Korean J. Chem. Eng., 33(2), 559-566 (2016)
DOI: 10.1007/s11814-015-0137-4

pISSN: 0256-1115
eISSN: 1975-7220

Simultaneous removal of Cr(VI) and phenol from synthetic binary solution using consortium culture of *Bacillus* sp. and *E. coli* immobilized on tea waste biomass in packed bed reactor

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(*Received 10 April 2015 • accepted 26 June 2015*)

Abstract−A continuous bio column reactor was designed for the simultaneous bioaccumulation of Cr(VI) and biodegradation of phenol from their binary synthetic solution with the ratio of (2 : 1). Consortium culture of *Bacillus* sp. and *Escherichia coli* was immobilized onto tea waste biomass in the packed bed column. The metabolites formed during the biodegradation of phenol by *Bacillus* sp. were utilized by *Escherichia coli* for the bioaccumulation of Cr(VI). The considerable effect of empty bed contact time (EBCT), bed height (cm) and flow rate (mL/min) was investigated onto the simultaneous removal of Cr(VI) and phenol in the column reactor. However, after 3-4 days of continuous treatment of Cr(VI) and phenol the effect of these process parameters was not significant. Dissolved oxygen (DO) of effluent has been found to decrease with run time of packed bed column. The pH of the effluent decreased initially for 2 days but after that it became the same as the influent. A mass transfer study was carried out to calculate the pseudo-first-order rate constant for Cr(VI) and phenol, which was in good agreement with experimental results.

Keywords: Consortium Culture, Bio Column, *Escherichia coli*, *Bacillus* sp., Mass Transfer

INTRODUCTION

Both Cr(VI) and phenol are carcinogenic and mutagenic to the living organism. The maximum permissible limit of discharge from industrial waste water as per WHO (World health organization) recommendations for Cr(VI) and phenol is 0.05 and 1 mg L^{-1}, respectively [1,2]. A number of batch studies have been performed in the literature on metals and organic compounds biosorption [3]. Therefore, in the present study for the needs of the demand in process industries continuous simultaneous removal of Cr(VI) and phenol using bio column reactor has been carried out. A continuous-flow packed bed column was used for the full-scale biosorption process using immobilized cell, since it can be operated easily, high percentage removal of pollutant can be achieved, and it can be easily scaled up from laboratory pilot plant to industrial scale [4]. In literature various studies have been performed on single toxic heavy metals removal, but organic pollutants like phenol, naphthalene, trichloroethylene are also discharged simultaneously from industrial waste water, such as leather, paints and pigments, refining of petroleum, manufacturing of automobiles and agricultural activity. Therefore, simultaneous removal of heavy metals and organic compounds is needed [5]. Both chromium and phenol are used in tanneries in the tanning process for the manufacturing of leather; therefore, simultaneous removal of Cr(VI) and phenol from waste effluent is required for this industry. In this study simultaneous removal of Cr(VI) and phenol is carried out in a continuous bio column reactor because it can be easily applied to industries for the removal of toxic pollutants like Cr(VI) and phenol. Some of the bacteria which consume chromium are *Pseudomonas* sp. [6] *E. coli* ATCC 3456 [7] *Bacillus* sp. [8] *Acinetobacter haemolytius* [9] *Bacillus coagulans* [10] *Escherichia coli* [11] *Acinetobacter* [12] *Providencia* sp. [13] *Anacystis nidulans* [14] *Arthrobacter* sp. [15] *Candida* sp. [16] *Arthrobacter viscosus* [17] *Bacillus subtilis* [18] and phenol reducing microorganism are *Bacillus stearothermophilus* [19] *Pseudomonas putida* [20] *Pseudomonas flurorescens* [21] *Acinetobacter* sp. [22] *Rhodococcus erythropolis* [23] *Acinetobacter* sp. PCP3 [24] *Pseudomonas putida Migula* [25] *Bacillus cereus* MTCC9817 [26] *Bacillus* sp. [27]. The advantage of using a bio column reactor is that it does not require regeneration of the adsorbent bed, because the toxic pollutant adsorbed by the packing material is consumed by the microbes [28]. In a bio column reactor a thin layer of bacterium (biolayer) is developed onto the surface of adsorbent bed which works as a biofiltration unit. The suitability of technology developed in batch study is tested in a continuous reactor. In this study mixed culture of *Escherichia coli* and *Bacillus* sp. immobilized on tea waste biomass was used for simultaneous removal of Cr(VI) and phenol. The effect of EBCT, bed height onto the percentage removal of Cr(VI) and phenol was investigated. A mass transfer study was performed for the calculation of pseudo-first-order rate constant for Cr(VI) and biodegradation rate constant of phenol.

MATERIALS AND METHOD

1. Growth and Acclimatization of *Escherichia coli* and *Bacillus* sp.

The bacterium *Bacillus* sp. MTCC No. 3166 and *Escherichia coli*.

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NCIM No. 5051 was purchased from the Institute of Microbial Technology (IMTECH) Chandigarh and National Collection of Industrial Microorganisms (NCIM) Pune, India, respectively. The *Bacillus* sp. and *Escherichia coli* were stored at 4 °C in nutrient agar medium (Beef extract 1.0 g, yeast extract 2.0 g, peptone 5.0 g, NaCl 5.0 g, Agar 15 g in 1 L of distilled water) [29]. The acclimatization of *Escherichia coli* and *Bacillus* sp. was carried out at different concentrations of Cr(VI) and phenol, respectively. For acclimatization the bacterium *Escherichia coli* and *Bacillus* sp. was grown at different concentrations of Cr(VI) and phenol with increment of 1 mg L^{-1} in (2 : 1) ratio. Based on the tannery industry waste water composition Cr(VI) and phenol in synthetic waste water was taken in (2 : 1) ratio because generally the concentration of chromium in waste effluent is more than phenol [1].

2. Experimental Setup

The experimental setup used for the simultaneous bioaccumulation of Cr(VI) and biodegradation of phenol was described by Mondal et al. [28]. A line diagram of the experimental setup is given in Fig. 1. The bioreactor consists of stainless steel pipe of 100 cm height, 8 cm internal diameter, 5 L net empty working volume, and 4 equidistant ports of 1.25 cm diameter. The S.S reactor was steam sterilized at 121±0.5 °C and 15 psig pressure for 30 minutes. The biocolumn was packed with tea waste biomass of size 2-2.5 mm immobilized with consortium culture of *Bacillus* sp. and *Escherichia coli*. Tea waste was obtained from local shop Roorkee INDIA. Tea waste was boiled for 30 min for the removal of soluble matter, dirt and colored components. After boiling, tea waste biomass was washed with distilled water in triplicate and then dried in hot air oven at 50 °C for 12 h. When all the moisture and volatile material was removed from tea waste biomass, it was sieved to obtain a constant particle size [29]. The physical and chemical properties of tea waste biomass are given in Table 1. Nutrient broth was pumped through the bioreactor at the feed flow rate of 2.08 mL min^{-1} for the conditioning of adsorbent bed. After the conditioning of the adsorbent bed, it was filled with consortium culture of *Escherichia coli* and *Bacillus* sp. and left as such for two days for the immobilization of bacterium onto the surface of adsorbent bed. When the bacterium *Escherichia coli* and *Bacillus* sp. was immobilized onto the surface of adsorbent bed in packed bed column, influent of desired concentration of Cr(VI) and phenol was passed through the column.

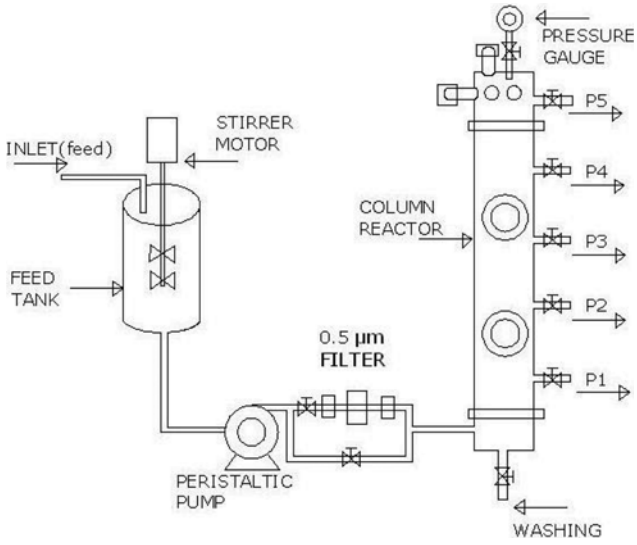

Fig. 1. Line diagram of experimental setup.

Table 1. Physical and chemical properties of tea waste biomass used as adsorbent bed in packed bed reactor

Physical properties	
BET surface area m^2/g	22.658
Total pore volume m^3/g	0.0255
Monolayer volume cm^3/g	4.387
Chemical properties	
Moisture (wt%)	8.01
Volatile matter	5.09
Ash content	12.45
Fixed carbon	74.45

RESULTS AND DISCUSSION

1. Effect of Operating Parameters

1-1. Effect of EBCT on Removal of Phenol and Cr(VI)

Experiments were conducted to study the effect of EBCT onto the removal of Cr(VI) and phenol in a continuous reactor. The detailed procedure of the experiments conducted has been described above. EBCT affects the efficiency of removal of pollutant in a biocolumn reactor. Longer EBCT confers longer contact time, and as a result the outlet concentration of pollutant decreases with the increase in EBCT [28,30,33]. It could be due to the fact that with increasing the EBCT, the RPM of peristaltic pump decreases due to which the flow rate (mL min^{-1}) of the influent waste water passed through the reactor decreases [31,32,34]. By increasing the EBCT the residence time of the pollutant Cr(VI) and phenol in packed bed reactor was increased. A curve of normalized concentration vs. time was drawn at various EBCT (4 h, 8 h, 12 h), called breakthrough curve or S curve. The percentage removal of Cr(VI) and phenol varying with time was determined through the breakthrough

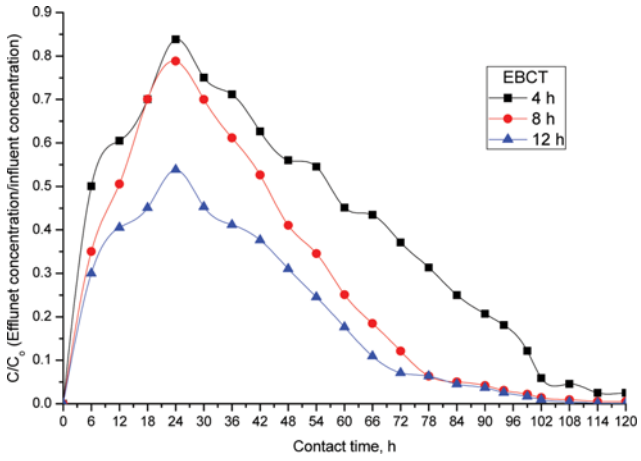

Fig. 2. Effect of EBCT on the Cr(VI) removal in the bio column reactor (sample collected from the top of the reactor).

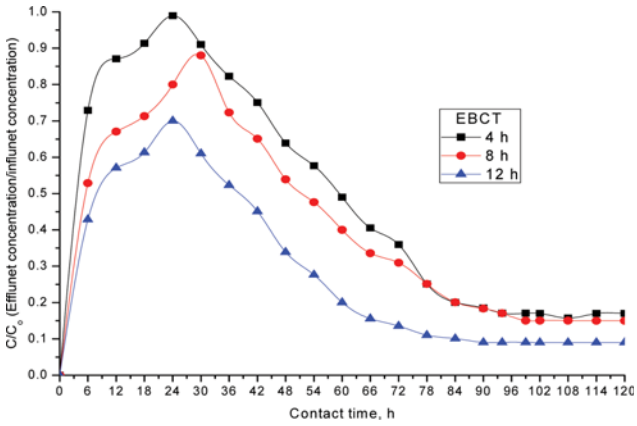

Fig. 3. Effect of EBCT on the phenol removal in the bio column reactor (sample collected from the top of the reactor).

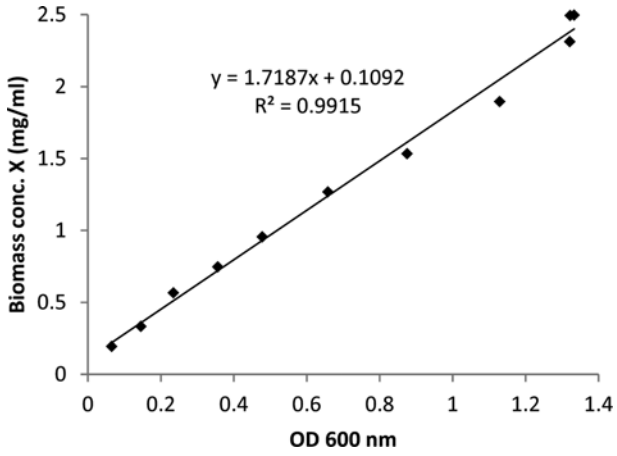

Fig. 5. Plot for the conversion of OD to biomass concentration.

curve shown in Fig. 2 and Fig. 3, respectively. The normalized concentration was calculated as given below.

$$\text{Normalized Concentration} = C/C_0$$

Where, C_0=Influent concentration, (mg/L)
C=Effluent concentration, (mg/L)

From Fig. 2 and Fig. 3, for all EBCT values, the concentration of both Cr(VI) and phenol increases initially, reaches a maximum value and decreases thereafter with the run time. The possible reason behind this fact is that initially all the active sites available for the adsorption of Cr(VI) and phenol onto the surface of tea waste biomass were vacant, but as time passed the active sites available for adsorption decreased, but with the increase in time bacterial biomass immobilized onto the surface of tea waste accommodated themselves into the toxic environment or became active. Therefore, in the initial stage of experimental run the adsorption was more in comparison to bacterial bioaccumulation and biodegradation of toxic pollutant, but as time passed the vacant sites of biosorbent available for adsorption were occupied by the pollutant. Therefore, initially the effluent concentration was very less and then started to increase up to 30 h and then decreased. Because after 30 h of experimental run bacterial biomass was completely adjusted in the toxic environment; therefore after 30 h of contact time effluent concentration was decreased [28]. The growth of biomass with time at different EBCT is given in Fig. 4. Fig. 4 shows that the biomass growth initially decreased and then started to increase after 30 h of contact time at all EBCT values. For the measurement of biomass concentration, 5 mL samples of different concentrations of Cr(VI) and phenol were centrifuged at 10,000 rpm and the bacterial biomass attached to the centrifuge tube was dissolved in 2 mL millipore water, and its OD was taken at 600 nm [35,36]. The calibration curve was plotted for converting the OD to biomass concentration according to the method of [37]. A curve of dry weight biomass per liter against optical density was plotted for the conversion of optical density to biomass concentration shown in Fig. 5. Also, within 102 h of operation the concentration of Cr(VI) in treated water reached the minimum and achieved the regulatory levels (mg L^{-1} of Cr(VI)) at higher EBCT of 12 h. The effluent concentration of phenol reached the minimum within 114 h at EBCT of 8 h and 12 h, while it reached to minimum within 96 h at EBCT of 4 h. Therefore, for the achievement of regulatory limit of phenol (mg L^{-1} of phenol) in the effluent, recycling of waste effluent is required. Similar observation has been made by [28,38] for the treatment of arsenic contaminated waste water. Therefore, longer EBCT time and low flow rate is suggested for the maximum removal of Cr(VI) and phenol [39]. The maximum adsorption capacity (mg/g) of tea waste biomass in packed bed reactor for Cr(VI) and phenol was found to be 5238.61 and 2145.99, respectively.

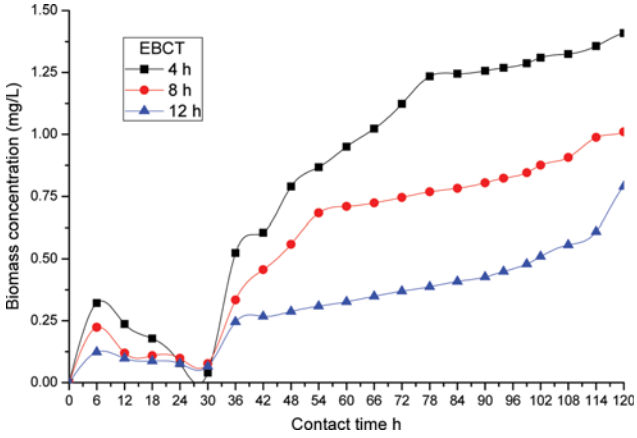

Fig. 4. Growth of biomass with time.

1-2. Effect of Bed Height on Removal of Cr(VI) and Phenol

From Fig. 6 and Fig. 7, for all bed height concentration of Cr(VI) and phenol in effluent decreases in the initial stage of the operation of packed bed column in the following order:

$$P_5<P_4<P_3<P_2<P_1$$

P_5, P_4, P_3, P_2 and P_1 denote the bed height of the column at 93, 72, 54, 36 and 18 cm respectively. However, after a run of four days, Cr(VI) and phenol concentration in the treated effluent collected from all the five ports became constant and less than the regulatory levels. It could be that with the increase in bed height of the reactor the contact time of the pollutant Cr(VI) and phenol with the bacterial biofilm increased, leading to the increase in the per-

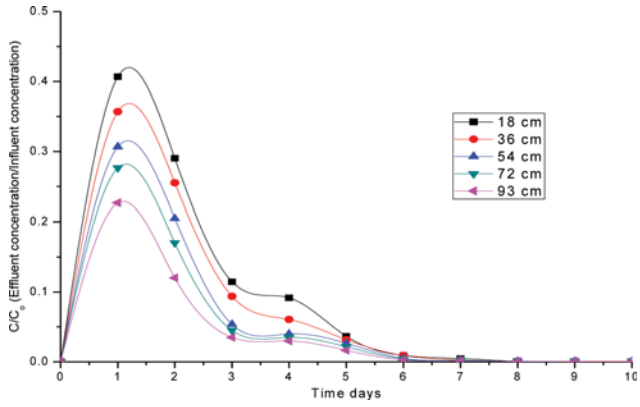

Fig. 6. Effect of bed height onto the percent removal of Cr(VI).

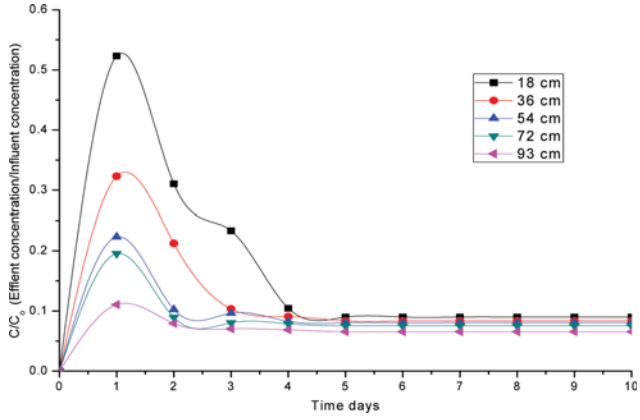

Fig. 7. Effect of bed height on the percent removal of phenol.

centage removal of both Cr(VI) and phenol [40,41]. It could also be because at higher bed depth large surface area and free active sites were available for the simultaneous removal of Cr(VI) and phenol. However, it was also observed that after four days of operation, the effect of bed height onto the residual concentration of Cr(VI) and phenol became negligible.

1-3. Studies on the Change in pH and DO (Dissolved Oxygen) with Runtime

The change in pH and dissolved oxygen with run time is given in Fig. 8 and Fig. 9, respectively. The initial pH of the synthetic waste water was 7 and DO was 8. To find the variation in pH and DO with the run time, samples were collected at port P_5 for seven days. From Fig. 8 it is evident that initially the pH of effluent decreased, but after two days of operation the pH increased and then became constant with time. The initial decrease in pH may be attributed to the adaptation of bacteria to the toxic environment. The decrease in pH may be due to the fact that as the bacteria used for the toxic pollutant removal was aerobic, therefore they utilized the oxygen available at the surface of biosorbent for the degradation of toxic pollutant. The bacterium used for the bioaccumulation and biodegradation of phenol formed the complex of carbon and oxygen as phenol was used as toxic pollutant. The complex of carbon and oxygen was combined with water producing hydroxyl anions, then these hydroxyl ions combined with water to produce

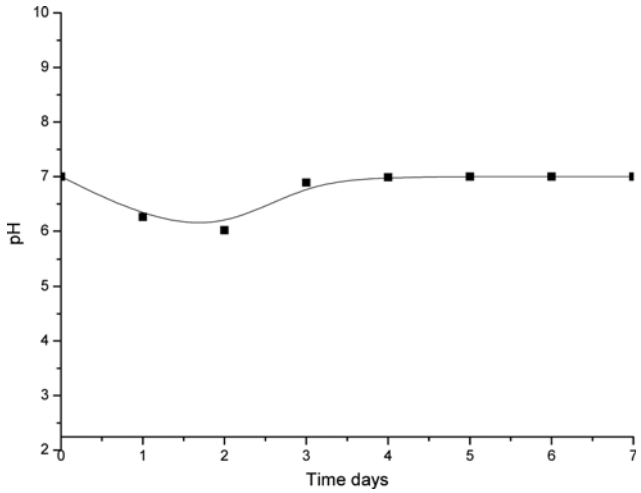

Fig. 8. Change in pH with run time.

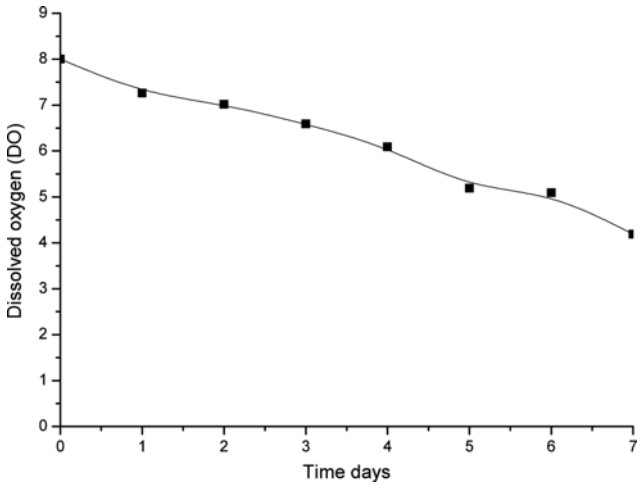

Fig. 9. Change in DO (dissolved oxygen) with run time.

H^+ ions; therefore, the pH of the effluent was increased [28]. The decrease in DO with time shown in Fig. 9 is attributed to the fact that the bacteria used for the continuous operation were aerobic.

1-4. Removal Mechanism of Cr(VI) and Phenol from Binary Solution

It was observed that the kinetic of Cr(VI) reduction was improved by coupling the Cr(VI) reduction to other energy yielding reactions such as phenol degradation because the metabolites formed during the biodegradation of phenol by *Bacillus* sp. were utilized by *Escherichia coli* for the reduction of Cr(VI). Phenol is a carbon or energy source for the bacterium, which was utilized by the bacteria for the bioaccumulation of Cr(VI). The following reaction is given for the simultaneous removal of Cr(VI) and phenol by the consortium culture of bacteria [5]:

$$C_6H_6O + 9\frac{1}{3}CrO_4^{2-} + 40\frac{2}{3}H^+ \rightarrow 9\frac{1}{3}Cr^{3+} + 6HCO_3^- + 20\frac{1}{3}H_2O \quad (1)$$

The above reaction shows that phenol is used as an electron donor for the reduction of Cr(VI) and was completely degraded to HCO_3^- and H_2O.

Table 2. The percentage removal and uptake capacity of tea waste biomass immobilized packed bed reactor at different flow rates

Component	Q (ml/min)	t_{total} (min)	V_{eff} (ml)	m_{total} (mg)	q_{total} (mg)	q_{eq} (mg/g)	Removal %	Total removal %
Cr(VI)	5.46	7200	39312	78624	78579.18	5238.61	99.94	97.16
Phenol	5.46	6480	35380.8	35380.8	32189.81	2145.99	90.99	
Cr(VI)	8.19	4680	38329.2	76658.4	76262.08	5084.14	99.48	95.16
Phenol	8.19	3960	32432.4	32432.4	27552.95	1836.86	84.96	
Cr(VI)	16.3	2160	35208	70416	67231.08	4482.07	95.48	91.79
Phenol	16.3	1800	29340	29340	24335.77	1622.39	82.94	

MATHEMATICAL DESCRIPTION

The mathematical description of mass balance for simultaneous percentage removal of Cr(VI) and phenol in packed bed reactor, i.e., the amount of Cr(VI) and phenol passed through the reactor, amount adsorbed and passed through the effluent of the packed bed reactor for the initial concentration of 2,000 and 1,000 mg/L of Cr(VI) and phenol in binary solution can be calculated as follows [4]:

The effluent volume can be calculated as

$$V_{eff}=Qt_{total} \tag{2}$$

where V_{eff}=Effluent Volume, (mL)
Q=Volumetric flow rate, (mL/min)
t_{total}=Total flow time, (min)

Total quantity of pollutant simultaneously adsorbed and consumed by the bacterium, q_{total} (mg), in a packed bed reactor is given below:

$$q_{i,\,total}=Q\int_{t=0}^{t=t_{total}} c_{ad,\,i}\,dt$$

Total amount of pollutant passed through the inlet, m_{total} (mg), and the removal percentage can be calculated from the equation given below.

$$m_{i,\,total}=C_0\,Qt_{i,\,total} \tag{3}$$

$$\text{Removal }\%_{,\,i}=\frac{q_{i,\,total}}{m_{i,\,total}}\times 100 \tag{4}$$

Equilibrium pollutant uptake (q_{eq}) for the simultaneous removal of Cr(VI) and phenol can be calculated as given below:

$$q_{eq,\,i}=\frac{q_{i,\,total}}{x} \tag{5}$$

where X is the amount (g) of adsorbent packed in the column

Total simultaneous percentage removal of Cr(VI) and phenol in a packed bed reactor can be estimated as follows:

$$\text{Total removal }\%=\left(\Sigma_{i=1}^{i=N}\frac{q_{i,\,total}}{m_{i,\,total}}\right)\times 100 \tag{6}$$

where, N=Number of components in the mixture

The total amount of Cr(VI) and phenol passed through the reactor at different flow rates (mL/min) and amount of pollutant simultaneous accumulated and consumed by the bacterium is given in Table 2. From Table 2, the amount of Cr(VI) and phenol passed through the reactor, amount adsorbed and percentage removal decreased with the increase in flow rate, because with the increase in flow rate the contact time between pollutant and bacterium immobilized onto the surface of packed bed was decreased [42].

1. Mass Transfer Studies

1-1. Calculation of First-order Biodegradation Rate Constant for Phenol and Bioaccumulation Rate Constant for Cr(VI)

The first-order biodegradation rate constant for phenol and first order bioaccumulation rate constant for Cr(VI) was calculated for the consortium culture of *Bacillus* sp. and *Escherichia coli.* immobilized onto tea waste biomass using the assumption of plug flow, no axial dispersion, and steady state condition [43].

The material balance for the Cr(VI) and phenol in continuous packed bed bio column reactor is given according to following equation [30,44].

$$\left[\frac{HQ}{W}\right]\frac{dc}{dz}\times 6\times 10^{-2}=-r=-k_pC \tag{7}$$

On integrating the above equation using the boundary conditions for Cr(VI) and phenol

$$\text{Cr(VI): z=0, C=C}_{oCr(VI)}\ln\left(\frac{C_{oCr(VI)}}{C_{Cr(VI)}}\right)=\frac{W}{Q}k_{p1}\times\frac{10^3}{60} \tag{8}$$

z=H, C=$C_{Cr(VI)}$

$$\text{Phenol: z=0, C=C}_{o\,phenol}\ln\left(\frac{C_{o\,phenol}}{C_{phenol}}\right)=\frac{W}{Q}k_{p2}\times\frac{10^3}{60} \tag{9}$$

z=H, C=C_{phenol}

where r is the biodegradation and bioaccumulation rate of phenol and Cr(VI) (mg g^{-1} h^{-1}), Q is the volumetric flow rate (mL min^{-1}), H height of the column (cm), W is the total amount of dried cells immobilized on biosorbent (g), dC/dz is the concentration gradient along the length of reactor (mg L^{-1} cm^{-1}). $C_{oCr(VI)}$ and $C_{Cr(VI)}$ are the initial and the final concentration of Cr(VI) and $C_{o\,phenol}$ and C_{phenol} is the initial and the final concentration of phenol. k_{p1} is the first-order bioaccumulation rate constant for Cr(VI) (L g^{-1} h^{-1}) and k_{p2} is first-order biodegradation rate constant for phenol (L g^{-1} h^{-1}).

1-2. Combined Mass Transfer and Simultaneous Biodegradation and Bioaccumulation of Phenol and Cr(VI)

The rate of mass transfer from the bulk fluid to the immobilized tea waste biomass surface is directly proportional to the area of mass transfer and concentration difference between bulk fluid and immobilized tea waste biomass surface.

$$r=k_m a_m\,[C-C_s]\times 10^{-3} \tag{10}$$

where r is the external mass transfer rate (mg g^{-1} h^{-1}), k_m is the exter-

nal mass transfer coefficient (cm h^{-1}), a_m is the surface area per unit weight of microorganism cells for mass transfer (cm^2 g^{-1}), C is the substrate concentration in the bulk liquid, C_s is the substrate concentration at the surface of immobilized particle (mg L^{-1}).

For the packing in a biocolumn reactor, the value of a_m can be calculated as given by the following equation:

$$a_m = \frac{6(1-\varepsilon)}{\rho_p d_p} \tag{11}$$

where d_p is the particle diameter (cm), ρ_p is the density of particle (g cm^{-3}).

Mass flux G (g cm^{-2} h^{-1}) and Reynold number (Re) can be calculated as

$$G = \frac{60Q}{A\varepsilon} \tag{12}$$

$$R_e = \frac{d_p G}{\mu} \tag{13}$$

At steady state $r = k_m a_m C_s$

where k is the first order intrinsic rate constant (L g^{-1} h^{-1})

$$C_s = \frac{k_m q_m C}{k + k_m a_m} \tag{14}$$

$$k_p = \frac{k\, k_m a_m}{k + k_m a_m} \tag{15}$$

The mass transfer coefficient is given by the following equation according to the mass transfer correlation [44-47].

$$K_m = \left(\frac{k}{\rho}\right)\left(\frac{\mu}{\rho D_f}\right)^{-2/3}\left(\frac{d_p}{\mu}\right)^{n-1} G^n \tag{16}$$

On substituting the value of k_m from Eq. (16) in Eq. (15).

$$\frac{1}{k_p} = \left(\frac{1}{AA_m}\right)\left(\frac{1}{G^n}\right) + \left(\frac{1}{k_s a_m}\right) \tag{17}$$

The experimental values of first-order biodegradation rate constant (L g^{-1} h^{-1}) k_{p1} for Cr(VI) and k_{p2} for phenol were calculated and are given in Table 3 and Table 4, respectively. A plot of $1/k_p$ vs $1/G^n$ for different value of n was depicted for Cr(VI) and phenol is given in Table 5 and Table 6, respectively. Experimentally measured val-

Table 3. Ratio of outlet Cr(VI) concentrations to the inlet Cr(VI) concentrations and pseudo first order biodegradation rate constants evaluated from Eq. (4) at various flow rates at 2,000 mg L^{-1} of inlet Cr(VI) concentration in the packed bed

Q (mL min^{-1})	C/C_o	$k_p \times 10^3$ (Lg^{-1} h^{-1})
5.49	0.00057	80.0501
8.19	0.0057	84.9448
16.3	0.02507	117.9257

Table 4. Ratio of outlet phenol concentrations to the inlet phenol concentration and pseudo first order biodegradation rate constants evaluated from Eq. (4) at various flow rates at 1,000 mg L^{-1} of inlet phenol concentration in the packed bed

Q (mL min^{-1})	C/C_o	$k_p \times 10^3$ (L g^{-1} h^{-1})
5.49	0.09	25.7819
8.19	0.15	30.495
16.3	0.17	56.689

Table 5. Calculated values of mass fluxes, Reynolds numbers, $1/k_p$ and $1/G^n$ at various flow rates at 2,000 mg L^{-1} of inlet Cr(VI) concentration in the packed bed

G (g cm^{-2} h^{-1})	N_{Re}	$1/k_p$ g h L^{-1}	$1/G^{0.22}$	$1/G^{0.33}$	$1/G^{0.41}$	$1/G^{0.53}$	$1/G^{0.65}$	$1/G^{0.73}$	$1/G^{0.82}$	$1/G^{0.94}$	1/G
299.62	14192.53	12.4922	0.2852	0.1523	0.0965	0.0487	0.0246	0.0156	0.0093	0.0047	0.0033
449.43	21288.79	11.7724	0.2609	0.1332	0.0817	0.0393	0.0189	0.0116	0.0067	0.0032	0.0022
894.46	42369.15	8.4799	0.2242	0.1062	0.0616	0.0273	0.0121	0.0070	0.0038	0.0017	0.0011

Table 6. Calculated values of mass fluxes, Reynolds numbers, $1/k_p$ and $1/G^n$ at various flow rates at 1,000 mg L^{-1} of inlet phenol concentration in the packed bed

G (g cm^{-2} h^{-1})	N_{Re}	$1/k_p$ g h L^{-1}	$1/G^{0.22}$	$1/G^{0.33}$	$1/G^{0.41}$	$1/G^{0.53}$	$1/G^{0.65}$	$1/G^{0.73}$	$1/G^{0.82}$	$1/G^{0.94}$	1/G
299.62	14192.53	38.7869	0.0257	0.1523	0.0965	0.0487	0.0246	0.0156	0.0093	0.0047	0.0033
449.43	21288.79	32.7918	0.0305	0.1332	0.0817	0.0393	0.0189	0.0116	0.0067	0.0032	0.0022
894.46	42369.15	17.6401	0.0567	0.1062	0.0616	0.0273	0.0121	0.0070	0.0038	0.0017	0.0011

Table 7. Comparison of experimental k_p values obtained from Eq. (3) and calculated k_p values obtained from Eq. (16) at various flow rates and n values for Cr(VI)

Q mL min^{-1}	Experimental k_p (L g^{-1} h^{-1})	n=0.41 k_p (L g^{-1} h^{-1})	n=0.53 k_p (L g^{-1} h^{-1})	n=0.65 k_p (L g^{-1} h^{-1})	n=0.73 k_p (L g^{-1} h^{-1})	n=0.82 k_p (L g^{-1} h^{-1})	n=0.94 k_p (L g^{-1} h^{-1})	n=1 k_p (L g^{-1} h^{-1})
5.49	0.0801	0.0853	0.0683	0.0163	0.0041	0.0008	8.76×10^{-5}	2.89×10^{-5}
8.19	0.0849	0.1003	0.0779	0.0168	0.0041	0.00078	8.76×10^{-5}	2.89×10^{-5}
16.3	0.1179	0.1316	0.0949	0.0174	0.0042	0.00079	8.77×10^{-5}	2.9×10^{-5}

Table 8. Comparison of experimental k_p values obtained from Eq. (3) and calculated k_p values obtained from Eq. (16) at various flow rates and n values for phenol

Q mL min^{-1}	Experimental k_p (L g^{-1} h^{-1})	n=0.73 k_p (L g^{-1} h^{-1})	n=0.82 k_p (L g^{-1} h^{-1})	n=0.94 k_p (L g^{-1} h^{-1})	n=1 k_p (L g^{-1} h^{-1})
5.49	0.0258	0.0249	0.0249	0.0248	0.0248
8.19	0.0305	0.0331	0.0333	0.0335	0.0336
16.3	0.0567	0.0531	0.0528	0.0524	0.0521

ues of $1/k_p$ vs. $1/G^n$ were plotted for the different values of n and from the slope and intercept A_m, k_m and k is calculated from Eq. (17) and it was compared from experimental values of k_p for different values of n is given in Table 7 and Table 8 for Cr(VI) and phenol, respectively. The effect of flow rate on the biodegradation and bioaccumulation rate of phenol and Cr(VI) was investigated by calculating the biodegradation and bioaccumulation rate constant k_p for phenol and Cr(VI). From Table 3 and Table 4 it is evident that the first-order rate constant for Cr(VI) and phenol increased with increasing the flow rate (mL min^{-1}), while the removal of Cr(VI) and phenol decreased. The effect of film diffusion on the biodegradation rate was determined by calculating the Reynolds number and mass flux for Cr(VI) and phenol by putting the value of μ=0.0076 g cm^{-1} s^{-1}, ρ=0.976 g cm^{-3}, D_f=2.808×10^{-5} cm^2 sec^{-1}, ε=0.5 and A=3.79 cm^2. Not all values of n for Cr(VI) and phenol provide satisfactory values of first-order rate constant due to negative slope and intercept. For Cr(VI) the flow rate of 5.49 and 16.3 mL min^{-1}, n=0.41 and for 8.19 mL min^{-1}, n=0.53 provides a satisfactory value of k_p when compared with experimental values. For phenol all the flow rate values for n=0.73, 0.82, 0.93 and 1 provide a satisfactory value of k_p. For n<0.41 for Cr(VI) and n<0.73 for phenol gives the negative slope; therefore, it could not be considered for further analysis.

CONCLUSION

1. Simultaneous removal of Cr(VI) and phenol was achieved in a continuous bio column reactor packed with consortium culture of *Escherichia coli* and *Bacillus* sp. immobilized tea waste biomass.

2. For simultaneous removal of Cr(VI) and phenol from binary synthetic waste water an EBCT of 4 h was found to be sufficient to bring the effluent concentration below the prescribed regulatory level of Cr(VI) and phenol.

3. Effect of bed height onto the simultaneous removal of Cr(VI) and phenol was investigated, which shows that percentage removal of both Cr(VI) and phenol was increased with the increase in bed height, but after 4 days of operation percentage removal became constant at all bed heights.

4. DO (dissolved oxygen) was found to decrease with time, while pH was decreased initially and then became constant.

5. A mass transfer study was carried out to find the first-order degradation rate constant for phenol and bioaccumulation rate constant for Cr(VI), which was in good agreement with experimental data.

ACKNOWLEDGEMENT

The author gratefully acknowledges financial support provided by the MHRD assistantship by Government of India and Chemical engineering Department IIT Roorkee for the facility provided for conducting research work.

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