Front. Environ. Sci. Eng. 2018, 12(4): 12
https://doi.org/10.1007/s11783-018-1073-4

RESEARCH ARTICLE

Metallic wastewater treatment by sulfate reduction using anaerobic rotating biological contactor reactor under high metal loading conditions

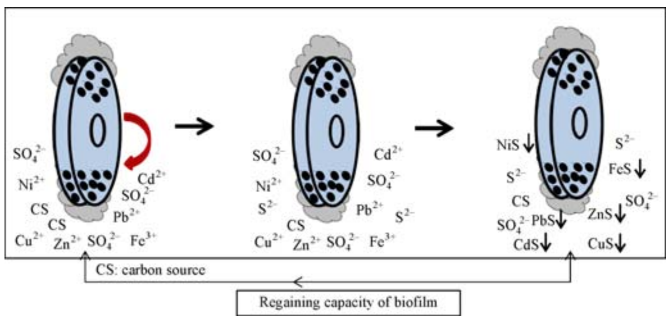
Mothe Gopi Kiran[1], Kannan Pakshirajan (✉)[1,2], Gopal Das[1,3]

1 Centre for the Environment, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
2 Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
3 Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India*

HIGHLIGHTS

- An-RBC reactor is highly suited to treat metallic wastewater.
- Metal removal is due to sulfide precipitation via sulfate reduction by SRB.
- Cu(II) removal was the best among the different heavy metals.
- Maximum metal removal is achieved at low metal loading condition.
- Metal removal matched well with the solubility product values of respective metal sulfide salts.

GRAPHIC ABSTRACT



ABSTRACT

This study was aimed at investigating the performance of anaerobic rotating biological contactor reactor treating synthetic wastewater containing a mixture of heavy metals under sulfate reducing condition. Statistically valid factorial design of experiments was carried out to understand the dynamics of metal removal using this bioreactor system. Copper removal was maximum (>98%), followed by other heavy metals at their respective low inlet concentrations. Metal loading rates less than 3.7 mg/L·h in case of Cu(II); less than 1.69 mg/L·h for Ni(II), Pb(II), Zn(II), Fe(III) and Cd(II) are favorable to the performance of the An-RBC reactor. Removal efficiency of the heavy metals from mixture depended on the metal species and their inlet loading concentrations. Analysis of metal precipitates formed in the sulfidogenic bioreactor by field emission scanning electron microscopy along with energy dispersive X-ray spectroscopy (FESEM-EDX) confirmed metal sulfide precipitation by SRB. All these results clearly revealed that the attached growth biofilm bioreactor is well suited for heavy metal removal from complex mixture.

ARTICLE INFO

Article history:
Received 10 January 2018
Revised 29 April 2018
Accepted 10 July 2018
Available online 4 August 2018

Keywords:
Factorial design analysis
Sulfate reducing bacteria
Multi-metal solution
Heavy metal removal
Anaerobic rotating biological contactor reactor
High metal loading

1 Introduction

Natural water bodies, such as rivers, lakes, marine environment, etc. encounter heavy metal toxicity either due to natural or human developmental activities (Sema et al., 2012). Among the many developmental activities, industries that discharge metallic wastewater, e.g. acid mine drainage (AMD), are responsible for the various soluble metals and sulfate in the environment (Min et al., 2008). Performance of wastewater treatment system is often prone to deterioration owing to an elevated contamination of sulfate and heavy metals in the waste-

✉ Corresponding author
E-mail: pakshi@iitg.ac.in

*Special Issue—Bio-based Technologies for Resource Recovery (Responsible Editors: Aijie Wang & David Stuckey) water, thereby posing serious threat to the environment and the ecosystem (Sema et al., 2012; Kiran et al., 2016).

Metabolic activity of sulfate reducing bacteria (SRB) can reduce sulfate present in metallic wastewater to sulfide (Kaksonen and Puhakka, 2007), and microbial sulfate reduction coupled to the high affinity of sulfide to form insoluble metal sulfides with metal ions is marked as a key substitute to treat wastewater containing both metals and sulfate (Bai et al., 2008; Jiménez-Rodríguez et al., 2009; Teclu et al., 2009; Velasco et al., 2008). Metals in wastewater invariably occur as mixture and often interfere with biological treatment systems to a great extent by manifesting toxic effects than the individual metals (Wang et al., 2009).

The collective effect of a mixture of metals can be either superior than the sum of each metal effect (synergy) or equivalent to the summation of each individual metal effect (additive effect) (Utgikar et al., 2004; Gikas, 2007) or lesser than their sum due to antagonistic effect (Utgikar et al., 2004). Hence, metals in mixture present a different effect on microorganisms in a biological treatment system than the individual metals themselves (Sema et al., 2012). Furthermore, it is well reported that results from batch experiments cannot be extrapolated to comprehend the collective effect of two or more heavy metals on their continuous removal by SRB (Chen et al., 1997; Hu et al., 2004; Sen et al., 2007).

In a study using packed bed bioreactor (PBR) for treating mine wastewater with a novel marine waste extract (MWE) as an alternative nitrogen source for the growth of SRB, Dev et al. (2016) reported 62%–66% of sulfate removal and 66%–75% of metal removal (Fe, Cu, Zn, Mn, Mg and Ni) at a very high hydraulic retention time (HRT) of 120 h. The authors also demonstrated that MWE can serve as a cheap and alternative source of nitrogen for enhancing SRB growth compared with any commercially available sources. However, the removal efficiency values of metal and sulfate removal are low when compared with the values reported in the literature (Robinson-Lora and Brennan, 2009), which can be attributed to differences in the (i) heavy metals used and its initial concentration, (ii) microbial community, (iii) reactor operating conditions followed and (iv) reactor configuration (Kaksonen and Puhakka, 2007; Kiran et al., 2017a).

Dev et al. (2017) followed Taguchi design of experiments to optimize batch process parameters (pH, HRT, MWE, sulfate and total organic carbon (TOC)) involved in heavy metal removal by sulfate reduction. At an optimum level of these parameters, the authors operated a PBR with SRB biomass for nearly 150 days to treat acid mine drainage (AMD) collected from two different sources. More than 94%–98% removal efficiency of heavy metals (Fe, Cu, Zn, Mg and Ni) was reported except in the case of Mn, for which, the removal was 28%–38% (Dev et al., 2017). Guo et al. (2017) conducted bioassessment of heavy metal toxicity and investigated heavy metal removal from single and multi-metal solutions using by SRB or SRB with zero valent iron. The SRB with zero valent iron showed better results than SRB in terms of high sulfate reduction and heavy metal removal (Cr, Mn, and Zn) values (Guo et al., 2017). These results also showed that zero valent iron synergistically supported high level of sulfate reduction which further enhanced the metal removal (Guo et al., 2017).

Zhang and Wang (2016) used an up-flow anaerobic packed bed bioreactor (UAPBR) filled with immobilized SRB granules for treating AMD containing high concentration of heavy metal (Fe, Cu, Zn, Mn and Cd). More than 99.9% removal of Fe, Cu, Zn and Cd and 42.1%–99.3% of Mn were reported. Maximum removal of Fe, Zn, Cu and Cd was obtained due to their low solubility product constant values with sulfide as compared with low removal of Mn which has a high sulfide solubility product value. Moreover, Mn removal was mainly attributed to hydroxide or carbonate precipitation (Zhang and Wang, 2016). Similar to this study, Zhang et al. (2016) reported a high removal of sulfate (61%–88%) and heavy metal (>99.9%) from synthetic AMD containing high concentrations of Fe, Cu, Cd and Zn using an UAPBR filled with novel immobilized SRB beads. Using this reactor system, not only very high removal of the heavy metals was achieved, but also it demonstrated very good tolerance of the immobilized SRB beads to high concentrations of heavy metals in the wastewater (Zhang et al., 2016).

For continuous metal removal from mixture by SRB, reactor configuration is a very important factor affecting the SRB activity and therefore, its performance. Among the different sulfidogenic reactors, anaerobic rotating biological contactor (An-RBC) reactor has been recently shown to be effective for removing metals under sulfate reducing conditions (Kiran et al., 2017a). However, its ability to treat wastewater containing a mixture of heavy metals under high metal loading condition has not been investigated so far. Hence, this study is aimed at investigating the performance of An-RBC reactor for continuous metal removal from mixture in which, the SRB exist mainly as passively attached biofilm onto its rotating discs. The bio-precipitates formed in the reactor were further characterized using field emission scanning electron microscope integrated with energy dispersive X-ray spectroscopy (FESEM-EDX) to understand the metal removal mechanism involved.

2 Materials and methods

2.1 Biomass source and wastewater composition

Mixed SRB consortia used in this study was acquired from a laboratory scale 10 L capacity packed bed reactor treating sulfate rich wastewater (Brahmacharimayum and Ghosh, 2014; Kiran et al., 2016; Kiran et al., 2017b). Microbial community analysis of the biomass showed that the SRB consortium consisted of *Desulfovibrio.* species (Brahmacharimayum and Ghosh, 2014). Modified Postgate medium for the SRB growth consisted of (g/L): 1 NH_4Cl, 1.47 Na_2SO_4, 0.1 ascorbic acid, 0.2 tri-sodium citrate, 0.1 $CaCl_2 \cdot 2H_2O$, 0.2 bromo ethane sulfonic acid (BESA) (Jin et al., 2007), 0.2 EDTA (Saifullah et al., 2009), 0.5 KH_2PO_4, 0.15 $FeSO_4 \cdot 7H_2O$ (Postgate, 1984). Sodium lactate (60% v/v) was used as the carbon source. Concentrations of sulfate and chemical oxygen demand (COD) in the influent were adjusted so as to obtain a COD/sulfate ratio of 0.67±0.08 (Rinzema and Lettinga, 1998). The influent pH was adjusted to 7 using 1N NaOH. All the reagents and chemicals used in this study were of analytical grade.

2.2 Heavy metal removal experiments

The details of An-RBC reactor (passive biofilm bioreactor) used to study heavy metal removal from mixture under high inlet loading condition can be found in our previous work (Kiran et al., 2017a). Schematic of the An-RBC reactor used in this study is provided in Fig. 1a. Photograph of experimental setup showing the An-RBC reactor with the immobilized SRB on its discs is shown in Fig. 1b. Owing to its attached/immobilized form in the reactor, the amount of biomass present could not be precisely determined, for example, as mixed liquid suspended solids (MLSS) or mixed liquor volatile suspended solids (MLVSS), which are however, commonly reported for characterizing the amount of suspended biomass present in any biological system. The statistically valid fractional factorial design (FFD) was employed for carrying out the experiments involving different combination levels of Cd(II), Fe(III), Zn(II), Cu(II), Pb(II) and Ni (II) in the influent wastewater.

Table 1 presents the FFD experiments with different metal combination levels highlighting high metal loading conditions. Heavy metal concentration levels shown in Table 1 are chosen in view of the results obtained from a previous study using SRB immobilized An-RBC bioreactor (Kiran et al., 2017a). The results of heavy metal removal under high metal loading conditions were statistically analyzed in terms of student's *t* test and analysis of variance (ANOVA) to comprehend the collective effect of mixture of metals on each other removal. Low metal combination levels in the mixture always yielded maximum removal (90%) of the corresponding metals in mixture except Ni(II), which is more than 66%. Minitab (Version 16, PA, USA), a statistical software was employed for the FFD and for statistical analysis of the results.

Metal stock solutions each of 100 g/L concentration were prepared using $CuCl_2 \cdot 2H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $Cd(NO_3)_2 \cdot 4H_2O$ and $PbNO_3$. Desired concentration of the metals as per the FFD was obtained by adding corresponding metal stock solution to the modified Postgate medium mentioned earlier. All experiments were carried out at 48 h hydraulic retention time (HRT) and at an ambient temperature of 25±2°C (Kiran et al., 2017a). Samples collected from outlet of the reactor at regular time intervals were analyzed for sulfate, COD, metal and sulfide concentrations.

Sulfate, metal and COD concentration in the samples

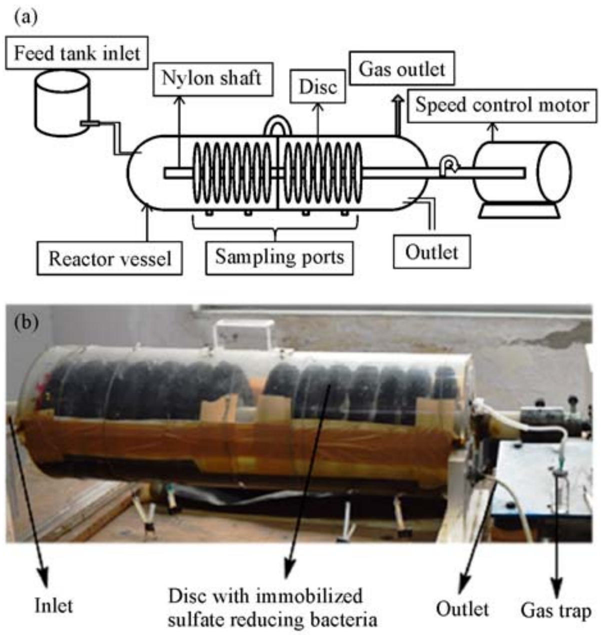


Fig. 1 (a) Schematic of the An-RBC reactor; (b) photograph of experimental setup showing the An-RBC reactor with the immobilized SRB on its discs

Table 1 Fractional factorial design of experiments presenting different heavy metal combination levels highlighting high metal loading condition in the study

Experimental runs	Cd	Cu	Ni	Fe	Pb	Zn
1	**90**	**175**	**90**	**50**	**90**	**50**
2	50	175	90	50	50	50
3	50	100	90	50	90	75
4	**50**	**100**	**90**	**90**	**90**	**50**
5	**50**	**100**	**50**	**50**	**50**	**50**
6	**50**	**175**	**50**	**90**	**90**	**50**
7	**90**	**100**	**90**	**90**	**50**	**50**
8	**90**	**100**	**50**	**90**	**90**	**75**
9	**70**	**137.5**	**70**	**70**	**70**	**62.5**
10	50	100	50	90	50	75
11	90	100	50	50	90	50
12	**50**	**175**	**90**	**90**	**50**	**75**
13	50	175	50	50	90	75
14	90	100	90	50	50	75
15	**90**	**175**	**90**	**90**	**90**	**75**
16	**70**	**137.5**	**70**	**70**	**70**	**62.5**
17	**70**	**137.5**	**70**	**70**	**70**	**62.5**
18	**90**	**175**	**50**	**90**	**50**	**50**
19	**90**	**175**	**50**	**50**	**50**	**75**

were determined as per the methods explained in American Public Health Association (APHA) (APHA, 2005). Sulfide concentration in the samples was measured as per the method outlined by Cord-Ruwisch (1985). Each experimental run as per the FFD was carried out for a period until three steady-state values of effluent heavy metal concentration were obtained at 48 h HRT interval. Results reported are average of three steady-state values.

2.3 Metal bio-precipitates characterization using FESEM-EDX

Metal precipitates obtained from experimental run 5, which yielded a maximum heavy metal removal efficiency in this study were analyzed for its elemental composition and morphology using FESEM-EDX (Zeiss, Sigma, Germany) as per the method described by Cao et al. (2013).

3 Results and discussion

3.1 Removal of metal, sulfate and COD from wastewater

This study was mainly carried out to comprehend the collective effect of mixture of metals commonly present in metallic wastewater on each other removal using the An-RBC reactor (passive biofilm bioreactor). Simultaneous removal of Cu(II), Zn(II), Fe(III), Ni(II), Cd(II) and Pb(II) using the An-RBC reactor is presented in Fig. 2(a–b). Copper removal was maximum (98.4%), which is followed by Zn(II) (96%), Fe(III) (95%), Pb(II) (93.5%), Cd(II) (91%), and Ni(II) (65.7%) at their respective low inlet concentrations i.e., experimental run 5 with 50 mg/L each of Ni(II), Pb(II), Fe(III), Cd(II) and Zn(II) and 100 mg/L of Cu(II) (Fig. 2(a-b)). Metal removal was minimum at their respective high inlet combinations in mixture (experimental run 15; Table 1; Fig. 2(a–b)). These results were slightly lower than the results of metal removal from wastewater containing the individual metals in solution (Kiran et al., 2017a). Hence, it is clear that the reactor performance to treat wastewater is based on the metal and their inlet loading concentrations. To gain further insight into the high metal loading effect on the reactor performance, metabolic activity of SRB was evaluated in terms of wastewater COD removal, sulfate reduction efficiency and sulfide generation.

Figure 2c presents the results of COD removal, sulfate reduction efficiency and sulfide generated consequent to the experimental runs using the An-RBC reactor. Maximum sulfate reduction efficiency was obtained at low metal loading condition i.e., in experimental run 5 and in experimental run 1 (>71%) (Fig. 2c). Similarly, maximum COD removal was obtained in experimental runs 5 and 2

(>78%) (Fig. 2c) and sulfide generated was maximum in experimental runs 4, 11, 8 and 5, respectively. However, these values were reduced at high metal loading condition, particularly in experimental run 15. Thus, the removal of different heavy metals correlated well with sulfate removal, confirming that the metal removal by SRB is due to sulfide precipitation (Fig. 2). Copper removal was maximum among the different heavy metals owing to its low sulfide solubility product value (Hill et al., 2005). Removal of the other metals was also consistent with the their respective sulfide solubility product values (Kiran et al., 2017a).

At low metal loading condition, the SRB activity is unaffected as evident from the sulfate reduction and COD

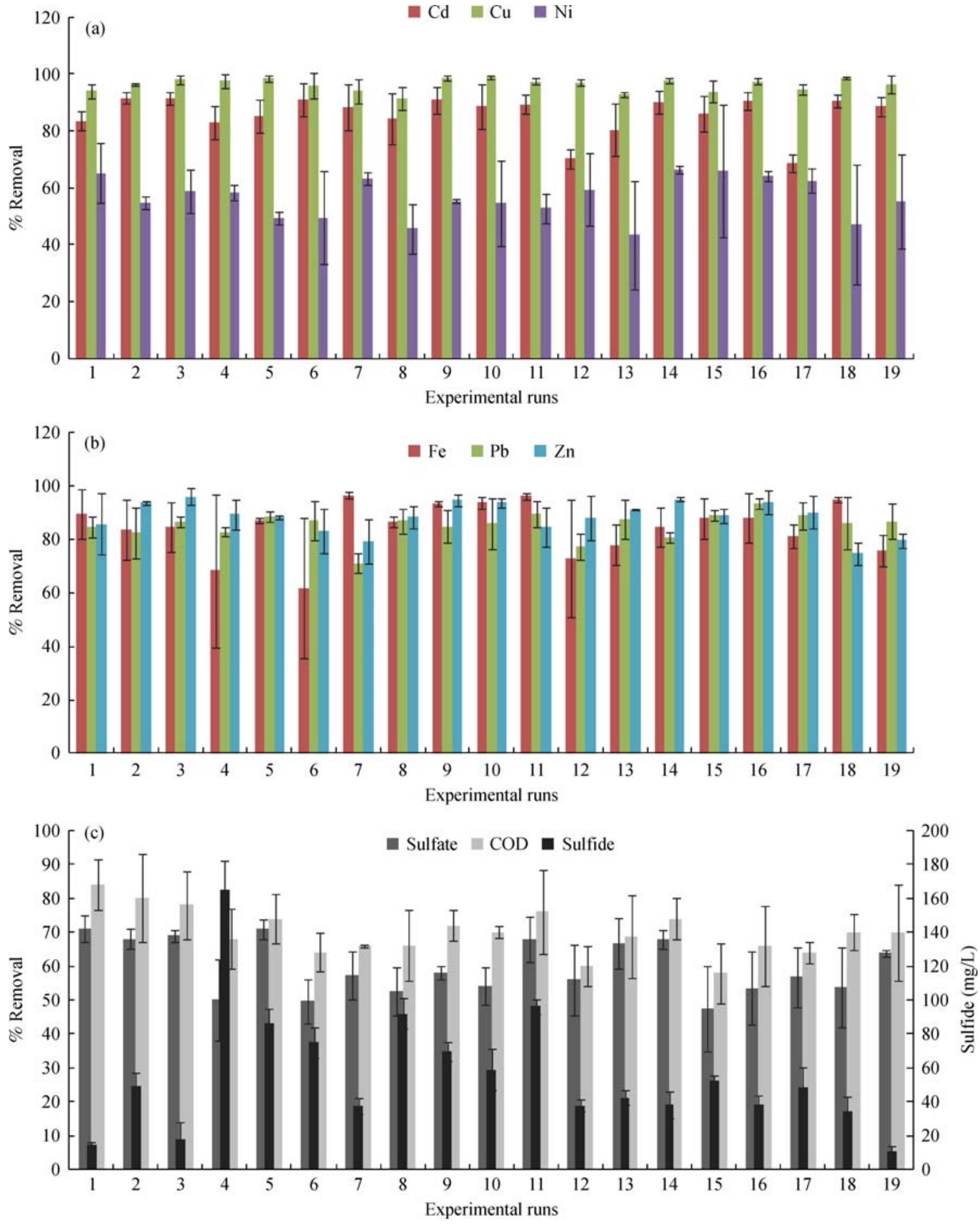


Fig. 2 Heavy metal removal: (a) Cd(II), Cu(II), Ni(II), (b) Fe(III), Pb(II) and Zn(II) and (c) COD removal, sulfate reduction and sulfide generation using the An-RBC reactor

removal values (Fig. 2c). It is clear from Fig. 2b that sulfide produced through sulfate reduction is high at a low metal to sulfide (M/S^{2-}) ratio value of less than 1, which aided in metal removal by sulfide precipitation (Fig. 2(a–b)). Moreover, it is apparent that any toxic effect posed by the low residual metal concentration in solution on the sulfate reduction process becomes extraneous. Metal removal was reduced at a high loading condition due to an increase in the M/S^{2-} ratio value (Fig. 2c) (Villa-Gomez et al., 2015). A M/S^{2-} ratio value greater than 1 results in an elevated residual concentration of metal, which is toxic to the microorganism, thus hindering the sulfate reduction activity and, consequently, its own removal (Fig. 2c) (Villa-Gomez et al., 2015). Furthermore, a high residual COD in the effluent is attributed to the formation of acetate from lactate (Omil et al., 1996; Lens et al., 1998; Widdel, 1998; Nagpal et al., 2000).

3.2 Bio-precipitates characterization and metal removal mechanism

Bio-precipitate analysis using FESEM-EDX was performed to determine the elemental composition and morphology of the bio-precipitates produced in the reactor. Figure 3a represents the EDX spectrum of the precipitate collected from experimental run 5 with its FESEM image in the insert. X-ray dot mapping of the precipitates obtained from experimental run 5 collected from the An-RBC reactor is presented in Fig. 3b. The presence of sulfur peak in the FESEM-EDX spectra (Fig. 3a) is attributed to metal sulfide precipitation. Figure 3 confirms that the metal removal mechanism is attributed to the precipitation of metals as metal sulfide salts via sulfate reduction by SRB in the reactor along with the occurrence of other elements present in the liquid medium.

From FESEM-EDX results, it is evident that sulfide peak is more prominent compared to other elements peaks, revealing that metals were precipitated as metal sulfides (Fig. 3), whereas the other forms, such as MCO_3, $M(OH)_x$, etc. are not considerable, where M is metal. X-ray dot mapping of the precipitates (Fig. 3b) further reveal the qualitative information about the presence and distribution of different metals present in the wastewater. Sulfate and COD reduction along with metal sulfide formation illustrated by FESEM-EDX substantiate sulfidogenesis as the prevailing mechanism for metal removal by SRB in the reactor (Jin et al., 2007). Based on all these results, schematic of the metal removal mechanism is depicted in Fig. 4.

The combined effect of inlet metal concentration and HRT on metal removal was examined by determining the inlet metal loading rate (ILR) (mg/L·h) and the corresponding metal removal rate (mg/L·h) as described in Kiran et al. (2017a). Metal removal performance of the An-RBC reactor as a function of ILR is depicted in Fig. 5, which shows that low ILR values yielded maximum metal removal efficiency. Whereas, at high ILR values of the metals (metals at high inlet concentration), an inhibitory effect on SRB activity was observed as evident from the decrease in the metal removal rate and removal efficiency (Fig. 5).

Stable performance of a reactor is generally indicated by a line passing through the origin of the figure plotted between metal removal rate and different inlet loading conditions. The metal removal rate values that deviate from the line passing through the origin signify that ILR values beyond this point are unfavorable for achieving a high metal removal using the An-RBC reactor (Kiran et al., 2017a). Therefore, metal loading rates greater than 3.7 mg/L·h in case of Cu(II); more than 1.69 mg/L·h, in case of other metals seem to be toxic and inhibitory to SRB activity. Compared to the metal removal results from single metal solutions (Kiran et al., 2017a), the removal rates of Ni(II), Fe(III)and Zn(II) from mixture was reduced (Figs. 5c, 5d and 5f), which can be attributed to their competitive effect for sulfide precipitation due to their similar solubility product constant values. Whereas the removal rate of the other metals was stable and unaffected in both single (Kiran et al., 2017a) and multi-metal containing solutions (Fig. 5).

Utgikar et al. (2004) in their study demonstrated that the toxic effect of Zn and Cu in mixture was notably greater than their individual toxic effect. In an another study using an anaerobic semi continuous stirred tank reactor containing SRB for metal removal from mixture, Kieu et al. (2011) reported about 94%–100% metal removal each of Cu, Ni, Zn and Cr without any effect on the reactor performance. It is evident from the results obtained from different studies that the toxic and inhibitory effect of different metals on SRB are controlled by parameters, such as pH, temperature, HRT, type of metal and its concentration, microbial community and the bioreactor configuration (Kieu et al., 2011).

Dev et al. (2016) reported that use of MWE enhanced the growth rate of the bacteria in PBR and the metal removal followed the order: Ni>Mg>Fe>Cu>Zn>Mn which matched with the solubility product values of the corresponding metal sulfide salts. The authors further attributed the incomplete removal of metals at low sulfate reduction efficiency, which strongly indicates the dependence of metal removal on SRB activity in the PBR (Dev et al., 2016).

Optimization of the operational parameters using Taguchi design revealed that pH and MWE concentration was the most significant followed by sulfate concentration, HRT and TOC. At an optimum level of these process parameters, the PBR system performed well for sulfate and metal removal thereby, ensuring that these levels were below the permissible limit for discharge of treated wastewater into the environment (Robinson-Lora and

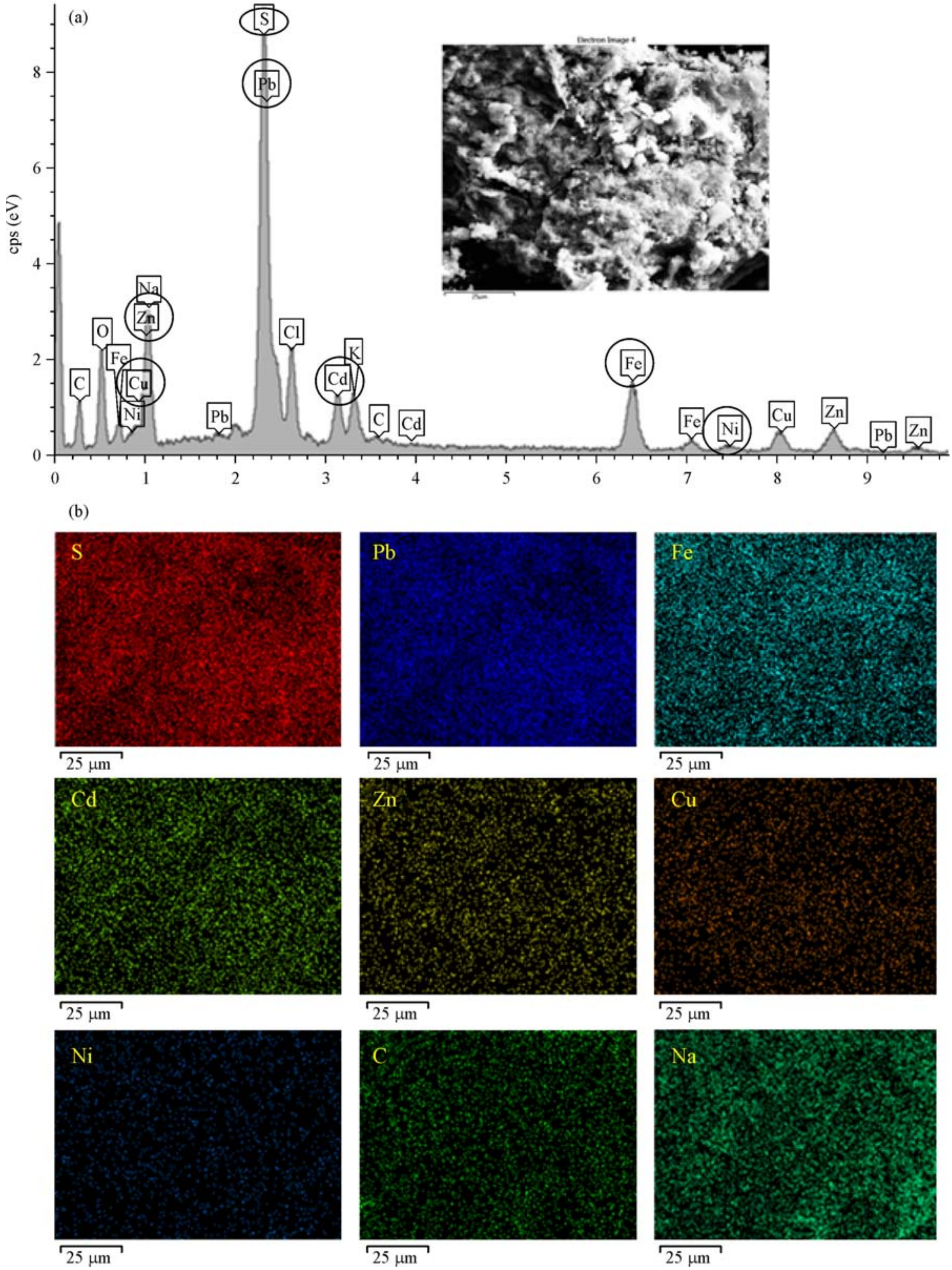


Fig. 3 (a) EDX spectra and (b) X-ray dot mapping of the bio-precipitate collected from the An-RBC reactor during experimental run # 5

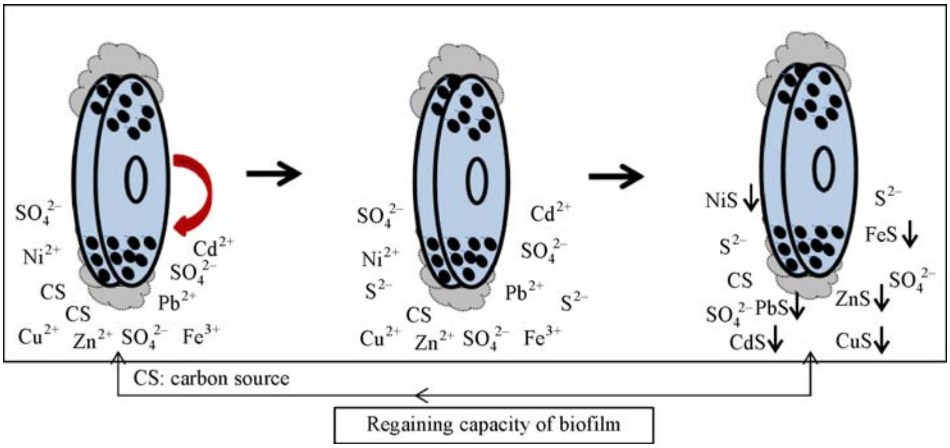


Fig. 4 Schematic showing metal removal mechanism by SRB in the An-RBC reactor

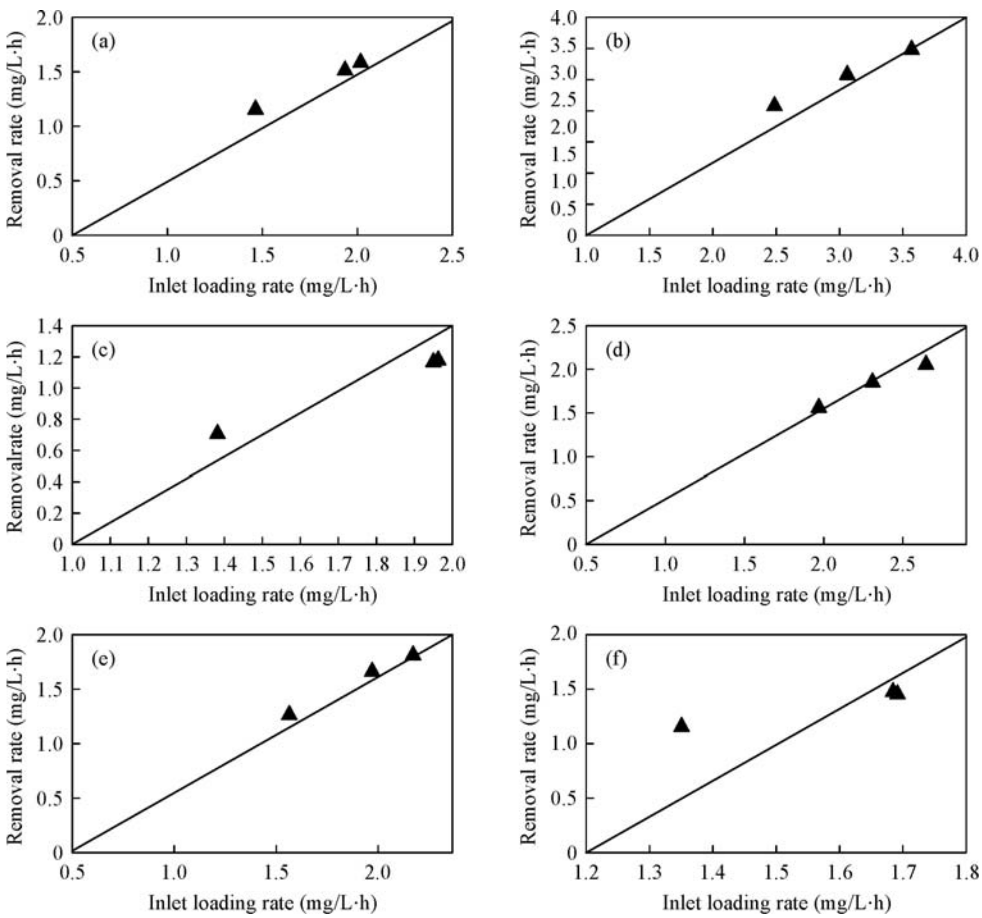


Fig. 5 Metal removal performance of the An-RBC reactor as a function of inlet metal loading rate: (a) Cd(II), (b) Cu(II), (c) Ni(II), (d) Fe (III), (e) Pb(II) and (f) Zn(II) (▲: Metal removal rate)

Brennan, 2009). This study which was carried out at an ambient temperature for more than 150 days also showed that the PBR can successfully treat AMD even with high concentrations of Cu and Zn; the metal removal order followed was Zn>Cu>Ni>Fe>Mg>Mn which as well matched with the results reported by Dev et al. (2016). It is reported that combined toxicity of heavy metals in mixture was not very strong in contrast to toxicity exhibited by individual metals and the metal removal order followed in the study was Zn>Mn>Cr (Guo et al., 2017).

In the present study, the better results obtained than those found from the literature can be attributed to the use of a different community of metal resistant SRB consortium (Kiran et al., 2016, Kiran et al., 2017a; 2017b) and a different reactor configuration. This main advantage of the An-RBC reactor is that it offers a high interfacial area on its rotating discs for biomass growth and attachment, thereby providing sufficient contact between the microbes and contaminants present in the wastewater (Pakshirajan and Kheria, 2012). Along with this attached biomass, the suspended biomass present in the system further improves the wastewater treatment efficiency, viz. heavy metals, COD and sulfate removal (Kiran et al., 2017a).

3.3 Statistical analysis

Analysis of variance (Table 2) and student *t* test (Table 3) were employed to bring out the collective effect of different metals on the performance of the An-RBC reactor at high metal loading conditions. In this ANOVA table, a low probability (P) value and a high Fisher's (F) value of the regression model signify the model precision in explaining the variations in the results (Montgomery, 2004). In this study, a low P value ($P < 0.2$) for the factors and their effects is considered as significant (Montgomery, 2004; Madamba and Liboon, 2001). Therefore, from Table 2, low P values (< 0.1) of the main effects for Pb(II) and Zn(II) removal reveal a very high significance of these responses over the others. A high Ni(II) concentration in the mixture favored its own removal (P value of 0.167; Table 3) even though its overall Ni(II) removal efficiency was less when compared with the removal efficiency of the

Table 2 ANOVA of heavy metal removal from wastewater using the An-RBC reactor

Variable	Ni		Fe		Pb		Zn	
source	F	P	F	P	F	P	F	P
Main Effects	2.78	0.288	2.23	0.341	**9.2**	**0.101**	**10.34**	**0.091**
Cd	0.89	0.445	0.01	0.929	0.13	0.752	26.54	0.036
Cu	0.18	0.709	0.34	0.616	3.68	0.195	0	0.974
Ni	**4.54**	**0.167**	**4.17**	**0.178**	**7**	**0.118**	**4.86**	**0.158**
Fe	**4.17**	**0.178**	**5.86**	**0.137**	**5.34**	**0.147**	**19.55**	**0.048**
Pb	0.62	0.513	0.09	0.793	1.01	0.421	**9.36**	**0.092**
Zn	0.75	0.477	1.09	0.406	**8.38**	**0.102**	2.83	0.235
2-Way Interaction effect	0.37	0.786	2.7	0.282	1.4	0.443	5.62	0.155
Cd*Cu	0.4	0.591	1.31	0.371	2.23	0.274	0.46	0.567
Cd*Ni	0	0.982	0.66	0.503	0.03	0.886	0.64	0.508
Cd*Fe	0.24	0.67	7.04	0.118	0.19	0.705	15.24	0.06

Table 3 Student *t* test of heavy metal removal from wastewater using the An-RBC reactor

Variable	Ni		Fe		Pb		Zn	
term	T	P	T	P	T	P	T	P
Constant	22.39	0.002	25.01	0.002	67.37	0	65.07	0
Cd	–0.94	0.445	–0.1	0.929	–0.36	0.752	**-5.15**	**0.036**
Cu	–0.43	0.709	0.59	0.616	–1.92	0.195	0.04	0.974
Ni	**2.13**	**0.167**	**2.04**	**0.178**	**-2.65**	**0.118**	**2.2**	**0.158**
Fe	**-2.04**	**0.178**	**-2.42**	**0.137**	**-2.31**	**0.147**	**-4.42**	**0.048**
Pb	–0.79	0.513	0.3	0.793	–1	0.421	**3.06**	**0.092**
Zn	–0.87	0.477	–1.04	0.406	**-2.89**	**0.102**	1.68	0.235
Cd*Cu	–0.63	0.591	–1.14	0.371	–1.49	0.274	–0.68	0.567
Cd*Ni	–0.03	0.982	–0.81	0.503	–0.16	0.886	–0.8	0.508
Cd*Fe	0.49	0.67	2.65	0.118	0.44	0.705	3.9	0.06

other metals (Fig. 2a). A high Fe(III) concentration in the mixture showed inhibitory effect on its own removal as well as on Ni(II) removal owing to similar solubility product values of these metals ($P < 0.137$; Table 3).

Presence of Fe(III), Cu(II), Ni(II) and Zn(II) in the metallic wastewater inhibited Pb(II) removal ($P < 0.195$), whereas Pb(II), Fe(III), Cd(II) and Ni(II) inhibited Zn(II) removal ($P < 0.158$), both due to the competitive effect of these ions for metal sulfide precipitation (Table 3). All these effects of heavy metals on each other removal from the wastewater is associated with the solubility product constant values of the respective metal sulfide salts (Hill et al., 2005). The solubility product values of CuS, PbS, CdS, ZnS, NiS, and FeS are 6×10^{-37}, 3×10^{-28}, 8×10^{-28}, 2×10^{-25}, 3×10^{-19} and 6×10^{-19}, respectively (Hill et al., 2005). Metal removal results obtained in this study is also attributed to the competitive/interaction effect posed by simultaneous presence of different heavy metals on sulfate reducing activity of the SRB which is the main mechanism for metal removal. It is also reported that metals present in different proportion exert more toxic effect to SRB than when presented as single metal solution (Utgikar et al., 2004).

4 Conclusions

This study evaluated the performance of the An-RBC reactor for metallic wastewater treatment under high loading conditions. The reactor performance depended on the metallic species present and their concentration, i.e., maximum removal efficiencies of the metals were achieved at low loading condition than at high loading condition. Metal removal rates of Ni(II) and Fe(III) were lower than that of the other metals. Metal removal efficiency order followed in the mixture study was Cu>Zn>Cd>Pb >Fe>Ni which closely matched with the solubility product values of respective metal sulfide salts. FESEM-EDX analysis of the metal precipitates revealed metal sulfide precipitation as the governing mechanism of metal removal in the reactor. The passive biofilm bioreactor (An-RBC reactor) could be chosen for large scale treatment of complex sulfate and metal rich wastewater owing to its excellent performance under different metal loading conditions.

Acknowledgements Authors gratefully acknowledge Centre for the Environment, IIT Guwahati, Assam, India, for providing the facilities to carry out the reactor studies and the Central Instruments Facility, IIT Guwahati, Assam, India, for FESEM-EDX analysis of the samples. Authors also thank Mr. Partha Protim Bakal for his help with heavy metal analysis.

References

American Public Health Association (APHA) (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed. American Water Works Association (AWWA) & Water Environment Federation (WEF), Washington D.C.

Bai H J, Zhang Z M, Yang G E, Li B Z (2008). Bioremediation of cadmium by growing *Rhodobacter sphaeroides*: Kinetic characteristic and mechanism studies. Bioresource Technology, 99(16): 7716–7722

Brahmacharimayum B, Ghosh P K (2014). Sulfate bioreduction and elemental sulfur formation in a packed bed reactor. Journal of Environmental Chemical Engineering, 2(3): 1287–1293

Cao J, Li Y, Zhang G, Yang C, Cao X (2013). Effect of Fe(III) on the biotreatment of bioleaching solutions using sulfate-reducing bacteria. International Journal of Mineral Processing, 125: 27–33

Chen C Y, Lin K C, Yang D T (1997). Comparison of the relative toxicity relationships based on batch and continuous algal toxicity tests. Chemosphere, 35(9): 1959–1965

Cord-Ruwisch R (1985). A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. Journal of Microbiological Methods, 4(1): 33–36

Dev S, Roy S, Bhattacharya J (2016). Understanding the performance of sulfate reducing bacteria based packed bed reactor by growth kinetics study and microbial profiling. Journal of Environmental Management, 177: 101–110

Dev S, Roy S, Bhattacharya J (2017). Optimization of the operation of packed bed bioreactor to improve the sulfate and metal removal from acid mine drainage. Journal of Environmental Management, 200: 135–144

Gikas P (2007). Kinetic responses of activated sludge to individual and joint nickel (Ni(II)) and cobalt (Co(II)): An isobolographic approach. Journal of Hazardous Materials, 143(1-2): 246–256

Guo J, Kang Y, Feng Y (2017). Bioassessment of heavy metal toxicity and enhancement of heavy metal removal by sulfate-reducing bacteria in the presence of zero valent iron. Journal of Environmental Management, 203(Pt 1): 278–285

Hill J W, Petrucci R H, McCreary T W, Perry S S (2005). General Chemistry. 4th Edition, Pearson Prentice Hall, Upper Saddle River.

Hu Z, Chandran K, Grasso D, Smets B F (2004). Comparison of nitrification inhibition by metals in batch and continuous flow reactors. Water Research, 38(18): 3949–3959

Jiménez-Rodríguez A M, Durán-Barrantes M M, Borja R, Sánchez E, Colmenarejo M F, Raposo F (2009). Heavy metals removal from acid mine drainage water using biogenic hydrogen sulphide and effluent from anaerobic treatment: Effect of pH. Journal of Hazardous Materials, 165(1-3): 759–765

Jin S, Drever J I, Colberg P J (2007). Effects of copper on sulfate reduction in bacterial consortia enriched from metal-contaminated and uncontaminated sediments. Environmental Toxicology and Chemistry, 26(2): 225–230

Kaksonen A H, Puhakka J A (2007). Sulfate reduction based bioprocesses for the treatment of acid mine drainage and the recovery of metals. Engineering in Life Sciences, 7(6): 541–564

Kieu H T Q, Müller E, Horn H (2011). Heavy metal removal in anaerobic semi-continuous stirred tank reactors by a consortium of sulfate-reducing bacteria. Water Research, 45(13): 3863–3870

Kiran M G, Pakshirajan K, Das G (2016). Heavy metal removal using sulfate reducing biomass obtained from a lab scale upflow anaerobic packed bed reactor. Journal of Environmental Engineering, 142(9):

C4015010

Kiran M G, Pakshirajan K, Das G (2017a). A new application of anaerobic rotating biological contactor reactor for heavy metal removal under sulfate reducing condition. Chemical Engineering Journal, 321: 67–75

Kiran M G, Pakshirajan K, Das G (2017b). Heavy metal removal from multicomponent system by sulfate reducing bacteria: Mechanism and cell surface characterization. Journal of Hazardous Materials, 324 (Part A): 62–70

Lens P N L, van den Bosch M C, Hulshoff Pol L W, Lettinga G (1998). Effect of staging on volatile fatty acid degradation in a sulfidogenic granular sludge reactor. Water Research, 32(4): 1178–1192

Madamba P S, Liboon F A (2001). Optimization of the vacuum dehydration of celery (*Apium graveolens*) using the response surface methodology. Drying Technology, 19(3-4): 611–626

Min X, Chai L, Zhang C, Takasaki Y, Okura T (2008). Control of metal toxicity, effluent COD and regeneration of gel beads by immobilized sulfate-reducing bacteria. Chemosphere, 72(7): 1086–1091

Montgomery D C (2004). Design and analysis of experiments, 7th ed. New York: Wiley

Nagpal S, Chuichulcherm S, Livingston A, Peeva L (2000). Ethanol utilization by sulfate-reducing bacteria: an experimental and modeling study. Biotechnology and Bioengineering, 70(5): 533–543

Omil F, Lens P, Hulshoff Pol L, Lettinga G (1996). Effect of upward velocity and sulphide concentration on volatile fatty acid degradation in a sulphidogenic granular sludge reactor. Process Biochemistry, 31 (7): 699–710

Pakshirajan K, Kheria S (2012). Continuous treatment of coloured industry wastewater using immobilized *Phanerochaete chrysosporium* in a rotating biological contactor reactor. Journal of Environmental Management, 101: 118–123

Postgate J R (1984). The Sulphate-reducing Bacteria. Cambridge: Cambridge University Press, 107–152

Rinzema A, Lettinga G (1998). Anaerobic treatment of sulfate containing wastewater. In: Biotreatment Systems, 3: (Wise, DL, Ed). Boca Raton: CRC Press, Inc., 65–109

Robinson-Lora M A, Brennan R A (2009). Efficient metal removal and neutralization of acid mine drainage by crab-shell chitin under batch and continuous-flow conditions. Bioresource Technology, 100(21): 5063–5071

Saifullah, Meers E, Qadir M, de Caritat P, Tack F M, Du Laing G, Zia M H (2009). EDTA-assisted Pb phytoextraction. Chemosphere, 74: 1279–1291

Sema S S, Gikas P, James G M, Brent M P, Timothy R G (2012). Comparison of single and joint effects of Zn and Cu in continuous flow and batch reactors. Journal of Chemical Technology and Biotechnology (Oxford, Oxfordshire), 87(3): 374–380

Sen M, Dastidar M G, Roy Choudhury P K (2007). Biological removal of Cr(VI) using *Fusarium solani* in batch and continuous modes of operation. Enzyme and Microbial Technology, 41(1-2): 51–56

Teclu D, Tivchev G, Laing M, Wallis M (2009). Determination of the elemental composition of molasses and its suitability as carbon source for growth of sulphate-reducing bacteria. Journal of Hazardous Materials, 161(2-3): 1157–1165

Utgikar V P, Chaudhary N, Koeniger A, Tabak H H, Haines J R, Govind R (2004). Toxicity of metals and metal mixtures: Analysis of concentration and time dependence for zinc and copper. Water Research, 38(17): 3651–3658

Velasco A, Ramírez M, Volke-Sepúlveda T, González-Sánchez A, Revah S (2008). Evaluation of feed COD/sulfate ratio as a control criterion for the biological hydrogen sulfide production and lead precipitation. Journal of Hazardous Materials, 151(2-3): 407–413

Villa-Gomez D K, Pakshirajan K, Maestro R, Mushi S, Lens P N L (2015). Effect of process variables on the sulfate reduction process in bioreactors treating metal-containing wastewaters: Factorial design and response surface analyses. Biodegradation, 26(4): 299–311

Wang W, Lampi M A, Huang X D, Gerhardt K, Dixon D G, Greenberg B M (2009). Assessment of mixture toxicity of copper, cadmium, and phenanthrenequinone to the marine bacterium *Vibrio fischeri.* Environmental Toxicology, 24(2): 166–177

Widdel F (1998). Microbiology and ecology of sulfate-and sulfur-reducing bacteria. In: Zehnder A, ed. Biology of Anaerobic Microorganisms. New York: Wiley

Zhang M, Wang H (2016). Preparation of immobilized sulfate reducing bacteria (SRB) granules for effective bioremediation of acid mine drainage and bacterial community analysis. Minerals Engineering, 92: 63–71

Zhang M, Wang H, Han X (2016). Preparation of metal-resistant immobilized sulfate reducing bacteria beads for acid mine drainage treatment. Chemosphere, 154: 215–223