - 0.74, CaO - 3.01, MgO - 0.06, TiO_2 - 0.90. Cation exchange capacity and exchangeable cations are respectively: CEC – 112,75, K^+ - 33.88, Na^+ - 21.01, Ca^{2+} - 63.48, Mg^{2+} - 2.68. The heavy metals in the synthetic solution are in equimolar concentratios (each of 15 mM) respectively in the forms of $CuSO_4.5H_2O$, $FeSO_4.7H_2O$, $NiSO_4.7H_2O$ и $ZnSO_4.7H_2O$ to a total concentration 60 mM. The pH of the solution is adjusted in the range of 1.8 – 1.9 with 1 N H_2SO_4.

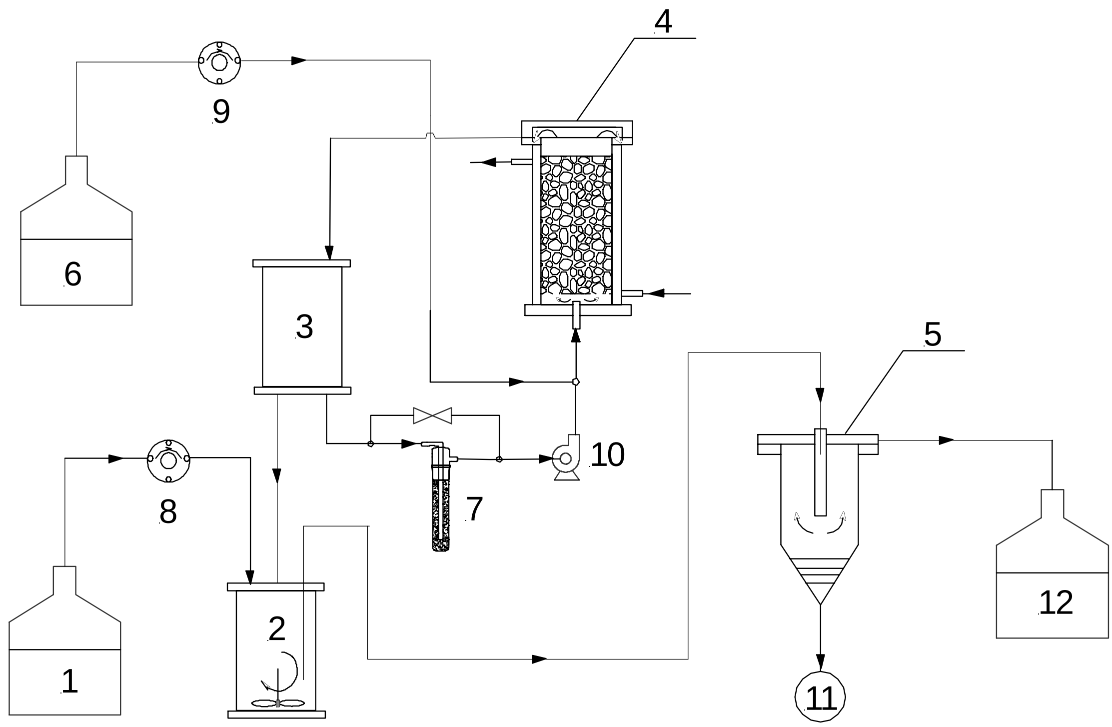

Fig.1. Schematic diagram of the laboratory installation for active treatment of wastewaters polluted with heavy metals. 1 – Heavy metals solution tank, 2 – chemical reactor, 3 - mix tank, 4 - anaerobic fixed-bed biofilm reactor*, 5 – settler, 6- nutrient medium tank, 7- sand filter, 8 and 9 – peristaltic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

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.

Table 1.
Nutrient media and cultivation conditions used for enumeration of main physiological groups of microorganisms

Physiological groups	Nutrient media	Cultivation regime
Aerobic heterotrophic bacteria	nutrient agar	37°C, 48 h
Fermenting sugars bacteria with gas production	nutrient broth + 1% glucose + liquid paraffin	37°C, 5 days
Sulphate-reducing bacteria	Postgate medium	30°C, 5 days

Numbers of sulphate-reducing, aerobic 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 (Table 1) (*Postgate, 1984; Parks at al, 1997)*.

Results and discussion

Formation of active biofilm containing SRB in the anaerobic fixed-bed biofilm reactor

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 mixed SRB culture, obtained from a laboratory anaerobic cell for treatment of synthetic acid mine waters (Fig.2a, 2b). The microbial composition of the inoculum is presented in Table 2.

Table 2.
Number of main physiological groups of microorganisms of the so-used inoculum

Physiological group	Number, cells/ml
Aerobic heterotrophic bacteria	$6,0.10^5$
Fermenting sugars bacteria with gas production	$2,5.10^4$
Sulphate-reducing bacteria, using lactate	$1,3.10^6$

The formation of active biofilm is carried out through repeated periodic replacement of 50% of the liquid phase of the bioreactor with fresh medium. Replacement of the liquid phase is performed after the concentration of sulphates reduces below 0.2 g/l. In the end of the this period started continuous feeding of the anaerobic bioreactor with the modified nutrient Postgate medium. For a period of 2 months progressively is decreased the residence time (at 240 to 41.4 h) and respectively increases volume loading rate of the bioreactor with sulphates (at 0.025 to 0.145 g/l.h).

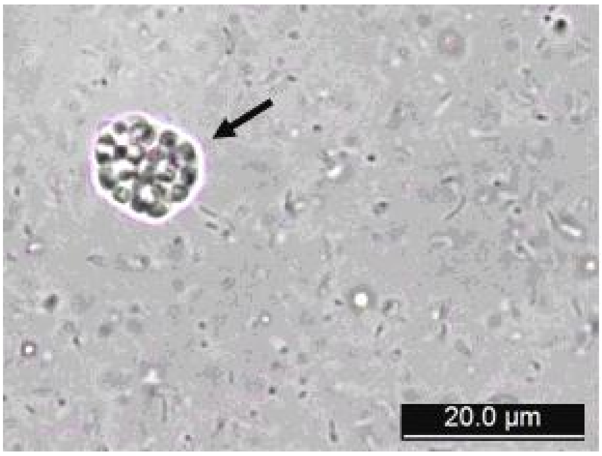

Fig.2a. Photomicrograph by phase contrast of bacteria in the liquid phase

Maintaining sulphates volume loading rate at about 0.145 g/l.h the rate of microbial sulphate-reduction is in the range of 130 - 133 mg/l.h as its efficiency is 90.0 – 91.8. The data of number of main physiological groups of microorganisms in the liquid phase of the anaerobic bioreactor during this period and during the treatment of waters, polluted with Cu, Fe, Ni and Zn ions are presented in Table 3.

Table 3.
Number of main physiological groups of microorganisms in the liquid phase of the anaerobic bioreactor

Physiological group	Number, cells/ml
Aerobic heterotrophic bacteria	$5,0.10^6$ - $5,9.10^6$
Fermenting sugars bacteria with gas production	$1,3.10^7$ - $6,0.10^7$
Sulphate-reducing bacteria, using lactate	$6,0.10^7$ - $1,3.10^8$

From the obtained data is obvious that after the period of 5 months the ratio between different microorganisms changes. Number of fermenting sugars bacteria with gas production highly increases. The dominants in the formed mixed culture are sulphate-reducing bacteria, using lactate as a carbon and energy source. The concentrations of all investigated groups of microorganisms are higher with orders in the microbial biofilm. For this reason the process of microbial dissimilative sulphate-reduction carries out with high rates.

At Figure 2 is shown photography of the bacteria in the liquid phase of the anaerobic bioreactor at sulphates volume loading rate at about 0.145 g/l.h.

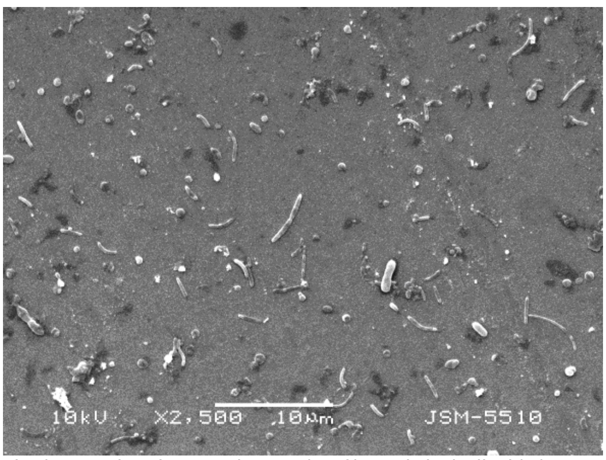

Fig.2b. Scanning electron micrographs of bacteria in the liquid phase

Influence of heavy metals volume loading rates of the chemical reactor to the removal of pollutants

Influence of heavy metals volume loading rates of the chemical reactor to the removal ratio of pollutants is established through operating the installation in 4 different technological regimes. For them is changed the flowrate in the chemical reactor with feed solution and are held a constant concentrations of incoming H_2S. For this purpose is maintained a flowrate of feeding with nutrient medium the anaerobic bioreactor at which the sulphates loading rate ranges to 0.145 g/l.h. Under these conditions the concentration of H_2S in effluent waters is at about 1.34 to 1.67 g/l.

Summarized data in Table.4 shows that at the highest tested volume loading rate of the chemical reactor with ions of heavy metals - 3.23 mM/l.h is achieved only 65.0 – 65.5 % of removal of the pollutants from water. Heavy metals react with microbial produced hydrogen sulphide in chemical reactor and precipitates in the settler of the laboratory installation as respective insoluble sulphides. Mainly are removed the copper ions (at about 99 %) according the chemical properties of sulphides. Effluents from the whole set have pH in the range of 4.25 - 4.65 as the treated solution, containing ions of Cu, Fe, Ni and Zn, is extremely acid with pH at about 1.8-1.9. The sulphates content in the effluents is high - 4.04 - 4.15 g/l, because ions of metals are imported into the synthetic solution in the form of $CuSO_4.5H_2O$, $FeSO_4.7H_2O$, $NiSO_4.7H_2O$ and $ZnSO_4.7H_2O$ as sulphuric acid is used to adjust the pH to values below 1.9. The concentration of sulphates in this solution ranges 5.97 - 6.18 g/l.

The removal of pollutants from the solutions in ranges of 71 – 72 % is due to the decrease of volume loading rate of the chemical reactor with heavy metals to 1.97 mM/l.h (Table 5). The effluents have pH at about 5.36 – 5.95 and content high concentrations of heavy metals ions.

Table 4.

Basic parameters measured in sample points at the outlets of main facilities of the laboratory installation at volume loading rate of chemical reactor with heavy metals 3.23 mM/l.h

Parameter	Solution of heavy metals	Outlet of the anaerobic bioreactor	Outlet of the chemical reactor	Outlet of the settler
pH	1.80 – 1.90	7.56- 8.30	4.37 – 4.86	4.25 – 4.65
Eh, mV	-	- 407 - -439	+31 - +46	+57 - +85
SO_4^{2-},g/l	6.35 – 6.53	0.49 – 0.65	3.95 – 4.02	4.04 – 4.15
H_2S, g/l	-	1.34 – 1.67	0	0
Cu, mg/l	954.8 – 967.3	-	9.67 – 10.59	9.61 – 10.21
Fe, mg/l	847.9 – 850.1	-	380 - 391	376 - 365
Ni, mg/l	887.8 – 896.2	-	524 - 529	520 - 530
Zn, mg/l	975.7 – 982.3	-	311 - 323	307 - 317
Removal of heavy metals ratio, %				65.0 – 65.5

Table 5.

Basic parameters measured in sample points at the outlets of main facilities of the laboratory installation at cupric volume loading rate of chemical reactor of 1.97 mM/l.h

Parameter	Solution of heavy metals	Outlet of the anaerobic bioreactor	Outlet of the chemical reactor	Outlet of the settler
pH	1.80 – 1.90	7.56- 8.30	5.47 – 6.16	5.36 – 5.95
Eh, mV	-	- 407 - -439	+1 - +35	+8 - +15
SO_4^{2-},g/l	6.35 – 6.53	0.49 – 0.65	3.07 – 3.13	2.96 – 3.15
H_2S, g/l	-	1.34 – 1.77	0	0
Cu, mg/l	954.8 – 967.3	-	5.12 – 6.65	5.08 – 6.13
Fe, mg/l	847.9 – 850.1	-	264 - 275	260 – 274
Ni, mg/l	887.8 – 896.2	-	452 - 460	447 – 453
Zn, mg/l	975.7 – 982.3	-	270 - 276	258 - 270
Removal of heavy metals ratio, %				71.0 – 72.8

In the course of the third technological regime (volume loading rate is 1.67 mM/l.h – Table 6) the removal of heavy metals ratio from polluted waters ranges at 92.1 - 93.4%. In the settler the redox conditions are favorable for microbial sulphate-reduction but probably due to relatively high concentrations of ions of heavy metals (mainly nickel) the process does not occur or occurs at very low rate.

In the course of the lowest investigated volume loading rate of the chemical reactor 1.0 mM/l.h is reached a complete removal of the ions of the heavy metals and their concentrations in the effluent flows are below the permeable level for water intended for use in the agriculture and/or industry (Table 7). i.e. there is realized 99.99% precipitation of the pollutants in forms of insoluble sulphides. In this mode, however, the concentration of microbial produced hydrogen sulfide in chemical reactor exceeds the required equimolar quantity for precipitation of heavy metals with a total content of 60 mM.

Excess amounts of H_2S are determined in the sample points after the chemical reactor and the settler (Table 7). Higher amounts of hydrogen sulphide in the outlets of the settler are consequence of the process microbial sulphate-reduction. 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.

Summarized data for the removal under the studied operational regimes are presented in Figure 3.

Conclusions

In this study are provided conditions under which in the anaerobic bioreactor, filled with natural occurred zeolite as a solid substrate, is achived formation of active biofilm, containing SRB. In chemical reactor in which is provided optimal mixing of the flows are treated waters with a total content of 60 mM of Cu, Fe, Ni and Zn ions.

Based on the obtained results it can be concluded that through the installation constructed that way is achieved complete removal of heavy metals in the anaerobic bioreactor in a mode with sulphates loading rate of 0.145 g / lh (1.51 mM / lh). A very small fraction of sulfates in water is used as a sulphur source for the bacteria. When choosing a volume loading rate of the chemical reactor with heavy metals 1 mM / lh, 99.99% removal of contaminants is achieved, but the difference in molar ratios leads to high concentrations of hydrogen sulphide in output flows. For as much as the copper ions form a sulphide with the lowest solubility, the selective precipitation of the element of poly-metallic solutions is possible. For this purpose it is necessary to provide a controlled two-stage mixing of solutions, containing metals, with water rich of H_2S and also a presence of two settlers in the installation.

Table 6.

Basic parameters measured in sample points at the outlets of main facilities of the laboratory installation at cupric volume loading rate of chemical reactor of 1.67 mM/l.h.

Parameter	Solution of heavy metals	Outlet of the anaerobic bioreactor	Outlet of the chemical reactor	Outlet of the settler
pH	1.85 – 1.99	7.56- 8.30	6.87 – 6.96	6.45 – 6.75
Eh, mV	-	- 407 - -439	-60 - 126	-81 - -147
SO_4^{2-},g/l	6.35 – 6.53	0.49 – 0.65	2.86 – 3.03	2.78 – 3.05
H_2S, g/l	-	1.34 – 1.77	0	0
Cu, mg/l	954.8 – 967.3	-	0.71 – 0.62	0.51 – 0.43
Fe, mg/l	847.9 – 850.1	-	112 - 121	105 - 110
Ni, mg/l	887.8 – 896.2	-	141- 153	135 - 138
Zn, mg/l	975.7 – 982.3	-	26 - 29	21 - 27
Removal of heavy metals ratio, %				92.1 -93.4

Table 7.

Basic parameters measured in sample points at the outlets of main facilities of the laboratory installation at cupric volume loading rate of chemical reactor of 1.0 mM/l.h.

Parameter	Solution of heavy metals	Outlet of the anaerobic bioreactor	Outlet of the chemical reactor	Outlet of the settler
pH	1.85 – 1.99	7.56- 8.30	7.37 – 7.74	7.21 – 7.53
Eh, mV	-	- 407 - -439	-133 - -196	-257 - -305
SO_4^{2-},g/l	6.35 – 6.53	0.49 – 0.65	2.27 – 2.43	2.14 – 2.35
H_2S, g/l	-	1.34 – 1.67	0.140 – 0.214	0.168 – 0.234
Cu, mg/l	954.8 – 967.3	-	<0.005	<0.005
Fe, mg/l	847.9 – 850.1	-	0.041 – 0.062	0.03 – 0.06
Ni, mg/l	887.8 – 896.2	-	0.17 – 0.19	0.18 – 0.19
Zn, mg/l	975.7 – 982.3	-	0.06 – 0.16	0.06 – 0.16
Removal of heavy metals ratio, %				99.99

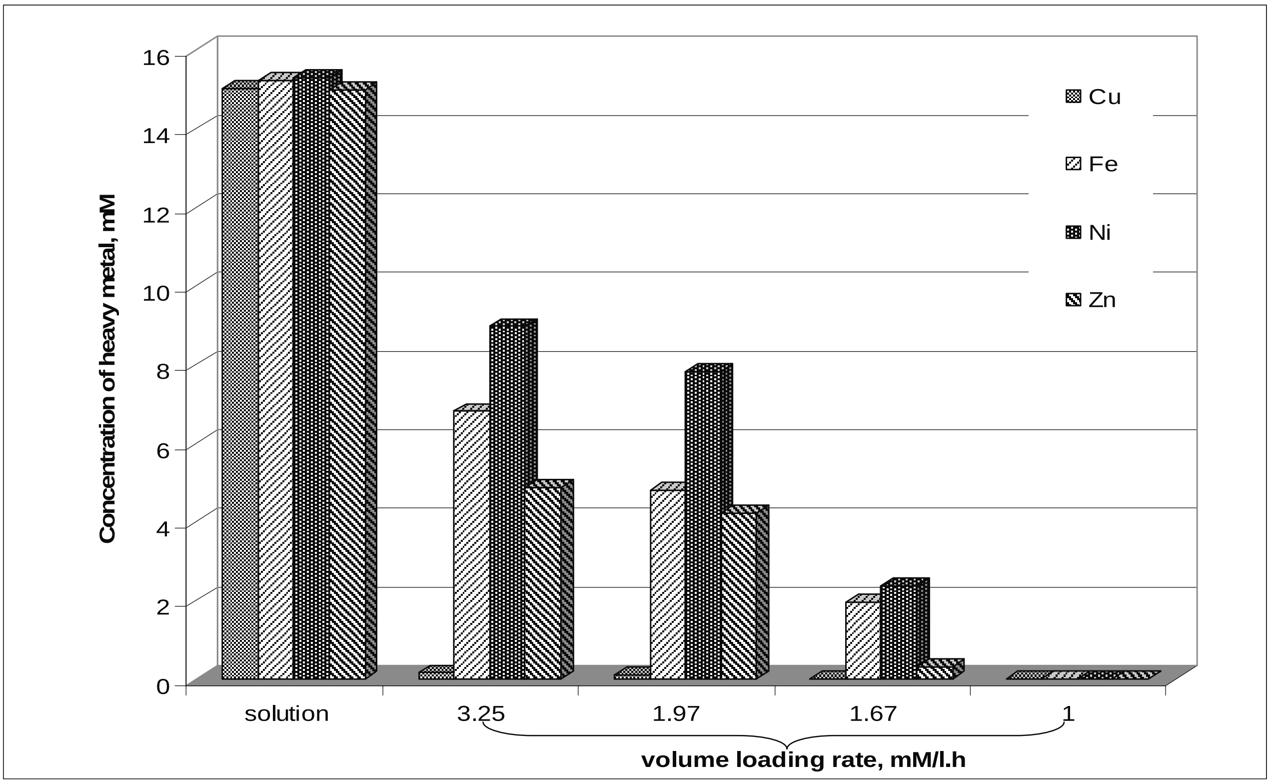

Fig. 3. Removal of Cu, Fe, Ni and Zn ions with initial concentrations 15 mM at different volume loading rates of the chemical reactor

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