*Full Length Research Paper*

# Biodegradation of phenol with immobilized *Pseuodomonas putida* activated carbon packed bio-filter tower

**A. Ullhyan\* and U. K. Ghosh**

Department of Paper Technology, Indian Institute of Technology Roorkee, Saharanpur Campus Saharanpur – 247001, India.

Comparative study on adsorption and simultaneous adsorption and biodegradation (SAB) of phenol using *Pseuodomonas putida* (MTCC 1194) in a biofilter tower packed with fresh granular activated carbon (GAC) or biological activated carbon (BAC) showed 37% higher breakthrough point in case of SAB. Maximum phenol degradation rate in case of SAB was 120 mg/L.h obtained at flow rate of 7.42 ml/min. Bioregeneration of bed material up to 63% was achieved in 12 h. The phenol degradation by *P. putida* (MTCC 1194) was immobilized on activated carbon. Higher surface area of activated carbon is suited as supporting materials for microorganism and shows a high adsorptive capacity for phenol. For biodegradation of phenol by *P. putida* at shake flask level Haldane's growth model fitted the best. Compared to GAC, 21% higher removal of phenol was observed in case of SAB in batch mode.

**Key words:** Biologically activated carbon, bioregeneration, phenol, *Pseudomonas putida*.

## INTRODUCTION

The effluent from various industries such as pulp and paper, oil refineries, polymeric resins, plastics, steel plants, insecticides, pesticides, textile, dyes, coal processing, pharmaceutical, etc. consist of phenolic compounds as their most important constituents (Pazarlioglu and Telefoncu, 2005). The phenol, a potential carcinogen, could be adsorbed through skin and upon ingestion can cause vomiting, paralysis, lung failure and cardiac arrest. It can be toxic or lethal to fishes at concentrations of 5 to 25 mg/L and imparts medicinal taste and odour at much lower concentration of 2 µg/L (Kumar et al., 2005; Dabhade et al., 2006). Adsorption of phenol by activated carbon is highly efficient, giving very low level of phenol in the effluent, though cost is high and regeneration is very difficult. However, biological activated carbon (BAC) is quite efficient in phenol removal and prolongs the operational period of activated carbon (Kumar et al., 2006). Several authors (Morsen and Rehm, 1987; Folsom et al., 1990;

Ehlers and Rose, 2005; Kumar et al., 2005; Fikret and Serkan, 2005; Ehardt and Rehm, 1985; Alvaro et al., 2000; Hank et al., 2010; Li et al., 2010; Sagar et al., 2004) have reported phenol uptake by *Pseudomonas putida* and other microorganisms. Biological degradation of chlorinated aromatic compounds has been studied by many investigators (Dapaah and Hill, 1992; Kim and Hao, 1999; Kim et al., 2002; Wang and Low, 2000; Fahr et al.,1999; Farrell and Quilty, 2002; Agarry et al., 2008; Allsop et al., 1993; De Laat, 1985; Schelgel, 1995; Powlowski and Shingler, 1994).

Different fungi and aerobic bacteria such as species of *Pseudomonas*, *Azotobacter*, *Alcaligenes* and *Acinetobacter* have been used for degradation of phenol and chlorophenols. Different types of reactors have been used for treatment of phenolic wastewater with immobilized microorganisms (Seker et al., 1997; Kim et al., 2002; Dabhade et al., 2006; Haigler et al., 1992). In spite of certain limitations, packed bed reactors are convenient for BAC treatment of wastewater because of ease of operation and lower operational costs (Fedra and Aska, 2003). For phenol degradation in fixed-bed biofilm reactor, Xiaojian et al. (1991) concluded that removal of substrate is affected by adsorption

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*Corresponding author. E-mail: miti_anupa@rediffmail.com or anupadpt@iitr.ernet.in.

and biological activity. Therefore, combination of both the processes in a single reactor has significant benefit. Hypothesis of desorption by exoenzymatic reaction related to the bioregeneration has been given by Perrotti and Rodman (1974).

The objective of this study was to investigate the performance of granular activated carbon (GAC) packed biofilter tower for phenol adsorption, immobilization and phenol degradation. Kinetic studies pertaining to microbial growth of acclimatized *P. putida* (MTCC 1194) cells and phenol biodegradation were performed at shake flask level in basal salt medium with phenol as inhibitory carbon source. Comparative performance of adsorption and simultaneous adsorption and biodegradation (SAB) was evaluated both in batch and in continuous mode.

## Theory

In a batch reactor, for low concentration of phenol, the dependence of specific growth rate, $\mu$, on substrate concentration, $S$, may be represented by Monod's Model.

$$\mu = \mu_{max} S/ (K_s + S) \tag{1}$$

This model is an over simplification and does not take care of inhibitory nature of substrate. For high concentration of phenol, Haldane's inhibitory growth kinetic equation can be used (Kumar et al., 2005).

$$\mu = \mu_{max} S/(K_s + S + S^2/K_i \tag{2}$$

where, $K_s$ and $K_i$ are half saturation coefficient and inhibitor constant, respectively. For very high concentration of phenol that is, for $S \gg K_s$, Linearized Haldane's equation may be used (Kumar et al., 2005).

$$\mu = \mu_{max} S/(S + S^2/K_i) \tag{3}$$

The above can be rearranged as,

$$(1/\mu) = (1/\mu_{max}) + ( S/K_i \mu_{max}) \tag{4}$$

For batch studies the specific growth rate may be expressed as

$$\mu = (1/t) \ln(OD_t/OD_o) \tag{5}$$

where, $OD_o$ is the initial optical density and $OD_t$ is the optical density at time t. In column studies, total amount of substrate sent into reactor, $m_{total}$, reactor performance, R, maximum specific uptake of substrate, q, and percentage regeneration of activated carbon, $P_{reg}$, may be calculated using the following expressions.

$$m_{total} = C_o Q_f t_{total}/1000 \tag{6}$$

$$R = (q_{total}/m_{total}) \times 100 \tag{7}$$

$$q = q_{total}/X_{ad} \tag{8}$$

$$P_{reg} = (q_{BAC}/q_{GAC}) \times 100 \tag{9}$$

where, $C_o$ is the initial concentration, $Q_f$ is the flow rate at inlet in ml/min, $X_{ad}$ is the amount of adsorbent in the reactor, $q_{total}$ is the total amount of substrate adsorbed, $q_{BAC}$ and $q_{GAC}$ are the total uptakes of substrate in BAC and GAC, respectively.

## MATERIALS AND METHODS

Phenol and other chemicals used were all of AR grade and supplied by M/s. S. D. Fine Chemicals Limited, India. Commercial grade granular activated carbon with size range 2 to 4 mm, was used as adsorbent. The GAC particles were cleaned with distilled water to remove the fines and were dried in an oven for 24 h at 110°C. Pure strain of *P. putida* (MTCC1194) was procured from Institute of Microbial Technology, Chandigarh, India. Nutrient agar medium and basal salt medium were used for microbial growth. Nutrient agar medium contained 1 g beef extract, 2 g yeast extract, 5 g peptone, 5 g NaCl and 15 g agar in one litre distilled water. Composition of BSM was 1.5 g $K_2HPO_4$, 0.5 g $KH_2PO_4$, 0.5 g $(NH_4)_3PO_4$, 0.5 g NaCl, 3 g $Na_2SO_4$, 2 g yeast extract 0.5 g glucose, 0.002 g $FeSO_4$ and 0.002 g $CaCl_2$ in 1 L distilled water.

### Acclimatization of culture

Acclimatization of culture was performed in batch mode in 250 ml conical flasks containing basal salt medium and bacterial inoculums to which stock solution of phenol was added in varied concentrations, ranging from 10 to 60 mg/L, with increment of 10 units in each stage. All the experiments were carried out at 30°C, 150 rpm and 24 h incubation period.

### Phenol determination

Measurement of phenol was done by UV-vis spectrophotometer (Model - Perkin Elmer) using standard 4-aminoantipyrene method at 510 nm (Clesceri et al., 2005). Broth samples containing biomass were centrifuged at approximately 12000 rpm for 20 min. The supernatants were used for phenol determination. The biomass attached to the tube walls for different samples were resuspended in distilled water and optical density of those suspensions were measured against distilled water as reference at 620 nm for kinetic studies.

### Batch study

Batch experiments were conducted in BSM with 20 ml inoculum of phenol acclimatized *P. putida* at 0.8122 g cell (dry wt)/L in 500 ml flasks at 30°C and 150 rpm with various initial concentrations of phenol to determine the parameters for different kinetic models for biodegradation. Unless specified otherwise, all the reaction mixtures in the batch experiments consisted of 100 ml BSM at pH 6.7. To vary the initial concentrations of phenol, aliquots of 40 ml were taken from different stock solutions of phenol and added to the reaction mixture. Effects of various parameters like initial concentration of phenol, pH, adsorbent dose, etc., on percentage
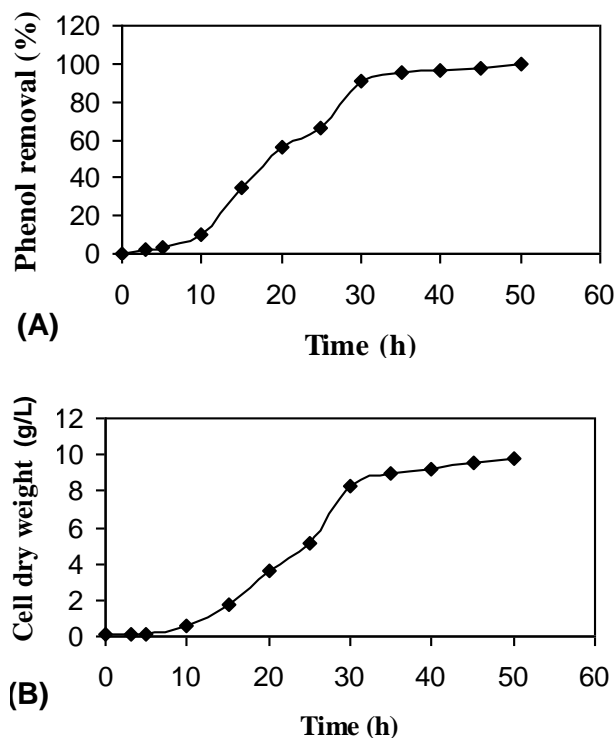
**Figure 1.** Biomass growth kinetics during phenol degradation by free *P. putida*. An aliquot of 20 ml from 500 mg phenol/L stock solution was mixed with 150 ml BSM in 500 ml flask.
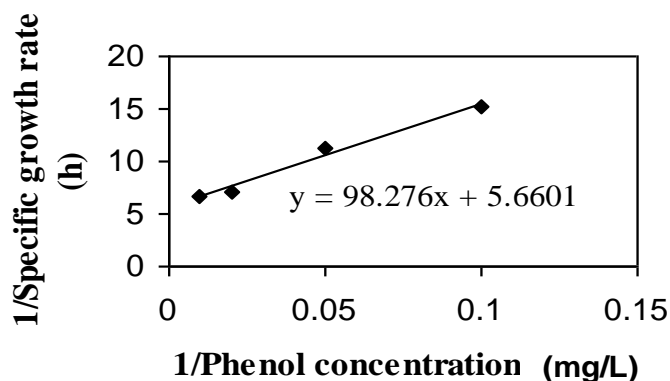


**Figure 2.** Biodegradation kinetics (Monod's model) for free *P. putida* at 10, 20, 50 and 100 mg phenol/L initial concentration in the reaction mixture.

removal of phenol were also investigated. In all the experiments with adsorbent, 2 to 4 mm GAC particles were used. To find the optimum dose of adsorbent different quantities of GAC were taken in 500 ml conical flasks containing BSM for removal of phenol by simple adsorption.

**Studies on bio-filter tower**

A glass column of height 80 cm and internal diameter 1.85 cm was

used to study adsorption of phenol on GAC and BAC. The column was filled with 218 g of GAC having particle size of 2 to 4 mm. The bed porosity was 0.457. For experiments with BAC, the acclimatized inoculum at 0.8122 g cell (dry wt)/L was added to the column at a flow rate of 10 ml/min until the column filled completely and was kept for 16 h. The stock solution with 200 mg phenol/L was introduced at various flow rates from the top of the tower filled with GAC or BAC. The temperature of the column was maintained at 30°C. Samples collected at the outlet were filtered with Whatman filter paper for further analysis. To calculate the bioregeneration capacity of the bed, the exhausted BAC column was kept idle for 12 h. After the end of 12 h, the aforementioned experiments were repeated under similar flow conditions to find the breakthrough curves to calculate the percentage regeneration.

**Statistical analysis**

Data were analyzed by Regression analysis and ANOVA for examining the statistical significance of the model parameters (Gujarati, 2004).

**RESULTS AND DISCUSSION**

**Kinetic studies of phenol biodegradation at batch study**

Figure 1A shows that for initial concentration of 500 mg phenol/L, 99.48% of phenol was removed in 50 h. A lag phase of 8 h followed by a steep log phase growth was evidenced till 30 h (Figure 1B). The long lag phase of 8 h shows inhibitory effect of phenol as substrate, which can possibly be decreased by application of larger amount of well acclimatized inoculums. Batch growth kinetics of *P. putida* on phenol removal was studied at shake flask level taking initial concentration as 10, 20, 50, 100, 200, 300 and 500 mg phenol/L at pH 6.75. The specific growth rate was calculated using equation (5) and the values were used to check the validity of various kinetic models like Monod's model, Haldane's model and linearized Haldane's model. Table 1 shows the values of constants for different kinetic models. Specific growth rate data from low concentration region (10 to 100 mg phenol/L) were fitted to Monod's model (Figure 2) and those from higher concentration region (100 to 500 mg phenol/L) fitted to linearized Haldane's model (Figure 3). Figure 4 shows that specific growth rate initially increases with the increase in the initial phenol concentration and then starts decreasing with the increase in phenol concentration which indicates inhibitory nature of phenol. In general, Haldane's growth kinetic model is used to represent growth kinetics data of an inhibitory substrate. Therefore, it is evident that the entire range of the present experimental data fits best to Haldane's growth kinetic model.

**Effect of mass of adsorbent**

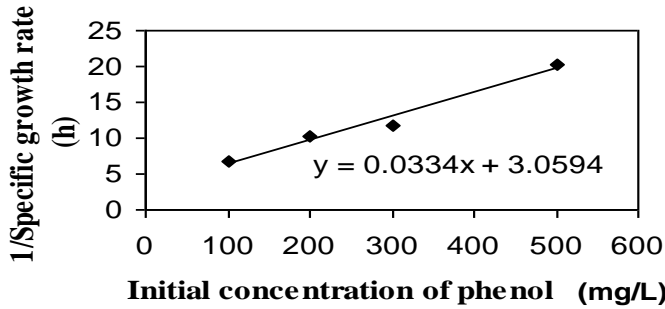From Figure 5, it is evident that the percentage removal

**Figure 3.** Biodegradation kinetics (Linearized Haldane's model) for free *P. putida* at 100, 200, 300 and 500 mg phenol /L initial concentration in the reaction mixture.
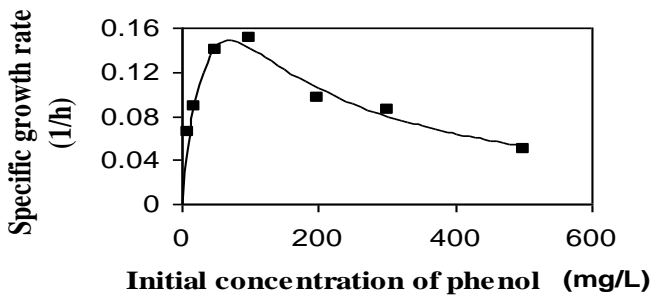


**Figure 4.** Biodegradation kinetics (Haldane's model) for free *P. putida* for initial concentration ranging from 10 to 500 mg phenol/L in the reaction mixture.
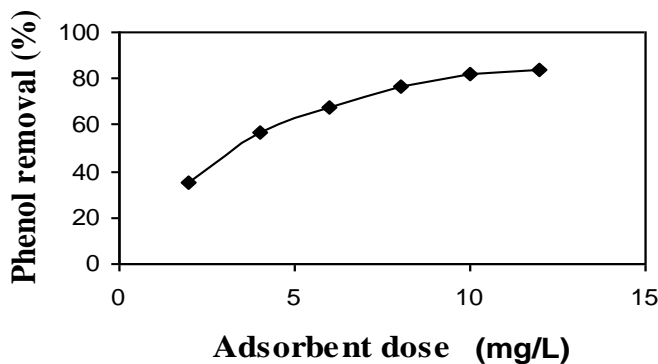


**Figure 5.** Effect of adsorbent dose on phenol removal. Initial concentration of phenol was 25 mg/L in the solution. Phenol concentrations were measured after 300 min.

of phenol reaches its constant value at the dose of around 8 g/L. However for phenol, the percentage removal increases beyond this dose and reaches maximum at the adsorbent dose of about 10 g/L. Result shows that increasing adsorbent dose beyond 10 mg/L has no significant effect on percentage of phenol removal. The uptake of solute markedly increased up to
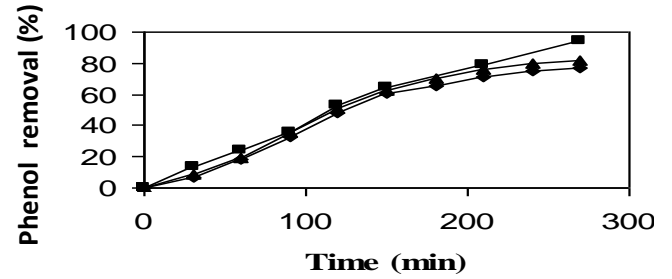


**Figure 6.** Removal of phenol by free *P. putida* at different pH values taking initial concentration of phenol as 25 mg/L in the reaction mixture. GAC dose was 10 mg/L. pH 6.75 (■); pH 9.01 (▲); pH 5.8 (♦).
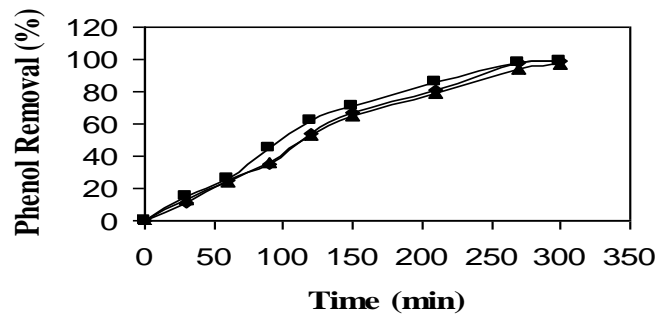


**Figure 7.** Removal of phenol by *P. putida* at various initial phenol concentration in the reaction mixtures. GAC dose was 10 mg/L. Suitable aliquots from stock solutions of 60, 80 and 100 mg phenol/L were added to the reaction mixture to obtain resulting concentrations. 15 mg phenol/l (♦); 20 mg phenol/l (■); 25 mg phenol/L (▲).

adsorbent dose of 10 g and thereafter no significant increase observed. The rate of phenol binding with the adsorbent increases more rapidly in the range of 2 to 10 g and after 10 g it becomes almost constant.Therefore, in subsequent experiments 10 mg/L GAC dose was applied.at 20 mg/L optimum phenol concentration.

**Effect of pH**

Figure 6 gives a comparative picture of effect of various pH values on corresponding phenol removal percentages, measured at regular time intervals. It reveals that pH values around 6 to 7 are favourable for higher removal percentage of phenol. Thus, phenol biodegradation occurs best near neutral pH due to neutrophilic behaviour of *P. putida,* and becomes less effective in highly acidic or alkaline conditions.

**Effect of initial concentration of phenol**

Figure 7 shows that the rate of phenol removal in the

**Table 1.** Values of constants for different kinetic models

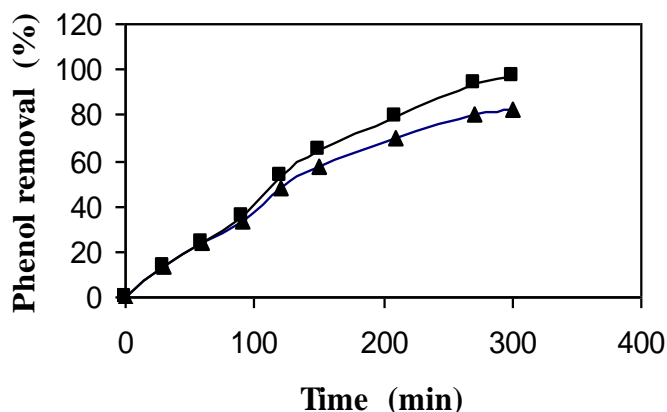| Monod's model | | Linearized Haldane's model | | Haldane's model | | |
|---|---|---|---|---|---|---|
| $\mu_{max}(h^{-1})$ | $K_s$(mg/L) | $\mu_{max}(h^{-1})$ | $K_i$(mg/L) | $\mu_{max}(h^{-1})$ | $K_s$(mg/L) | $K_i$(mg/L) |
| 0.182 | 17.03 | 0.291 | 106.11 | 0.3731 | 47.237 | 83.697 |



**Figure 8.** Comparison of phenol removal by simple adsorption and SAB. For both the experiments initial concentration of phenol was 25 mg/L in the reaction mixture. GAC dose was 10 mg/L. For SAB 20 ml *P. putida* inoculum was used. SAB (■); Adsorption (♦).

reaction mixture was highest in case of 20 mg phenol/L solution followed by 15 and 25 mg/L. The higher biodegradation found in case of 20 mg/L indicates that higher amount of available carbon source, possibly has increased the cell numbers, hence the phenol consumption is faster. Slower substrate degradation in case of 25 mg/L may be attributed to number of possible reasons. Growth inhibition may be caused by higher concentration of phenol as substrate or may be due to accumulation of toxic end products of phenol degradation cell leakage and cell lysis (Hugo, 1976) that might have caused blockage of pores. The unfavourable pH values arising during the course of degradation may also discourage the growth and biodegradation activities of *P. putida,* hence diminish the removal rate of phenol.

## Comparative study of adsorption and simultaneous adsorption and biodegradation (SAB)

Figure 8 shows the comparative patterns of phenol removal by adsorption (GAC) and SAB. It is evident from Figure 8 that initially up to 1 hour there is no variation in percentage removal of phenol in both processes, while after 1 h the variation starts where higher percentage removal of phenol has been observed in case of SAB (97.2%) with respect to adsorption (82.3%). Such an observation is evidenced in case of SAB due to occurrence of additional phenol removal by

biodegradation by *P. putida* cultures with progress of time resulting in higher phenol removal.

## Adsorption isotherms study

Adsorption and SAB are isothermal processes. Hence, these can be explained by adsorption isotherms. Adsorption equilibrium data which expresses the relationship between mass of adsorbate adsorbed per unit mass of adsorbent and the liquid phase equilibrium concentration of adsorbate are represented by adsorption isotherms and provides important design data for adsorption system. The sorption isotherm is probably the best method to determine the amount of sorbet that a sorbent can retain and that remaining in the solution at equilibrium. The concentration dependence of the sorption isotherm often confirms to one of the Langmuir or Freundlich isotherm. The Langmuir isotherm theory is based on the assumption that adsorption is 1st order chemical process and adsorbed layer is unimolecular. Equation 10 represents the Langmuir Isotherm.

$$\frac{1}{q_e} = (\frac{1}{Qb})(\frac{1}{C_e}) + \frac{1}{Q} \tag{10}$$

where, $qe$ is the amount of adsorbate adsorbed per unit mass of adsorbent (mg/g), $Ce$ is equilibrium concentration of adsorbate in aqueous solution (mg/L), $Q$ and $b$ are Langmuir constants related to the capacity and energy of adsorption (mg/g) and (l/mg) respectively. The magnitudes of these Langmuir constants can be determined from the linear plot of $1/qe$ vs. $1/Ce$. Where $b$ is Langmuir constant and Co is initial concentration of adsorbate. The $R$ value within 0 to 1 indicates the applicability of the Langmuir isotherm for describing the adsorption process. Equation 11 presents Freundlich isotherm.

$$\log q_e = \log K_f + 1/n \log C_e \tag{11}$$

where, $Kf$ and $1/n$ are Freundlich isotherm constants related to the adsorption capacity and adsorption intensity respectively. The $Kf$ and $n$ values can be determined by a linear plot of log $qe$ vs. log $Ce$. The values of dimensionless equilibrium parameter R Freundlich isotherm constant 1/n, and correlation coefficient ($R2$) for both Langmuir and Freundlich isotherm, for adsorption and SAB of phenol is given in

**Table 2.** Langmuir and Freundlich isotherm constants.

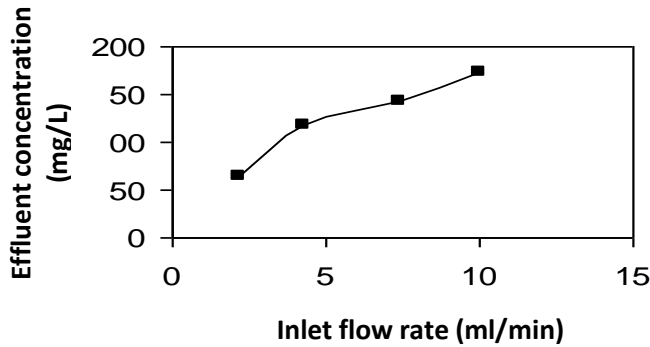| Process | Langmuir Isotherm constants | | | | Freundlich Isotherm Constant | | |
|---------|------|------|------|------|------|------|------|
| | Q ((mg/g) | b(l/mg) | R2 | R | Kf (mg/g)/(mg/L)1/n | 1/n | R$^2$ |
| SAB | 20.81 | 0.060 | 0.999 | 0.083 | 7.73 | 0.602 | 0.9797 |
| Adsorption | 36.78 | 0.040 | 0.986 | 0.146 | 13.37 | 0.508 | 0.9774 |



**Figure 9.** Variation of phenol concentration in effluent with change in inlet flow rate.
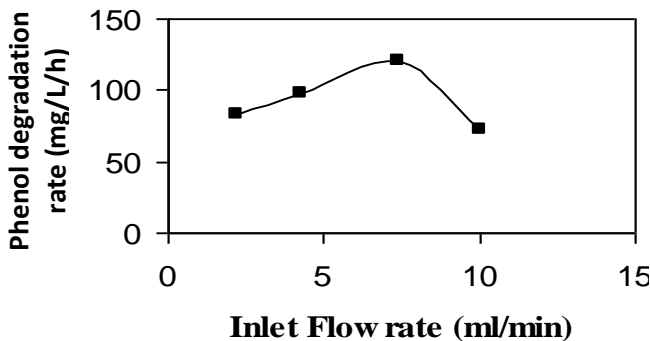


**Figure 10.** Variation of phenol degradation rate with change in inlet flow rate.
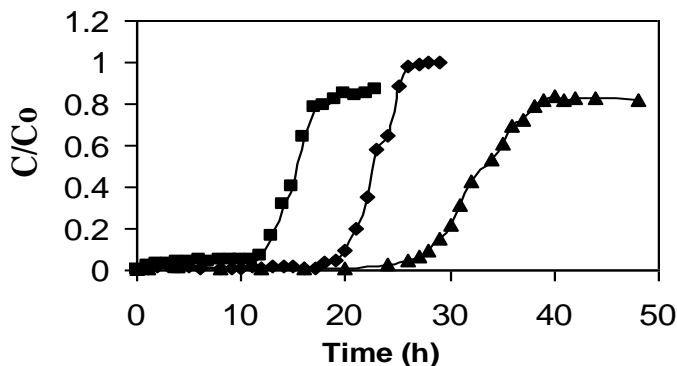


**Figure 11.** Break through curves for GAC, BAC and BAC (regenerated) beds. The inlet flow rate of 200 mg phenol/L solution was 10 ml/min at 30°C. GAC (♦); BAC (▲); Regenerated BAC (■).

Table 2. From Table 2, it is evident that *R* value lies within 0 to 1 for phenol and n value is also greater than 1 and lies within 1 to 5. Therefore both Langmuir and Freundlich isotherms can explain the adsorption as well as SAB processes. It is also evident that for the adsorption of both the compounds the correlation coefficient values for Langmuir isotherms are greater than those of Freundlich isotherms and *Q* values are also greater than the corresponding Kf values. This indicates that Langmuir isotherm is better fit for the description of adsorption process than the Freundlich isotherm. Recently, similar observation has also been reported (Bestamin, 2006) where decreasing order of the fitness of various adsorption isotherms for adsorption of phenol onto activated carbon has been explained as Langmuir > Toth > Redlich-Peterson > Freundlich. For the SAB of phenol compound, the *Q* values are less and *b* values are more than those of adsorption. This indicates less adsorption capacity of the adsorbents and more energy of adsorption. This may be due to the dominating role of SAB over adsorption. Again less values of Kf in case of SAB also indicates less adsorption capacity of the adsorbent and higher values of *1/n* (shift towards 1.0) indicates more adsorption intensity. This observation also favours the biodegradation along with adsorption in case of SAB for phenol.

**Phenol removal in bio-filter tower**

Column studies were performed for removal of phenol by adsorption and SAB at various flow rates of Phenol. Figure 9 shows the effect of inlet flow rate on stabilized concentration of phenol in effluent during SAB whereas Figure 10 shows variation of phenol degradation rate with change in inlet flow rate.

**Comparison of granular activated carbon (GAC), biological activated carbon (BAC) and BAC (regenerated) beds**

Figure 11 shows the breakthrough curves between effluent concentration and time for GAC, BAC and BAC (Regenerated) beds. The values of breakthrough point, reactor efficiency and specific uptake rate are given in Table 3. For SAB with BAC, up to 26 h (breakthrough point) phenol concentration in effluent was negligible. The

**Table 3.** Values of various parameters for phenol removal by GAC, BAC and BAC (regenerated) in biofilter tower.

| Parameter | GAC | BAC | BAC (Regenerated) |
|---|---|---|---|
| Reactor performance | 78.01 | 86.73 | 68.2 |
| Specific uptake (mg/g) | 13.26 | 19.51 | 9.023 |
| Regeneration (%) | - | - | 63.16 |
| Breakthrough point (h) | 19 | 26 | 12 |

effluent concentration then started to increase up to 39 h and stabilized at 164 mg/L which indicates that at this stage the phenol is being removed by biodegradation only. A 37% higher breakthrough point in case of BAC in comparison to GAC, suggests that BAC packed towers can be used more effectively for longer time. Table 3 shows that efficiency of the reactor with BAC is more than that with GAC by 11%. Due to its higher cost and complicated regeneration process, use of activated carbon in treatment of wastewater is not realistic. The present experimental results show that BAC packed towers can be regenerated very easily up to 63% in 12 h, which indicates its usefulness in the treatment of phenolic wastewater.

## Conclusions

It can be concluded that higher percentage removal of phenol has been possible in case of SAB (97.2%) with respect to adsorption (82.3%). It is also evident that for the adsorption of phenol the correlation coefficient values for Langmuir isotherms are greater than those of Freundlich isotherms and $Q$ values are also greater than the corresponding Kf values. This indicates that Langmuir isotherm is better fit for the description of adsorption process than the Freundlich .Haldane's growth kinetic model verifies the practically optimized parameters for the removal of the phenol. The breakthrough curves between effluent concentration and time for GAC, BAC and BAC (Regenerated) beds, show that BAC packed towers can be regenerated very easily up to 63% in 12 h, thus increasing the service life of GAC in the treatment of phenolic wastewater.

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