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Biodegradation of phenolic compounds using immobilized *Pseudomonas aeruginosa* on granular activated carbon*:* Effect of immobilization, kinetic study and microbial regeneration

Deepak M Jain* & Vasdev Singh

Department of Chemical Engineering, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Baroda 390 001, India

Current address: C-101, Jai Gurudeo Complex, Sector-17, Kamothe, Navi Mumabi 410 209, India

E-mail: deepak_mjain@yahoo.com

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Phenol and its derivatives are one of the major pollutants present in industrial effluents. In this work, *Pseudomonas aeruginosa* (ATCC 9027) has been effectively used to carry out biodegradation of phenol, 3-aminophenol, and catechol. In order to develop an industrially viable technology, *P. aeruginosa* has been immobilized on granular activated carbon (GAC). The biofilm thickness was found in the range 14 to 34 μm. The adsorbed *P. aeruginosa* on the GAC is found to be efficient for bio-degradation of the phenolic compounds and in addition they also helped in regeneration of the activated carbon systems which in turn reduce the operability cost. The extent of bio-regeneration varied between 33-66%. Haldane's growth kinetic equation is found to be suitable to fit the specific growth rate data of *P. aeruginosa.*

Keywords: Activated carbon, Biodegradation, Bio-regeneration, Industrial effluents, Kinetic study

Phenol and its derivatives are one of the major pollutants present in industrial effluents. Contamination of drinking water by phenolic compounds leads to sour mouth, diarrhea, excretion of dark urine and impaired vision. The toxic levels usually range between the concentrations of 10-24 mg/L for human and the toxicity level for fish between 9-25 mg/L. Lethal blood concentration of phenol is around 15 mg/L. US EPA has set a limit of 0.1mg/L of phenol in wastewater, while that in drinking water is 0.002 mg/L. Although efficient & economic removal of high concentrations (i.e upto 1000 mg/L) of phenolic compounds from effluent streams are well documented using hydrodynamic cavitation[1,2], the subsequent treatment to reduce their concentrations below 500 mg/L is not a very viable approach industrially[3]. On the other hand one of the common technique for removal of such concentrations of phenolic compounds from waste stream is adsorption on carbon based compounds. The rate and extent of adsorption are influenced by the properties of the adsorbate, adsorbent, and the system parameters such as concentration, temperature and *p*H of the phenolic solution. Nature of functional groups and surface area per unit weight are important parameters for adsorbent selection. The high surface area in the range 200-1500 m^2/g of activated carbon makes an adsorbent of practical utility. The costs associated with the use of activated carbon are prohibitive unless the carbon can be reused[4].

Usage of only activated carbon (both in batch and column operation) involves a large capital investment and high operating costs, mainly due to the need for regeneration. Various regeneration methodologies are reported in the literature[5-14]. These are mostly thermal, chemical and electrochemical regeneration processes. However, in most of the regeneration processes degradation of the adsorbed contaminants does not take place as well as there is a carbon loss by 5–10% due to oxidation and attrition. Biological regeneration of activated carbon is one of the promising technique. Bioregeneration is a process of metabolism of adsorbed organic compounds with microorganisms attached to the surface of carbon[15, 16]. The simultaneous adsorption and biological degradation can help to develop low cost technology for wastewater treatment[17-22].

Microbes can easily form a biofilm on the moist surface through adsorption and can degrade the phenolic compounds which pose a threat to the

environment. Formation of biofilm on activated carbon is one of the excellent examples of whole cell immobilization. The bacteria attach to the surface by this matrix and form a mass of tangled fibers, which is called as glyco-calyx. The most accepted view for attachment of microbes to the surface is that the production of a polysaccharide binding material is necessary[23]. Moreover, the bioregeneration of granular activated carbon (GAC) is widely studied[24-31] in the past few years.

The present work focus on biodegradation of phenolic compounds and microbial regeneration of granular activated carbon using *Pseudomonas aeruginosa* (ATCC 9027). Phenol, catechol and 3-amino phenol were taken up as model contaminant. The work was carried out in a systematic way as follows: (i) adsorption of phenolic compounds on activated carbon and fitting of adsorption isotherm models (ii) biodegradation of phenolic compounds using *P. aeruginosa* and fitting of kinetic model of specific growth rate (iii) Immobilization of *P. aeruginosa* on granular activated carbon (GAC) and bio-degradation of the phenolic compounds

Experimental Section

Chemicals

The granular activated carbon (IC 25) was obtained from Gujarat Industrial Carbon Ltd., Ankleshwar, was used. Phenol, catechol and 3- aminophenol were of AR grade and supplied by Sisco Research Lab, Mumbai and Qualigens Ltd., Mumbai, India. Stock solutions of all the phenols of 5000 ppm (0.5 g in 100 mL of distilled water) concentration were prepared and stored in amber glass bottles. Bushnell Hass Broth (magnesium sulfate 0.2 g/L, calcium chloride 0.02 g/L, mono potassium hydrogen phosphate 1.0 g/L, di potassium hydrogen phosphate 1.0 g/L, ammonium nitrate 1.0 g/L, and ferric chloride 0.05 g/1) of HiMedia Lab. Ltd, Mumbai, India was used. The *p*H of the medium was not adjusted as it was found to be 7.1. Sterilization was done in autoclave at steam pressure of 1.05 kgf/cm² for 15 min. A pure culture of *Pseudomonas aeruginosa* (ATCC 9027), procured from Food and Drug Administration (FDA) laboratory, Baroda, India was used as the microorganism for bioregeneration experiments.

Storage of culture, stock culture and inoculum preparation

Since the history of the culture was not known, it was not used directly in degradation studies. Therefore these cultures were transferred to Antibiotic assay medium No.1 (Seed Agar) of HiMedia Lab. Pvt. Ltd., Mumbai, India. The cultures were grown on solid seed agar petridishes and slants. Composition of storage medium is given in the Table 1. Spread plate and pour plate techniques were used for growing the colonies of *Pseudomonas aeruginosa* on petri dishes and some of the colonies were transferred to slants for proper growth. Later on, the test tubes and petri dishes were transferred to incubator, which was maintained at 30ºC temperature. After 24 h, colonial growth on slants and plates were observed. A loop-full of the culture obtained in the previous step, was spread on a newly prepared slant. This slant was kept in incubator at 30ºC for 24 h. After the growth was observed, this slant was transferred to the refrigerator.

Inoculum for use in experiments was developed by pouring 0.1% saline water into test tube of sub culture slant. This solution was inserted into minimal medium amended with 0.5% dextrose in Erlenmeyer flasks of 250 mL, with a working volume of 100 mL. After appreciable growth appeared, a small portion of broth of medium was used as inoculum for 25 ppm initial concentration of phenols. 5 ml broth of this solution obtained after complete degradation of 25 ppm concentration of phenolic solution was used as inoculum for the solution of 50 ppm initial concentration. Similarly for higher concentration solution of phenols inoculums were taken.

Phenol estimation and bacterial culture growth measurement

4-Amino antipyrine colorimetric method (5530 D APHA/ AWWA) has been used for estimation using visible spectrophotometer (Systronic make VISIMAX 167). Samples (5 mL) were withdrawn at regular time intervals and were centrifuged at around 8000 rpm for 15 to 20 min at room temperature. The supernatant layer was used to measure phenol concentration. The pellet was re-suspended in 5 mL of distilled water and the growth was determined by measuring the absorbance of bacterial culture at 660 nm wavelength against distilled water as reference using, Systronic make visible spectrophotometer (Model Visimax-

Table 1 — Medium for storage (final *p*H at 25 ºC was 6.6± 0.2)

Nutrient	Concentration g/L
Peptic digest of animal tissue	6.0
Casein enzymatic hydrolysate	4.0
Beef Extract	1.5
Yeast Extract	3.0
Dextrose	1.0
Agar	15.0

Visiscan-167). Pour plate technique with serial dilution for viable count of cell mass was used sometimes to check the growth and purity of bacterial strain.

Adsorbent and it characterization

The activated carbon used in this study was characterized for BET-surface Area, porosity and pore-size distribution. Sample was degassed at 150°C for about 1 hour to remove moisture and unwanted adsorbed species on the carbon surface. Nitrogen was introduced at pressure slightly below atmospheric pressure and at boiling point, i.e. 77.2 K. Amount of nitrogen adsorbed at equilibrium was measured for different nitrogen pressures. Thus, with the knowledge of amount of Nitrogen at monolayer adsorption, surface area can be determined. BET surface area analyzer of Micormeritices was used in this study. The pore size distribution analysis shows that this activated carbon has pore structure as follows: 18% with diameter < 0.01 μm, 41 % with diameter between 0.01 and 5 μm and 41 % with diameter > 5 μm. Mercury porosity meter was used for determination of porosity and pore-size distribution at SICART, V.V. Nagar. Pressure required for intrusion of mercury is inversely proportional to the diameter. Total volume of mercury introduced gives the apparent density and thus porosity of the material. Size analysis has been done using Laser particle size analyzer also at SICART.

Adsorption study

The equilibrium studies were conducted at 30±1°C for 48 h. The working solution (200 mL) at 800 mg/L concentration was obtained by adding the required amount of stock solution to the BSM. The pre-weighed quantities of activated carbon were contacted in a 250 mL stoppered conical flask with 200 mL of working solution for 48 h. The adsorption isotherm was compared with the existing adsorption models and it was found to follow the Freundlich adsorption isotherm as seen in Fig. 1. The data was fitted with a liner isotherm model to obtain the pseudo second order kinetics (Fig. 2) and subsequently determine the optimum dosage of activated carbon required. The optimum dosage of this activated carbon with different initial concentration of phenols was used in further experiments. In trial run, 48 h were found sufficient to attain equilibrium. The quantity of organic matter adsorbed was determined by weighing the filtered activated carbon from time to time and the percentage removal was plotted against time. The data for one set of activated carbon dosage and initial organic concentration was shown in Fig. 3. In some of the trial runs having higher dosage of activated carbon, bacterial growth was observed that affected the equilibrium concentrations. After 24 h, due to low concentration of the phenolic compounds in the solution with BSM, a favorable environment for bacterial growth was created. Therefore, it was decided to add $HgCl_2$ of 6 mg/L as bactericidal agent so that it will not affect the equilibrium of the phenolic compound. It was also tested that the presence of this agent does not interfere with any analytical procedure. After 48 h the flasks were taken out of the shaker for analysis.

Bio-degradation of phenolic compounds using Immobilized P. aeruginosa

The adsorption experiments of phenol, 3-aminophenol and catechol by activated carbon were carried out in cotton plugged conical flask of 250 mL. Each flask contained 100 mL of the final solution.

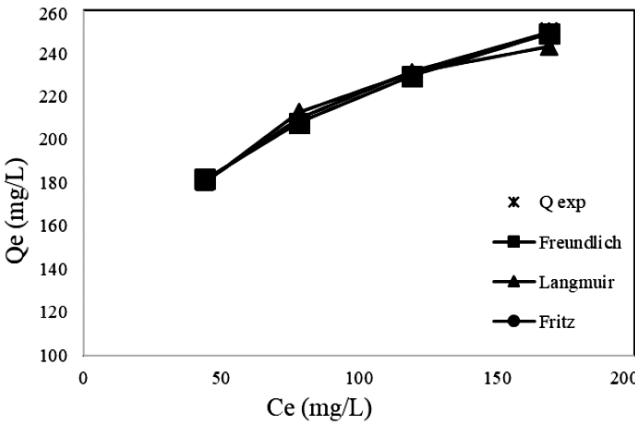

Fig. 1 — Comparison of different isotherm models for phenol adsorption on activated carbon with experimental results.

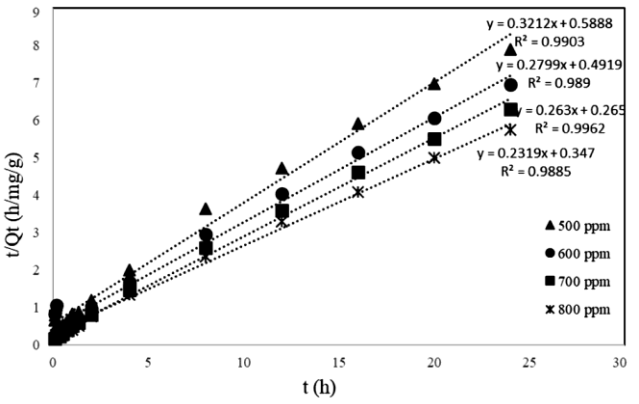

Fig. 2 — Plot to determine pseudo second order kinetics for adsorption of Phenol on Activated carbon.

Solutions were prepared by pouring the calculated volumes of Bushnell Hass Medium (BSM) solutions into the flask and subsequently, the required amount of the stock solutions of phenol, 3-aminophenol and catechol were added so as to get the solution of desired concentration. Broth obtained after complete biodegradation of 800 mg/L of phenol present in solution, was used for immobilization of bacteria on activated carbon. 50 mL of broth with 50 ml of fresh BSM solution was contacted with 0.25 g of oven dried granular activated carbon and turbidity of medium was observed at different levels. To monitor the immobilization process, visual observation has been made for turbidity of sample. After the turbidity of liquid medium stopped changing, it was decanted. The activated carbon was washed again with the fresh BSM solution, and then BSM solution with desired concentration of phenol was poured into 250 mL flask containing washed activated carbon. In case of 3-aminophenol and catechol, broth of completely biodegraded solution having initial concentration of 500 and 600 mg/L respectively was taken. For each concentration, two or more flasks were used. All the experiments were performed at 30ºC in orbital incubator cum shaker of Scigenics Make, Saksham Technologies, Mumbai, India.

The biological growth on the bioactive activated carbon increases the service life of activated carbon

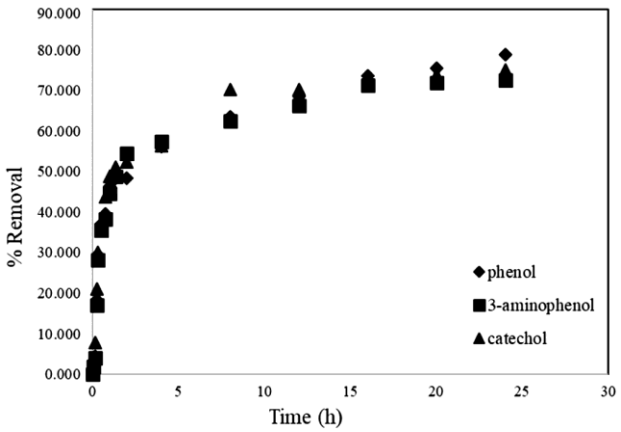

Fig. 3 — Effect of activated carbon dose on percentage removal of phenols [Temp = 30 ±1ºC, *p*H= 7.0, Initial phenol conc = 800 ppm, contact time = 48 h]

by degradation of phenolic compound in the liquid medium as well as those present on the activated carbon. Thus, active sites are regenerated at the activated carbon surface. This effect was investigated by conducting the experiments with BAC using initial concentrations of 800, 500 and 600 mg/L for aqueous solution of phenol, 3-aminophenol and catechol respectively. The BAC obtained at the end of these experiments was washed with distilled water and again contacted with fresh liquid medium containing phenol, 3-aminophenol and catechol of 800, 500 and 600 mg/L concentration respectively for 48 h. From material balance, the quantity adsorbed was calculated. Also, the liquid medium at same concentration was contacted with virgin activated carbon for 48 h, and the quantity of the phenolic compound adsorbed was calculated. The difference between the two quantities gives the extent of bio-regeneration. Table 2 presents the extent of bio-regeneration of the carbon saturated with phenol, 3-aminophenol and catechol. These experiments have been performed for higher concentrations also viz. for phenol 1000 and 1200 mg/L, for catechol 700 and 800 mg/L and for 3-aminophenol 600 and 700 mg/L to check bio regeneration at these concentrations.

Characterization of immobilized P. aeruginosa on GAC

Biofilm coated granular activated carbon particles from each run were examined using a JEOL scanning electron microscope to observe the morphology and to determine the thickness of the biofilm on them. Biofilm coated GAC particles obtained at the end of a run carried out with initial phenol concentration of 800 mg/L, 3-aminophenol concentration of 500 mg/L and initial catechol concentration of 600 mg/L were taken for SEM after drying at 50˚C for 15 min. SEM was also performed on the virgin activated carbon. A magnification was used which ensured that the immobilized bacteria and the macropore structure of the adsorbents could be analyzed in detail.

Kinetic study

Substrate utilization and bacterial growth kinetics studies were carried out in Erlenmeyer flasks. Initial concentrations were varied from 25 to 800 mg /L in

Table 2 — Thickness of biofilm and percentage bio-regeneration on bioactive activated carbon particles exposed to different initial concentrations

	Phenol			Catechol			3-Aminophenol		
	800 ppm	1000 ppm	1200 ppm	600 ppm	700 ppm	800 ppm	500 ppm	600 ppm	700 ppm
Biofilm thickness, (μm)	30	34	31	20	22	23	14	16	22
% Regeneration	58.1	48.8	40.8	62.9	47.5	40.6	66.4	37.8	33.1

case of phenol, 25 to 600 mg/L for catechol and 25 to 500 mg/L for 3-aminophenol. All the experiments have been performed in orbital incubator cum shaker of Scigenics make, Saksham Technologies, Mumbai at 30ºC. Haldane's model has mostly been utilized in describing the growth kinetics of microorganisms on phenolic compounds[29,30]. The Haldane's model equation (1930) is described by the (Eq.1).

$$\mu = \frac{\mu_{max} S}{K_s + S + \frac{S^2}{K_i}} \quad \ldots (1)$$

Where S is the substrate concentration, K_i is the value of inhibitory coefficient, μ_{max} (maximum achievable specific growth rate), K_s (half saturation coefficient) and $K_{i,}$ (inhibitory coefficient). The value of inhibitory coefficient, K_i reflects the degree of inhibition caused by a substrate. The higher the value, the less will be the degree of severity of inhibition. In the limiting cases, when the value of K_i reaches infinity, the Eq. (1) becomes the well-known growth kinetic equation of Monod for non-inhibitory type of substrates.

Results and Discussion

Acclimatization of Culture

Phenols being toxic compounds, degrading bacteria are required to be adapted to the phenolic environment. This is very important in cases where these bacteria are to be used to degrade aqueous solutions of intermediate to high concentrations of phenols[31]. In the present work, the phenolic compounds at low to intermediate concentration (500-800 mg/L) were used. Therefore, the acclimatization procedure was initiated for the phenolic compounds. Firstly, 0.1 % saline water containing bacteria from subculture slant was transferred to conical flask having BSM solution. To avoid problems due to contamination and non-growth, the bacteria was transferred to twelve flasks, four for each compound. After 72 h, significant bacterial growth was observed; the synthetic medium turned turbid. The stock solutions of phenol, catechol and 3-aminophenol were added to the flasks so as to give 100 mg/L concentration of the phenolic compounds in the BSM solution. When catechol was added to the BSM solution, its color changed from light pink to brownish to characteristic blackish. The samples were taken from these broths to determine degradation and growth rate.

In case of 3-aminophenol, the degradation did not start even in 30 days and solution turned black in 5 days indicating the declining phase of the culture. Probably it indicates that 3-aminophenol could not be degraded beyond the initial concentration of 500 mg/L. However, for phenol and catechol, the degradation was possible up to 800 mg/L and 600 mg/l concentrations respectively. In case of phenol, well acclimatized culture exposed to 1000 mg/L could not even start degradation in 23 days. It was also observed that culture started dying above 600 mg/L of catechol concentration. It merits mentioning that preliminary study with other target compounds like 4-aminophenol and resorcinol was also tried (data mot reported). Surprisingly, *P. Aeruginosa* could acclimatize up to 1.2% (i.e. 12000 mg/L) of 4- aminophenol in 33 days, and strain could not grow on resorcinol even at 50 mg/L. Different behavior and potential of the strain *P. Aeruginosa* indicated during adaptation reveals different degradative pathways for different phenolic compounds. This agrees well with the findings of Kumar (2004) using strain *Pseudomonas putida* MTCC 1194 and also that of Ehrhardt and Rehm (1985) with pure cultures of *Cryptococcus elinovii* H1 and *Pseudomonas putida* P8[34, 35].

Bioregeneration

The removal of phenol, 3-aminophenol and catechol were studied in batch mode. Fig. 4, show the concentration profile of phenol, catechol and 3-aminophenol respectively in course of time. All the plots indicate that the removal is initially faster followed by a slow phase. The initial faster rate removal may be due to the adsorption and then the slow removal is accomplished through degradation. This may be substantiated by the fact that there was no or little biomass growth during initial periods of removal and after that a phase of consistent biological growth was observed. The phenomenon of initial faster removal by adsorption followed by biological reduction may be regarded as characteristic of such systems. The similar trend was observed in batch as well as continuous reactors[35, 36].

As illustrated in Fig. 5, when phenol was removed by biodegradation alone, the well- acclimatized bacterial strain of *P. aeruginosa* (ATCC 9027) could metabolize phenol with initial concentration of 800 mg/L in 136 h with a lag phase of 3 days but with initial concentration of 1000 mg/L, it could not be metabolized even up to 23 days. Similarly, 3-

aminophenol and catechol were metabolized at an initial concentration of 500 mg /L in 160 h with lag phase of 2.5 days and at 600 mg/L in 85 h with 2 days lag phase respectively. They were not metabolized at

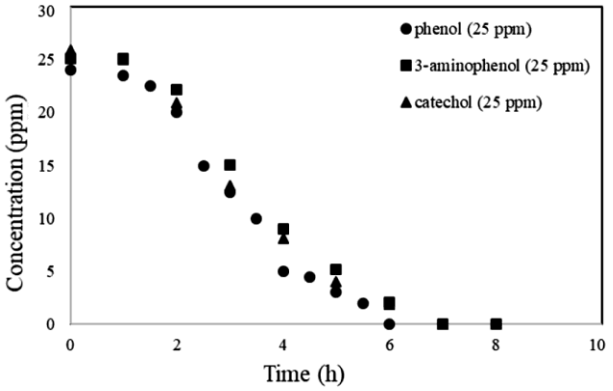

Fig. 4 — Degradation studies of phenol, 3-aminophenol and catechol by *P.aeruginosa* in batch reactor [Temp. = 30ºC, *p*H=7] at initial concentrations of 25 ppm.

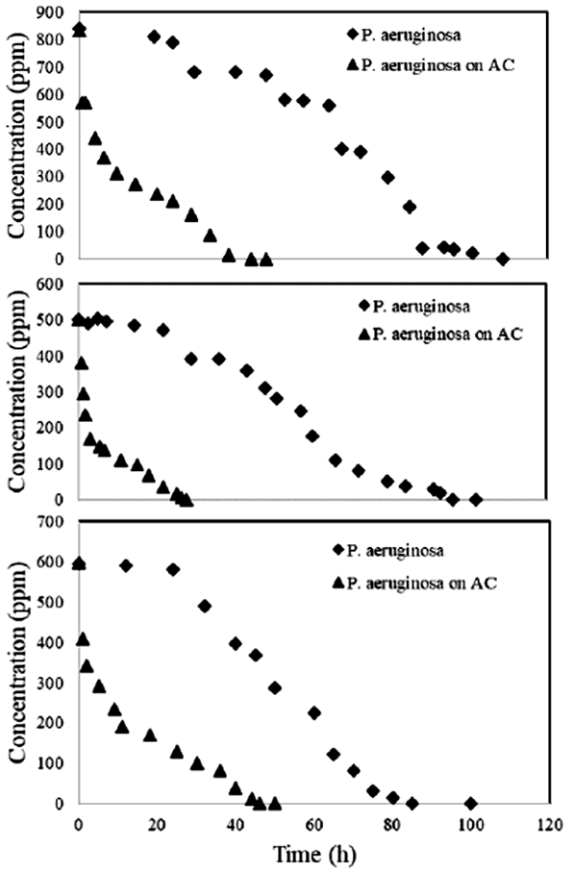

Fig. 5 — Comparative study of degradation of phenolic compounds by *P. aeruginosa* and by a coupled system of *P. aeruginosa* and activated carbon (AC) in batch reactor [Temp.= 30ºC, *p*H=7]: (a) Phenol (IC = 800 ppm); (b) 3- aminophenol (IC = 500 ppm) and (c) Catechol (IC = 600 ppm).

all even with a well acclimatized culture of *P. aeruginosa* (ATCC 9027) with initial concentration of 600 and 700 mg/L of 3-aminophenol and catechol respectively. Therefore, the removal behavior of phenol at initial concentration of 1000 and 1200 mg/L, of 3-aminophenol at 600 and 700 mg/L and of catechol at 700 and 800 mg/L were studied on bioactive activated carbon. For all compounds with the initial concentrations, stated earlier, removal was complete in bioactive activated carbon system. Also, no lag phase was observed. This suggests that the reactor using bioactive activated carbon can be operational with no start-up time. Moreover, the functioning of such reactors would not be disturbed in case of shock loads (at least up to additional 50% of design load). Initially, adsorption of phenols might have taken place and thereafter due to lowering of concentration of phenol / 3-aminophenol/catechol in liquid phase, a negative concentration gradient was established and phenol / 3-aminophenol / catechol desorbed back into the liquid phase. Presumably, the phenol was degraded either by the actively growing biofilm on activated carbon or by the bacteria suspended in liquid phase. Therefore, it may be concluded that creation of active sites took place as a result of desorption of the adsorbed phenol, 3-aminophenol or catechol.

As discussed earlier the biological growth on the bioactive activated carbon increases the service life of activated carbon by degradation of phenolic compound in the liquid medium as well as those present on the activated carbon. Thus, active sites are regenerated at the activated carbon surface. This effect was investigated by conducting the experiments with BAC using initial concentrations of 800, 500 and 600 mg/L for aqueous solution of phenol, 3-aminophenol and catechol respectively which can be seen in supplementary information. Table 2 indicates that when the concentration of the sorbed compounds in spent activated carbon is low, it gives slightly better regeneration than that of higher concentration. The percentage regeneration was in the range of 33-66% in 48 h contact time, which is not an appreciable result. Plausibly, the regeneration was not complete due to insufficient contact time.

According to Hutchinson and Robinson[31] the growth threshold concentration in the fermentation broth during regeneration prevents complete desorption of the adsorbate. Considerable amounts of phenol remain adsorbed even when the liquid phase concentration of phenol contacting the adsorber is

reduced to the growth threshold level. Intraparticle mass transfer resistance might have posed another problem to the complete bioregeneration of spent carbon. The rate of desorption of solute from the activated carbon decays exponentially as the regeneration proceeds. As the adsorbed phase concentrations decline, local driving force for intraparticle mass transfer decreases and intraparticle mass transfer rates become very low. The slow desorption rates in the later stages of regeneration prevent complete recovery of adsorptive capacity. Extremely long regeneration times would be required to approach the performance of virgin activated carbon, even if the phenol could be completely degraded in the bioreactor[15]. The use of smaller adsorbent particles could reduce the effect of intraparticle mass transfer, but this may again increase the hydrodynamic pressure drop across the packed bed. The use of an alternative organism or a mixed culture could reduce or eliminate the threshold concentration effect present in the bioreactor during regeneration. Therefore, it is obvious that effluent levels lower than the growth threshold concentration may not be achievable using bioregenerated activated carbon due to the residual adsorbate remaining following bioregeneration. The chances of microbial fouling of the pore openings and/or adsorption of microbially produced compounds cannot be ruled out[15].

Effect of Initial Concentration

Batch experiments were conducted to examine the effect of initial concentration on the degradation behavior of phenol, 3-aminophenol and catechol using bacterial strain *P. aeruginosa* at 30°C As stated earlier the initial concentration of phenol, 3-aminophenol and catechol were varied from 25 to 800 mg/L, 25 to 500 mg/L and 25 to 600 mg/L respectively. In all these experiments, the concentration of substrate and biomass in liquid medium were recorded till the substrate initially present was fully consumed. The degradation profiles of phenol, 3-aminophenol and catechol at their highest initial concentration (Phenol: 800mg/L; 3-amino phenol: 500 mg/L and catechol: 600 mg/L) are plotted in Fig. 5. In all these experiments, the concentration of substrate and biomass in liquid medium were recorded till the substrates initially present were fully consumed. The results of batch experiments showed that the initial concentration of phenol, 3-aminophenol and catechol concentration of 800, 500 and 600 mg/L respectively could be fully degraded in 136, 160 and 85 h. As expected it is clear from figures that higher the concentration of the phenol, more the time it takes to be fully consumed. Although, well-acclimatized inoculum was used in these experiments, the lag phase was observed. The lag phase of as high as one week have been reported to occur during degradation of phenol at initial concentration of 700 mg/L using well-acclimatized *P. putida* in literature.

The influence of phenols concentration on the duration of the lag phase is shown in Fig. 6 for phenol, catechol and 3-aminophenol respectively. It is observed that the length of lag phase increases exponentially with initial concentration of phenols. The exponential lag phase behavior has also been reported in literature by Monteiro[37] for phenol removal[37]. However, Vijayagopal[38] has reported linear lag phase behavior for degradation of phenol. It was observed from Fig.6, that at higher concentrations the rate of substrate removal becomes relatively less near the end of the substrate degradation curves. There are two possible reasons for this. The deficiency in the availability of oxygen as these experiments were done in cotton plugged conical flasks of 250 mL with 100 mL working volume. In this case there were two sources of oxygen: first, the air in headspace and second, the oxygen dissolved in the liquid medium. Furthermore, this may be due to the fall in *p*H of the solution. Lallai and Mura[39] have reported the similar observation for phenol metabolization by mixed culture composed of *Pseudomonadaceae*, *Vibrionaceae* etc. Therefore, low concentration of oxygen and low *p*H affect the kinetics of substrate consumption. In microbial growth curve after the lag

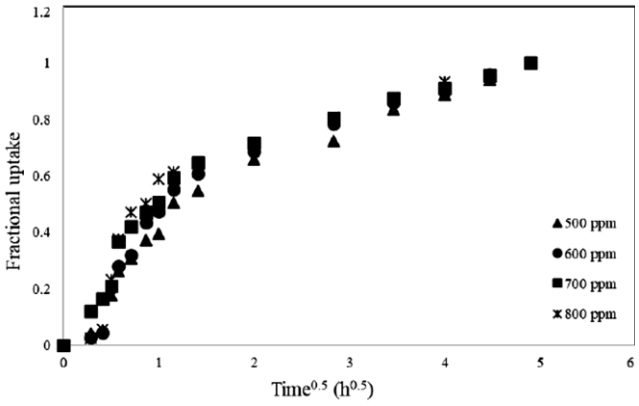

Fig. 6 — Typical Fractional up take curve (Weber-Morris plot) for phenol adsorption on activated carbon using different initial concentration.

phase as well as exponential phase, drop in microbial growth rate may be due to lower oxygen concentration in solution[32].

Kinetics of degradation

The analysis of the batch growth data for microorganisms metabolizing inhibitory carbon sources like phenols requires careful plotting of the semi logarithmic plot of biomass concentration versus time in order to determine the actual specific growth rate exhibited by the microorganisms for a particular initial substrate concentration. Such plots have earlier been used for the determination of specific growth rate for different initial concentration of phenols[32-33,40]. The specific growth rate μ was calculated from the slope of the ln (optical density) versus time plots for all concentrations (data not shown). The experimental values of specific growth rate μ derived from initial concentration have been plotted against initial constant concentration of phenols to determine kinetic constants using different kinetic models. The parameters of kinetic model of Haldane were determined by fitting the model to the experimental data using non-linear regression technique. However, there are almost infinite numbers of numerical combinations of the constants, which can provide equally adequate fits to the data, since all the parameters are obtained from the single curve fitting operation. Consequently, a unique set of values cannot be produced unless an independent evaluation of one of the parameters is obtained or unless numerical limits are placed on the input estimates and computed outputs from the parameter generated in the curve fitting algorithm. Range for the numerical limits can be discerned by utilizing both practical engineering insight and observation from previous work with non-inhibitory substrates. Hence, Biomass growth on phenol has been modeled both by inhibitory and non-inhibitory type of substrate kinetics. In the present study, specific growth rate data from low concentration region were fitted to Monod's model and those from high concentration region were fitted to Linearized Haldane's model as shown in Fig. 7. Table 2 and Table 3 present the parameter values obtained using these models respectively. The data of full concentration range cannot be correlated by either of the models in the case of phenol and catechol. However for 3-aminophenol, linearized Haldane model could represent the complete range of concentration. The values obtained by these expressions have been used as a base for the values of parameters for inhibitory kinetic models. Based on these parameters, the inhibitory model of Haldane was fitted to data using SPSS version 8.0 for Windows. In this procedure of data fitting, two criteria namely maximum percentage deviation and correlation coefficient (R^2) were met to get parameter values. Figure. 8, shows comparison of experimentally obtained specific growth rate, μ with those predicted by the model. This is evident from the figures that the growth kinetics of phenol, 3-aminophenol and catechol could be represented by Haldane's growth kinetic model very well. In the case of all the phenols, the maximum deviation was less than 10% and the coefficient of regression, R^2 in all the three cases was found to be more than 0.99.

The calculated values of the parameters were compared with the values available in literature. The maximum specific growth rates and the half saturation coefficients are within the range for phenol / mixed culture system [0.131 to 0.363 $h^{-1}$and 5 to 226 mg/L[41]]. The substrate inhibition coefficients are also

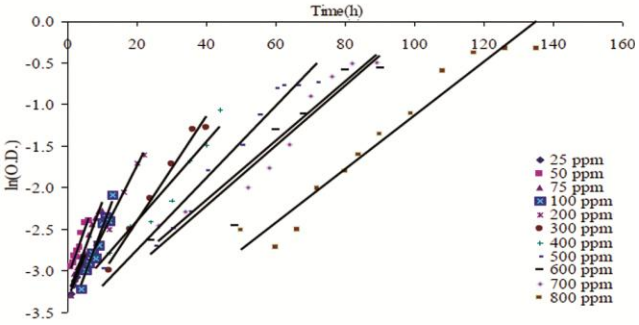

Fig. 7 — Determination of specific growth rate in the region of exponential phase of batch growth curve [Phenol/ *P. aeruginosa* system].

Table 3 — Haldane's kinetic parameter values for biodegradation of phenol, 3-aminophenol and catechol using *P. aeruginosa*

Compound	Haldane's model				
	μ_{max} (h^{-1})	K_s (mg/l)	K_i (mg/l)	Maximum % deviation	R^2
Phenol	0.221	20.89	124.61	6.21	0.996
3-Aminophenol	0.228	8.736	58.383	4.03	0.999
Catechol	0.287	17.82	128.95	10.25	0.991

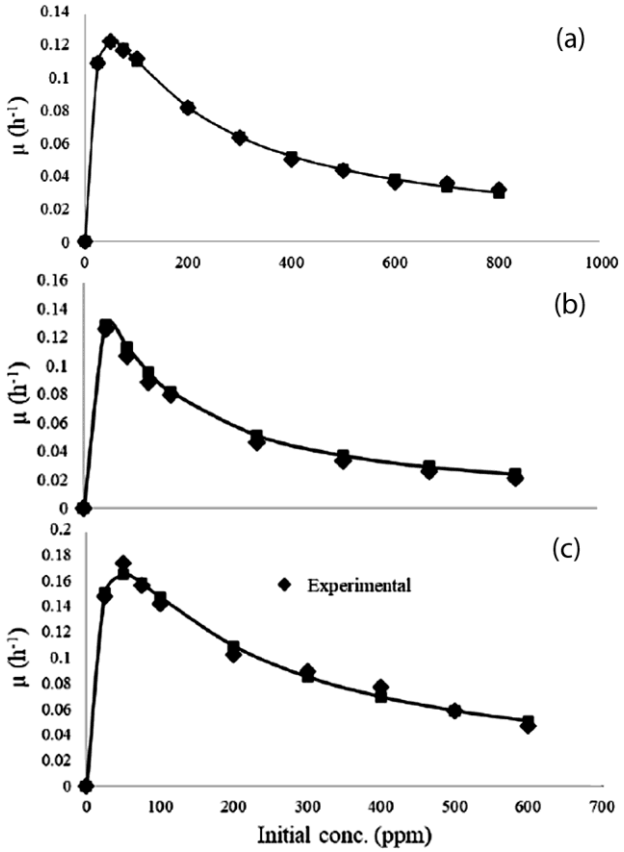

Fig. 8 — Haldane's kinetic model fitted to batch growth data to evaluate $\mu_{max,}$ K_s and K_i : (a) Phenol/ *P.aeruginosa* system, Conc. Range 0-800 ppm; (b) 3-aminophenol/ *P. aeruginosa* system, Conc. Range 0-500 ppm and (c) Catechol/ *P. aeruginosa* system, Conc. Range 0-600 ppm.

within the range when compared with different systems [K_i = 54.1 mg/L, K_i = 129.79[31]]. Nonetheless the calculated parameters are well within the range; these values ($\mu_{max,}$ $K_{s,}$ and K_i) in combination do not match exactly with other systems. Kotturi (1991) noted that the half saturation coefficient is influential on the growth kinetics in the low concentration region and at the same time the small changes in the biomass and substrate concentrations cannot be measured accurately in batch reactors[30].

Conclusion

The removal of phenol and its derivatives, namely catechol and 3-aminophenol, have been studied in bioactive activated carbon (BAC) systems in presence of *Pseudomonas aeruginos* ATCC 9027. The biofilm and GAC integration complement each other for the removal of impurities in aqueous solution. Scanning electron micrograph of activated carbon particles coated with biomass reveals the dense growth of bacterial mass in pits and crevices, and scattered bacteria over the smooth surface of activated carbon. Haldane's growth kinetic equation could fit the specific growth rate data of *P.aeruginosa* on all three phenols quite well over the concentration range studied. Statistical analyses indicate that the maximum deviation and the correlation coefficient, R^2 for the fit of this model to the data of all the three phenols are less than 10% and more than 0.99 respectively. The values of parameters of Haldane's model were found within the range given in the literature.

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References

1 Yuequn Tao, Jun Cai1, Bin Liu, Xiulan Huai & Zhixiong Guo, *Chem Eng Technol*, 39 8 (2016) 1363.
2 Gogate P, *Ultrason Sonochem*, 15 (2008) 1.
3 Pradhan A & Gogate P, *Chem Eng J*, 156 (2010) 77.
4 Tan I A W, Ahmad A L & Hammed B H, *J Hazard Mater*, 164 (2009) 473.
5 Zanella O, Bilibio D, Priamo W L, Tessaro I C & Féris L A, *Environ Technol*, 38 (2017) 549.
6 Mu'azu N D, Jarrah N & Zubair M, Alagha O, *Int J Env Res Pub He*, 14 (2017) 1094.
7 Duan F, Li Y, Cao H, Wang Y, Crittenden J C & Zhang Y, *Chemosphere*, 125 (2015) 205.
8 Cho J H, Kim Y S, Jeon S B, Seo J B, Jung J H & Oh K J, Korean *J Chem Eng*, 31 (2014) 1641.
9 Sun H, Liu Z, Wang Y & Li Y, *J Environ Sci China*, 25 (2013) S77.
10 Berenguer R, Marco-Lozar J P, Quijada C, Cazorla-Amorós D & Morallón E, *Energy Fuels*, 24 (2010) 3366.
11 Mourão P A M, Carrott P J M, Carrott M M L R & Marques L, *Mater Sci Forum*, 587 (2008) 844.
12 Cheng M Y, Yang S C & Hsieh C T, *Sep Sci Technol*, 42 (2007) 639.
13 Sabio E, González E, González J F, González-García C M, Ramiro A & Gañan J, *Carbon* 42 (2004) 2285.
14 Zhang H, Ye L & Zhong H, *J Chem Technol Biotechnol*, 77 (2002) 1246.
15 Aktas O & Cecen F, *Int Biodeter Biodegr*, 59 (2007) 257.
16 Nath K & Bhakhar M S, *Environ Sci Pollut*, 18 (2011) 534.

17 Islam M S, Zhang Y, McPhedran K N, Liu Y & Gamal El-Din M, *J Environ Manag*, 152 (2015) 49.
18 Dash R R, Balomajumder C & Kumar A, *J Hazard Mater*, 152 (2008) 387.
19 Mondal P & Balomajumder C, *Int J Chem React Eng*, 5 (2007) 1.
20 Lee K M & Lim P E, *Chemosphere*, 58 (2005) 407.
21 Lim P E, Ong S A & Seng C E, *Water Res*, 36 (2002) 667.
22 Sirotkin A S, Koshkina L Y & Ippolitov K G, *Water Res*, 35 (2001) 3265.
23 Marquez M C & Costa C, *Water Res*, 30 (1996) 2079.
24 Leong K Y, Adnan R, Lim P E, Ng S L & Seng C E, *Environ Sci Pollut*, 24 (2017) 20959.
25 Oh W D, Lim P E, Seng C E, Mohamed N, Adnan R, Leong K Y & Voon S Y, *J Chem Technol Biotechnol*, 88 (2013) 1157.
26 Toh R H, Lim P E, Seng C E & Adnan R, *Bioresour Technol*, 143 (2013) 265.
27 Li Y & Wang C, *World J Microbiol Biotechnol*, 24 (2008) 1843.
28 Putz A R H, Losh D & Speitel Jr G E, *J Environ Eng*, 131 (2005) 196.
29 Nakano Y, Hua L Q, Nishijima W, Shoto E & Okada M, *Water Res*, 34 (2000) 4139.
30 Sa F A C de & Malina Jr J F, *Water Sci Technol*, 26 (1992) 2293.
31 Hutchinson D H & Robinson C W, *Water Res*, 24 (1990) 1217.
32 Yang, R D & Humphrey A E, *Biotechnol Bioeng*, 17 (1975) 1211.
33 Kotturi G, Robinson C W & Inniss W E, *Appl Microbiol Biotechnol*, 34 (1991) 539.
34 Kumar A, Kumar S & Kumar S, *Biochem Eng J*, 22 (2004) 151.
35 Ehrhardt H M & Rehm H J, *Appl Microbiol Biotechnol*, 21 (1985) 32.
36 Lin W & Weber A S, *Environ Prog*, 11 (1992) 145.
37 Monteiro A M G, Boaventura A R & Rodrigues A E, *Biochem Eng J*, 6 (2000) 45.
38 Vijayagopal V & Viruthagiri T, *Pollut Res*, 24 (2005) 157.
39 Lallai A & Mura Giampasio, *Water Res*, 23 (1989) 1335.
40 Livingston A G & Chase H A, *AIChE J*, 35 (1989) 1980.
41 D'Adamo, Rozich A F & Gaudy A F, *Biotechnol Bioeng*, 26 (1984) 397.