



# Copper remediation by *Eichhornia* spp. and sulphate-reducing bacteria

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## ABSTRACT

*Eichhornia* spp. biomass was collected from Chandola Lake, Ahmedabad, Gujarat, India. Point of zero charge of the biomass was pH 7.3. Flask study showed pH 5 and 2–3 h contact time as optimum conditions for copper sorption with 67.25% copper removal. At the end of 24 h of contact time, copper removal reached to 85.0%, from 100 ppm copper containing solution. Copper loading capacity of the biomass ranged between 9.9 and 28.5 mg g<sup>-1</sup> of biomass. To understand the interaction among pH, temperature, presence of nickel and zinc in the system, 2<sup>4</sup> factorial experiment was performed. Under the experimental conditions pH and interactions between pH–nickel, temperature–pH and temperature–pH–nickel–zinc were found to be significant with 60–74.7% copper removal. Langmuir isotherm was better fit as compared to Freundlich isotherm and pseudo-second order equation gave R<sup>2</sup> of 0.999 for biosorption kinetic of *Eichhornia* biomass. Reactor study showed 90% overall copper removal from 24 L of copper containing waste studied and sulphate-reducing bacteria played a significant role. SEM/quant element analysis showed increase from 41.66% to 53.93%, 1.02–19.73% and 0.0–12.39% of chloride, aluminium and copper respectively in the loaded biomass as compare to unexposed biomass.

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## 1. Introduction

High toxicity of heavy metal and its magnitude of contamination necessitate metal remediation from sources of pollution of the atmosphere [1]. The heavy metal concentrations are increased in concentration at every level of food chain because of its leaching from waste dumps, polluted soils and water [2]. These metals have the incomplete 'd' orbital, which have ability to form complex compounds that may or may not be redox active, thus conferring toxicity to the living cell beyond a permissible concentration [3]. Conventional processes some times are not viable because of expensive investment, operational costs, the potential generation of secondary pollution and may be ineffective for the solutions that are containing lower than 1–100 mg L<sup>-1</sup> of dissolved metals [4]. For water pollution control, metal ions sorption from aqueous solution is very important [5]. When the waste biomass is utilized, biosorption mechanism represents a cheap alternative to conventional techniques, because of the application of a low cost sorbent material [6]. In the biosorption process due to the higher affinity of sorbent, sorbate gets attracted and bound to sorbent with the different mechanisms [1].

The objectives of the present work were to investigate the metal biosorptive behaviour of *Eichhornia* spp., an aquatic plant. Optimization of copper sorption with dried biomass of *Eichhornia* spp.

at shake flask level and scale up of the process to laboratory level bioreactor was investigated.

## 2. Materials and methods

### 2.1. Biomass

Species of *Eichhornia* was collected from Chandola Lake, Ahmedabad, India belongs to Plantae kingdom that is commonly known as Water Hyacinth. The collected biomass was washed with tap water and distilled water. The washed biomass was sun dried and grounded in a home mixer at ~1200 rpm for 10 min. The grounded biomass was sieved with 120 and 230 # B.S.S. sieves. The biomass used in the study was of mixed particle size consisting of –125 + 88 μm (80%) and –80 + 63 μm (20%) particles.

### 2.2. Point of zero charge

Point of zero charge was determined for the dried grounded biomass. To a series of 250 mL Erlenmeyer flask, 45 mL of 1% KNO<sub>3</sub> solution was transferred. The pH<sub>i</sub> values of the solution were roughly adjusted to 2–10 by adding either 0.1N HCl or 0.1N NaOH. The total volume of the solution in each flask was made up to 50 mL by adding the KNO<sub>3</sub> of the same strength. The pH<sub>i</sub> of the solution was noted and 0.1 g of crushed biomass was added to flask. The suspensions were then manually shaken and allowed to equilibrate for 24 h with intermittent manual shaking. The pH values of the supernatant liquids were noted.

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**Table 1**  
2<sup>4</sup> factorial experimental setup and percent sorption response.

Factors		Levels								
		Low (-)	High (+)							
A	Temperature (°C)	30	55							
B	pH	3	5							
C	NiSO <sub>4</sub> (ppm)	0	100							
D	ZnSO <sub>4</sub> (ppm)	0	100							
Treatment combinations	Design levels				Factor levels				Sorption (%)	F-test value
	a	b	c	d	Temperature (°C)	pH	NiSO <sub>4</sub> (ppm)	ZnSO <sub>4</sub> (ppm)		
0	-	-	-	-	30	3.0	0	0	66.0	-
a	+	-	-	-	55	3.0	0	0	63.0	7.22
b	-	+	-	-	30	5.0	0	0	65.0	36.20
ab	+	+	-	-	55	5.0	0	0	67.5	8.77
c	-	-	+	-	30	3.0	100	0	63.1	6.58
ac	+	-	+	-	55	3.0	100	0	65.4	0.45
bc	-	+	+	-	30	5.0	100	0	74.7	16.11
abc	+	+	+	-	55	5.0	100	0	66.1	5.22
d	-	-	-	+	30	3.0	0	100	64.3	1.07
ad	+	-	-	+	55	3.0	0	100	62.9	0.37
bd	-	+	-	+	30	5.0	0	100	69.7	1.41
cd	-	-	+	+	30	3.0	100	100	60.6	1.72
abd	+	+	-	+	55	5.0	0	100	60.0	0.03
acd	+	-	+	+	55	3.0	100	100	63.0	5.97
bcd	-	+	+	+	30	5.0	100	100	73.4	1.68
abcd	+	+	+	+	55	5.0	100	100	70.1	7.38
ad <sup>a</sup>	+	-	-	+	55	3.0	0	100	59.6	} E <sub>A</sub> = 2.684
ad <sup>a</sup>	+	-	-	+	55	3.0	0	100	62.5	
ad <sup>a</sup>	+	-	-	+	55	3.0	0	100	63.6	
ad <sup>a</sup>	+	-	-	+	55	3.0	0	100	60.0	
ad <sup>a</sup>	+	-	-	+	55	3.0	0	100	61.0	
ad <sup>a</sup>	+	-	-	+	55	3.0	0	100	62.9	

<sup>a</sup> Repeat points; copper 100 ppm, 200 mg dried *Eichhornia* biomass.

### 2.3. Optimization of copper biosorption

The physicochemical parameters were optimised for pH 2, 3, 4 and 5; contact time 1, 2, 3, 4, 12, 18 and 24 h; biosorbent 2, 4, 6 and 8 g L<sup>-1</sup>; and sorbate 50, 100, 150, 200, 300 and 400 ppm. Influence of temperature, pH, nickel and zinc and their interactions were studied in 2<sup>4</sup> factorial experiments at two levels (Table 1). After adsorption 200 mg biomass was treated with 25 mL of 0.1N HCl and 0.1N HNO<sub>3</sub> for desorption of copper. It was stirred for 1 h and then copper was estimated from the filtrate.

If otherwise mentioned, copper biosorption studies were carried out in 250 mL Erlenmeyer flasks with 200 mg sorbent in 50 mL system containing 100 ppm (16 mM) adsorbate adjusted between pH 5.0 and 5.5. Flasks were kept on orbital shaker rotating at 150 rpm at 30 ± 2 °C for 2 h for adsorption study. Metal content in the solution was estimated at 0 h and at the end of the treatment time and the difference in the copper content was considered for metal sorption calculation. Absorbed metal content was also confirmed by estimation of copper content from the extract (0.1N HCl) of metal loaded biomass.

### 2.4. Reactor study

Interrupted flow sorption experiments were conducted in 3 L capacity indigenously designed rectangular plastic reactor having 26.3, 16.7 and 10.8 cm length, width and depth respectively with one outlet. Ungrounded dried 100 g of *Eichhornia* biomass, 2300 mL of Rajpardi lignite mine wastewater and 100 mL inoculum of mixed culture of SRB developed in mine water was added in the reactor. The inoculated reactor was kept for 15 days for microbial biomass and biofilm development. Aqueous monometallic copper solution and industrial wastewater were charged at flow rate of 25 mL h<sup>-1</sup>

for copper remediation under different conditions, which corresponds to residence time of 96 h.

### 2.5. Analysis

Copper was estimated by spectrophotometric method using diethyldithiocarbamate complex method, which can detect copper up to 250 µg mL<sup>-1</sup> [7] and also by atomic absorption spectrophotometer (Elico, India, model SL 194) at 324 nm with air acetylene flame having range up to 5 µg mL<sup>-1</sup> copper. SEMquant elemental analysis was done by Scanning Electron Microscope (Leica Leo, UK, model S440i) from distilled water washed copper exposed and unexposed *Eichhornia* dried biomass by using silicon detector. System resolution and voltage during the analysis were 72 eV and 20 kV, respectively.

## 3. Result and discussion

### 3.1. Point of zero charge

Chandola Lake is contaminated with domestic waste, which resulted in heavy eutrophication and dominating population of *Eichhornia* growth. The collected *Eichhornia* plant showed the presence of 0.0018%, 0.0026% and 0.020% of copper, chromium and zinc, respectively. The plot of change in solution pH (ΔpH) versus initial pH (pH<sub>i</sub>) is shown in Fig. 1. After the treatment with the biomass, the pH of the 1% KNO<sub>3</sub> solution was increased up to initial 7.0 pH of the KNO<sub>3</sub>, where as the final pH decreased beyond the initial pH 7.0. The increase or decrease in the final pH is directly proportional to the initial pH of the KNO<sub>3</sub>. Point of zero charge determined for the biosorbent was pH 7.3. Particle sizes of biomass used in the study could not affect the point of zero charge. Determination of point

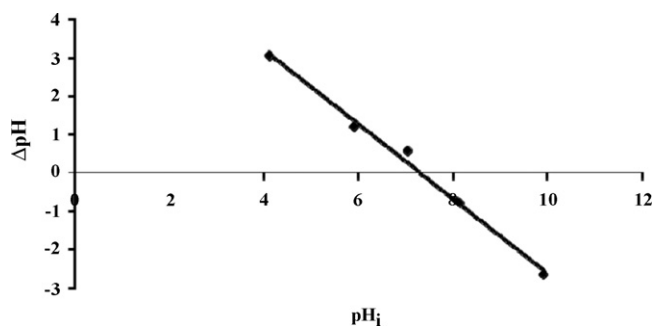


Fig. 1. Point of zero charge of *Eichhornia* biomass (system 50 mL volume containing 1% KNO<sub>3</sub>; 0.1 g biomass).

of zero charge of biosorbent is elucidating biosorption mechanism [8].

### 3.2. Influence of pH and temperature on copper sorption

Influence of pH and contact time on copper sorption by *Eichhornia* biomass is shown in Fig. 2. Copper sorption was found to be directly proportional to the pH of the reaction system. However, pH above 5 was responsible for chemical precipitation of copper from the system. The overall surface charge on the cells of *Eichhornia* biomass is positive at lower pH, that inhibit the sorption of positively charged metal cations, which may be due to protons combine with metal ions and thereby decrease the interaction of metal ions with biomass [9]. The lion share of copper was removed in 2–3 h of contact time and thereafter; removal was slower down indicated sorption as major mechanism. The rate of sorption was high in the beginning due to large available surface area of the biomass [10]. The highest sorption in flask study was 85% correspond to 21.25 mg g<sup>-1</sup> of biomass in 24 h.

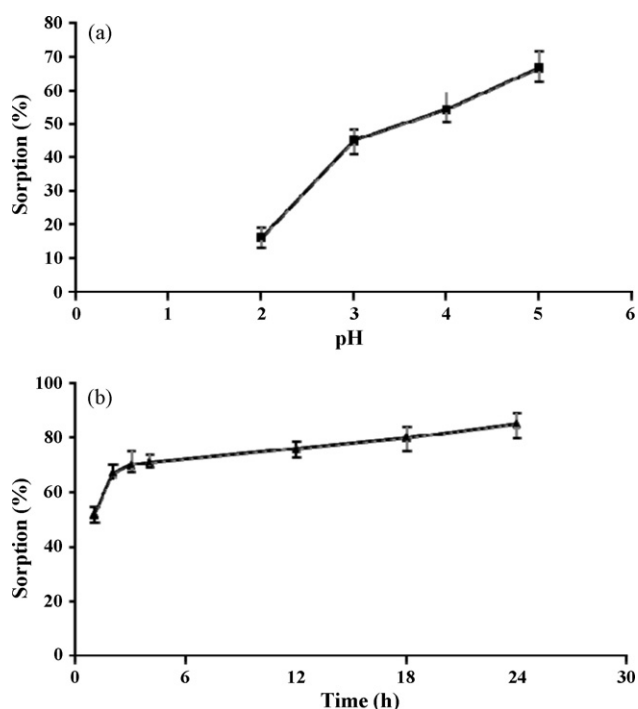


Fig. 2. Influence of (a) pH and (b) contact time on copper biosorption (working volume 50 mL; 200 mg biomass, 100 ppm copper).

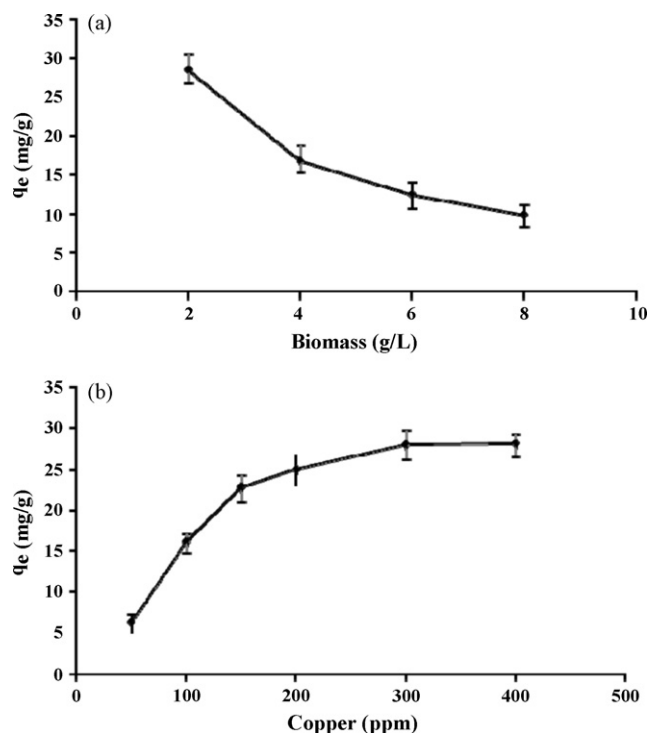


Fig. 3. Influence of (a) sorbent and (b) sorbate concentration (experimental setup with 50 mL system).

### 3.3. Effect of sorbent and sorbate concentration on copper sorption

The influence of sorbent and sorbate concentration is presented in Fig. 3. At a given equilibrium concentration the biosorbent adsorbed more metal ions per unit weight of biomass at low biomass concentration than at high concentration. Metal loading was directly proportional to sorbate concentration and inversely proportional to sorbent in the system. Under the experimental condition 28 mg g<sup>-1</sup> copper sorption was achieved for 4 g L<sup>-1</sup> biomass from 300 ppm copper containing solution, whereas, 28.5 mg g<sup>-1</sup> sorption was obtained with 2 g L<sup>-1</sup> of biomass from 100 ppm copper concentration. Significant role is played by sorbent to sorbate ratio in metal remediation and 13.3:1 to 20:1 biosorbent:biosorbate was found to be optimum under the experimental conditions.

### 3.4. Adsorption isotherms

Isotherm study indicates the adsorption capacity of sorbent for the removal of sorbate from the solution at constant condition [11]. These isotherm models (Eqs. (1) and (2)) are based on physicochemical concepts of adsorption, which explain the possible interactions of study in metal sorption on to the sorbent [12].

Langmuir isotherm

$$q_e = \frac{q_{\max} b C_e}{1 + b C_e} \quad (1)$$

where  $q_e$  is uptake of metal per unit weight of biosorbent,  $q_{\max}$  is the maximum amount of metal ion per unit weight of biomass to form a complete monolayer,  $C_e$  is the final equilibrium concentration of metal remaining in the solution and  $b$  is the Langmuir constant.

Freundlich isotherm

$$q_e = K_F (C_e)^{1/n} \quad (2)$$

where  $K_F$  and  $n$  are Freundlich constants.

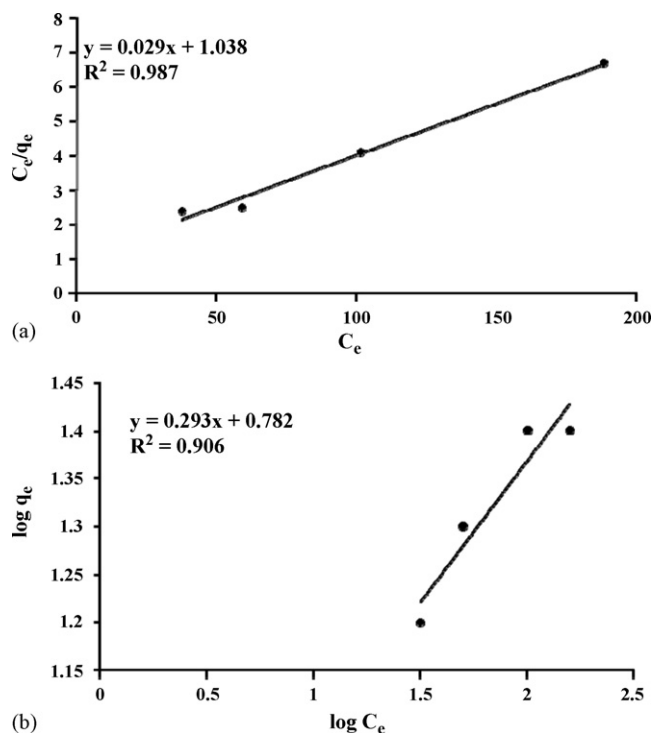


Fig. 4. Application of adsorption isotherms to experimental data: (a) Langmuir (b) Freundlich.

For copper sorption by *Eichhornia* the correlation coefficient obtained were 0.9878 and 0.906 for Langmuir and Freundlich isotherms, respectively (Fig. 4). This indicated that Langmuir isotherm was better fit than Freundlich isotherm. The other values and constants for sorption isotherms are shown in Table 2. The  $n$  value obtained in Freundlich isotherm is higher than 1 indicating that copper is favourably adsorbed by *Eichhornia* biomass. The obtained  $K_F$  value 6.03 indicates the high absorptive capacity and easy uptake of copper from the solution. Here, both isotherms depict different type of sorption. Langmuir model makes assumption of monolayer sorption where as Freundlich deals with heterogeneous surface sorption [9]. The obtained  $q_{max}$  for copper sorption from aqueous solution is  $33.4 \text{ mg g}^{-1}$  of *Eichhornia* biomass, which is more than 10-fold higher than reported for *Pycnoporus sanguineus* [5].

### 3.5. Kinetic models

To express the mechanism of sorbate sorption onto a sorbent, several models are used. To analyse the sorption rate of copper on *Eichhornia* biomass, pseudo-first order equation and pseudo-second order equation kinetic models were used. Kinetic equations are given as below (Eqs. (3) and (4)).

Pseudo-first order equation

$$\frac{dq_t}{dt} = k_{ad}(q_e - q_t) \quad (3)$$

Table 2  
Isotherm constants estimated from the fitting of experimental points of copper sorption.

Langmuir			Freundlich		
$q_{max}$	$B$	$R^2$	$n$	$K_F$	$R^2$
$33.4 \text{ mg g}^{-1}$	$0.028 \text{ dm}^3 \text{ mg}^{-1}$	0.9878	3.41	$6.05 \text{ mg g}^{-1}$	0.906

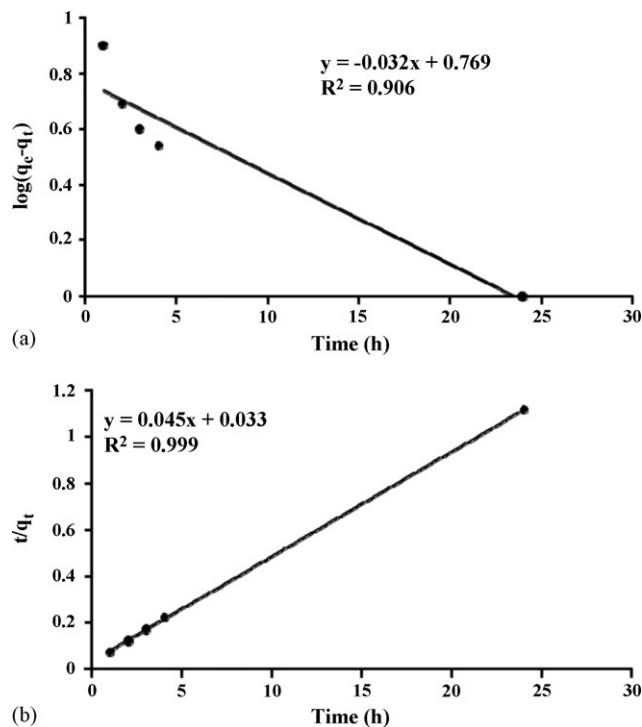


Fig. 5. Kinetic models: (a) pseudo-first order equation and (b) pseudo-second order equation.

where  $q_e$  is uptake of metal per unit weight of biosorbent ( $\text{mg g}^{-1}$ ),  $q_t$  is the amount of metal sorbed at any time ( $\text{mg g}^{-1}$ ), and  $k_{ad}$  is the rate constant ( $\text{min}^{-1}$ ).

Pseudo-second order equation

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (4)$$

where  $k_2$  is the equilibrium rate constant ( $\text{g mg}^{-1} \text{ min}^{-1}$ ) [5].

Both kinetic models are explained in Fig. 5 and different rate constants are shown in Table 3.

The values of correlation coefficient of pseudo-second order model is 0.999, which is higher than pseudo-first order model, moreover, all the points in pseudo-second order plot (Fig. 5) are more linear, which indicates a better fit of pseudo-second order model with the experimental data as compared to the pseudo-first order model.

### 3.6. Factorial experiment

Influence of temperature (a), pH (b), nickel (c), zinc (d) and their interaction was studied by  $2^4$  factorial experiments. The experiment design, obtained sorption results and  $F$ -test values are shown in Table 1. From the Yate's  $F$ -test table, the value of  $F$  at 95% confidence limit is  $F_{95}(1, 5)$  is 6.61 [13]. Obtained  $F$ -test value varied from 0.03 to 36.20 corresponds to more than 1200-fold variation. Based on  $F_{95}(1, 5)$  tabulated values,  $F$ -test of more than 6.61 consider being significant. Thus, pH (b) was found to be the most significant factor for copper remediation. Other significant factors or interactions in decreas-

Table 3  
Parameter constants estimated for kinetic study of copper sorption.

Pseudo-first order equation		Pseudo-second order equation	
$R^2$	$k_{ad}$	$R^2$	$k_2$
0.906	$1.26 \times 10^{-3} \text{ min}^{-1}$	0.999	$1.02 \times 10^{-3} \text{ g mg}^{-1} \text{ min}^{-1}$

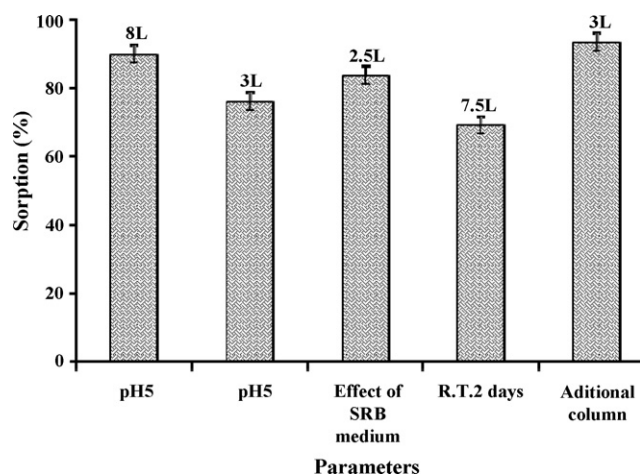


Fig. 6. Reactor study under different condition for copper bioremediation figures on the bars indicates volume of the waste treated.

Table 4  
SEMquant elemental analysis of *Eichhornia* biomass.

Elements	Element % concentration	
	Before sorption	After sorption
Na	5.29	2.09
Mg	3.66	0.34
Al	1.02	19.73
Si	2.44	0.77
P	2.29	Bd
S	1.31	4.55
Cl	41.66	53.93
K	22.41	1.07
Ca	19.91	2.59
Fe	Bd	2.55
Cu	Bd	12.39

Bd: below detectable limit.

ing order of significance are pH–nickel (bc) > temperature–pH (ab) > temperature–pH–nickel–zinc (abcd) > temperature (a). In the presence of nickel, copper sorption was found to be accelerated resulted in the highest sorption of 74.7%. The lowest sorption under the experimental condition was observed when temperature, pH and zinc concentrations were highest. However, result at highest pH, nickel and zinc conditions is consider, it is found that the highest temperature studied could be the reason for the observed lowest copper sorption in the study. This is also evident from the percent sorption result obtained from repeat points studied.

### 3.7. Reactor study

Results of laboratory scale reactor study are shown in Fig. 6. The copper removal ranged from 75.8% to 93.3% under various study conditions. In the initial cycles copper removal was as high as 91% and it reached to average value of 89.6% at the end of 8 L simulated waste loading in semi continuous mode. Thereafter, the average sorption dropped down to 75.8% when additional 3 L of simulated waste was added. Thus, SRB medium was added in the reactor and copper sorption rate increased and reached to 83.4%. When retention time was decreased from 4 to 2 days the percentage of sorption was also decreased. In two-stage experiment, when the reactor was equipped in series with additional *Eichhornia* containing column, the copper removal increased to 93.2% for 12 L of waste studied. The 100 ppm copper was used in single stage reactor experiment where as two-stage experiment was started when copper concentration was increased from 100 to 400 ppm in the system. When whole 24 L simulated waste was considered the average copper

removal reached to 82.1%. Even after the treatment of synthetic waste, the developed reactor process was able to remove 61% of copper and considerable amount of aluminium from the industrial waste in 3 days of contact time when it was passed through the reactor (data not shown). This indicates that even after treatment of 24 L waste, *Eichhornia* biomass and developed SRB culture have further potential to take care of more waste.

### 3.8. SEM-EDX analysis

As evident from SEMquant elemental analysis results depicted in Table 4, Al, Cl, Cu, K and Ca concentration changed from 1.02% to 19.73%, 41.66–53.93%, 0.0–12.39%, 22.41–1.07% and 19.91–2.59% in the *Eichhornia* untreated and treated biomass, respectively. This indicates the copper and aluminium from the waste are exchanged with potassium and calcium ions of *Eichhornia* biomass. Copper and iron elements were below the detectable limit in unexposed biomass, whereas, these two elements were absorbed from contaminated studied industrial waste.

## 4. Conclusion

Due to contamination of domestic waste, Chandola Lake showed heavy growth of *Eichhornia* spp. The Langmuir isotherm correlated better to the experimental data than the Freundlich adsorption isotherm. Pseudo-second order equation fit better to the data than pseudo-first order equation. System pH and the interaction with nickel, temperature and zinc showed significant effect on copper sorption. Activation of SRB in the reactor system and two-stage process were found to be more efficient and resulted in more than 90% copper removal. Copper and aluminium sorption were mainly exchange with potassium and calcium present in *Eichhornia* biomass. The used sorbent and SRB combination is found to be efficient, eco-friendly and can be used to remove copper ions from effluent generated at sulphidic mining sites.

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