Bioprocess Engineering 22 (2000) 493–501 © Springer-Verlag 2000

Adsorption and bio-degradation of phenol by chitosan-immobilized *Pseudomonas putida* (NICM 2174)

G. Annadurai, S. Rajesh Babu, K. P. O. Mahesh, T. Murugesan

Abstract Biodegradation of phenol by *Pseudomonas putida* (NICM 2174), a potential biodegradent of phenol has been investigated for its degrading potential under different conditions. *Pseudomonas putida* (NICM 2174) cells immobilized in chitosan were used to degrade phenol. Adsorption of phenol by the chitosan immobilized matrix played an important role in reducing the toxicity of phenol. In the present work, results of the batch equilibrium adsorption of phenol on chitosan from its aqueous solution at different particle sizes (0.177 mm, 0.384 mm, 1.651 mm) and initial concentration of phenol (20, 40, 60, 80, 100, 120, 140, 160, 180, 200 mg/l) have been reported. The adsorption isotherms are described by Langmuir, Freundlich and Redlich-Peterson types of equations. These indicate favourable adsorption with chitosan. From the adsorption isotherms, the adsorption capacity, energy of adsorption, number of layers and the rate constants were evaluated. In batch kinetic studies the factors affecting the rate of biodegradation of phenol, were initial phenol concentration (0.100 g/l, 0.200 g/l, 0.300 g/l), temperature (30 °C, 34 °C, 38 °C) and pH (7.0, 8.0, 9.0). Biodegradation kinetic data indicated the applicability of Lagergren equation. The process followed first order rate kinetics. The biodegradation data generally fit the Lagergren equation and the intraparticle diffusion rate equation from which adsorption rate constants, diffusion rate constants and diffusion coefficients were determined. Intraparticle diffusion was found to be the rate-limiting step. Cell growth contributed significantly to phenol removal rates especially when the degradation medium was supplemented with a utilizable carbon source.

List of symbols

a	Langmuir constant related to the energy of adsorption, (mg/g)
a_R	Redlich–Peterson isotherm constant
b	Redlich–Peterson isotherm exponent
C_e	Equilibrium liquid phase solute concentration, (mg/l)
D	Pore diffusion ($cm^2\ S^{-1}$)
K	Langmuir constant related to the adsorption capacity, (mg/g)
K_{ad}	Rate constant of adsorption, min^{-1}
K_f	Adsorption capacity, (mg/g)
K_p	Intraparticle diffusion rate constant and diffusion rate, g ($min^{0.5}$)
$1/n$	Adsorption intensity
q_e	Maximum amount of phenol degradation (mg/g)
q	Amount of phenol degradation at time (g)
t	Contact time, (h)
$t^{1/2}$	Time (h) for half adsorption
r_0	Radius of adsorbent

Received: 19 May 1999

G. Annadurai (✉), S. Rajesh Babu, K. P. O. Mahesh, T. Murugesan
Department of Chemical Engineering,
Alagappa College of Technology,
Anna University, Chennai – 600 025, India

Dr. G. Annadurai (R.A) is thankful to the Council of Scientific and Industrial Research, New Delhi, for the award of fellowship.

1 Introduction

The ever increasing demand for water has caused considerable attention to be focussed towards recovery and reuse of waste waters. The chemical environment consists of four million known chemicals and a very large number of unknown chemicals [1, 2]. More than two thousand chemical contaminants have been found in waste water, about 750 of which have been identified in drinking water. Of these, more than 600 are of organic origin. Phenols are among the most common water pollutants [2]. Phenolic derivatives are low-cost and broad spectrum pesticides which are used as algicides, bactericides, fungicides, herbicides, insecticides and molluscides in a variety of industrial, agricultural and domestic fields. They are present in the effluent of a number of industrial units such as oil refineries, coke oven plants, steel plants etc. The concentration of phenols in waste waters varies from 10 mg l^{-1} to 3000 mg l^{-1}. Phenol and their compounds impart taste and odour to water and are toxic to fixture, and other aquatic life [3]. Recent literature on the methods of removal of phenol and their compounds from waste water focuses on adsorption and microbial biodegradation process. Certain species of genera *Pseudomonas* sp. under very controlled condition of pH, temperature in presence of some specific nutrients can degrade phenol. Pseudomonas strain, capable of degrading pentachlorophenol was isolated around tannery soil and characterized as *Pseudomonas aeruginosa* [4, 5]. *Cyanobacterium phormidium val derianum* [6–8] are in more advantageous position than heterotophic bacteria because of their tropic independence from nitrogen as well as carbon. Phenol, the toxic constituent of several industrial effluents, was found to be effectively degraded.

Phenol uptake suggested that traditional kinetic model based on the Haldone, Monode equations may be inaccurate for describing the dynamics of phenol degrading systems [9–16]. Flavobacterium cells immobilized in polyurethane foam were used to degrade pentachlorophenol [17].

Many xenobiotic compounds thought to be toxic for microorganisms are being degraded under anaerobic, aerobic (or) anoxic conditions. Biodegradation of these compounds are being studied in soils, ground water, traditional activated sludge units, fixed film reactors and combinations of various processes. Biodegradation of phenol by chitosan immobilized *Pseudomonos putida* (NICM 2174) is versatile, inexpensive and has the potential to turn a toxic material into harmless products. Different techniques are commonly used for the immobilization process [18].

Immobilization of cells is one of the approaches in incorporating bacterial biomass into an engineering process. The advantages of the process based on immobilized biomass include, enhancing microbial cell stability, allowing continuous process operation and avoiding the biomass-liquid separation requirement. Physical entrapment of organisms inside a polymeric matrix is one of the most widely used techniques for whole-cell immobilization [18]. Utilizing the immobilization concept, phenol, *p*-nitrophenol etc., have been removed [19–26]. Immobilized living cell film system such as the *Citrobacter* sp. have been used in enzymatic mediated metal removal process [27–29]. Most of the previous research in this area has focused on entrapment of biomass in an insoluble material. Polyacrylamide silica gels have been the most extensively used immobilization materials for laboratory research studies [30, 31]. Wastewater having inconsistent pollution load has to be treated by protecting microbes from exposure to shock load [31]. Immobilization of microbial culture in porous supports increases the possibility of the microbes to confront the stock load [32]. The dissolved organics in wastewater first get adsorbed on the surface and then gradually penetrate through the immobilizing matrix [33]. Certain matrices like sodium alginate [34], polyacrylamide hydrazide gel [35, 36], activated carbon [37, 38] stintered glass [38] have been reported in the literature for immobilization of microbes.

The screening and selection of bacterial biomass entrapment matrices for *Pseudomonas putida* (NICM 2174) proposes a challenge. The immobilization matrix should be dense enough to restrain the biomass inside while being porous enough to allow phenol ions to be freely transported through the matrix. Further covalently linked polymeric materials, are believed to be less chemically stable in wastewater than the ionically cross-linked polymers such as chitosan. Biomass-containing chitosan matrices proved to be stable in authentic wastewater containing high concentration of anionic groups. High biomass utilization efficiency is a good indication of the phenol binding sites in biomass during immobilization, which excludes the contribution of immobilized matrix to phenol removal. The chitosan matrix show some capability in phenol binding, which may be due to the presence of active sites such as amino (-NH_2) groups in the chitosan. Biomass-immobilization, based on the criteria is that a suitable matrix should be stable both chemically and physically, and high in mechanical strength (or) resistance.

The objectives of this study are to investigate the above hypothesis and chitosan was used for the immobilization of the strain *Pseudomonos putida* (NICM 2174) for its high bulk density and quick settling which are considered as effective characteristics of chitosan in aqueous solution. To determine the chitosan to the uptake of phenol, to evaluate the mechanism and energetics of the uptake of phenol by chitosan, intraparticle diffusion and pore diffusion have been investigated.

2
Materials and methods

2.1
Chemicals

Phenol (99% pure, chemical grade), 4-amino antipyrine were got from Central Drug House Private Ltd., New Delhi, India. Chitosan to be used as the adsorbent was supplied by local manufacturing industry, M/s. Chemical Complex Pvt. Ltd., Ramapuram, Nellore, India. It was sieved into desired particle size ranges. The surface area of chitosan was determined by using BET (Bruner Emmett Teller) method. All other chemicals used were got from Central Drug House Private Ltd., New Delhi, India.

2.2
Source of organism

Pseudomonos putida (NICM 2174) species which degrades phenol was obtained from culture collection (NCl) Pune, India. The organism was maintained in a standard nutrient agar medium. The basal salt solution (minimal medium) used in this work composed of K_2HPO_4: 1.5 g/l; KH_2PO_4: 0.5 g/l; $(NH_4)_2SO_4$: 0.5 g/l; NaCl: 0.5 g/l; sodium sulphate: 3.0 g/l, yeast extract: 2.0 g/l, glucose: 0.5 g/l; ferrous sulphate: 0.002 g/l and calcium chloride: 0.002 g/l.

2.3
Batch equilibrium studies

Equilibrium adsorption isotherm was determined from the batch studies done in the above minimal medium with a portion of adsorbent material, namely, chitosan of known weight and varying amount of initial phenol concentrations: 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 mg/l at different particle size (0.177 mm, 0.384 mm, 1.651 mm) with constant pH (7.0), and temperature (30 °C) in conical flasks containing inoculated *Pseudomonos putida* (NICM 2174). The conical flasks were shaken at 180 rpm for 48 h to reach equilibrium.

2.4
Batch kinetic studies

Phenol solution of known concentration was treated with known weight of the adsorbent chitosan, at different parameters like (i) phenol concentration (0.100, 0.200, 0.300 g/l) with constant pH (7.0) and temperature (30 °C), (ii) pH (7.0, 8.0, 9.0) at constant phenol concentration 0.200 g/l and temperature 30 °C, (iii) Temperature (30 °C,

34 °C, 38 °C) at constant phenol concentration (0.200 g/l). Each experiment was carried out in conical flasks containing minimal medium and inoculated with *Pseudomonas putida* (NICM 2174) and kept at 180 rpm in a Lab-line orbit environ shaker for 48 h. For every 4 h the amount of phenol degraded was calculated by taking aliquot samples.

2.5
Estimation of phenol

Phenol was determined quantitatively by the spectrophotometric method (Beckman Du40 model) using 4-amino antipyrine as the colour reagent (λ_{max}: 500 nm) according to standard methods of analysis [39].

3
Results and Discussion

The equilibrium adsorption isotherm is of fundamental importance in the design of adsorption system. Knowledge of the adsorption capacity of an adsorbent material, such as immobilized chitosan matrix enables the design engineer to develop treatment systems for particular adsorbate/absorbent system [40–44]. There is a continuous diffusion of the solute from the liquid into the solid surface and back diffusion of solute into the liquid. At equilibrium the solute remaining in solution are in dynamic equilibrium with that in the surface. At this position of equilibrium, there is a defined distribution of solute between the liquid and solid phases which are generally expressed by a series of isotherm [40, 45].

Biological treatment using *Pseudomonas* sp. was the most effective method to degrade phenol from a variety of industrial effluents. It is also a time saving method than the other conventional methods for industrial effluents. General assumptions were made in order to develop a useful mathematical model which can predict the biodegradation system. The model describes the effect of adsorption, mass transfer, and cell properties of the phenol degradation system. Immobilized cell particles are spherical: Each particle has an inner homogeneous distribution initially. Most bacteria are present as micro colonies in the pore surface area. Cells are able to grow by consuming phenol; interfacial mass transfer resistance can be ignored when the external solution is mixed. Intraparticle mass transfer is assumed to occur only through liquid in the pores, and the effective diffusion coefficient of phenol is independent of concentration. Adsorption reaches equilibrium quickly depending on the diffusion rate. The growth and phenol degradation kinetic properties of immobilized cells are similar to that of free cells. The cell leakage can be ignored once cells are immobilized and prepared for phenol degradation. During the biodegradation process, phenol molecules are assumed to be transported from bulk liquid, through a static solid-liquid film on particle external surfaces and then into the porous solid matrix spheres. The biodegradation process is isothermal and may be characterized by either a Langmuir, Freundlich, Redlich–Peterson isotherm, rate constant study (Lagergren equation) and diffusion rate constant study.

3.1
Langmuir isotherm

Langmuir [46] suggested a theory to describe the adsorption of molecule onto adsorptive surfaces. The Langmuir adsorption isotherm has found successful application to many real sorption processes and it can be used to explain the sorption of phenol into chitosan. A basic assumption of the Langmuir theory is that sorption takes place at specific sites within the adsorbent. Theoretically, therefore, a saturation value is reached beyond which no further sorption can take place. To determine the adsorption capacity at different initial phenol concentration, and particle sizes, a study of sorptions isotherm is essential. The data obtained from the adsorption experiment conducted in the present investigation was fitted in different particle sizes in isotherm equation (C_e vs q) as shown in Fig. 1. The saturation value can be represented by the expression:

$$q = \frac{KaC_e}{1 + aC_e} \quad . \tag{1}$$

A plot of ($1/C_e$ vs $1/q$) resulted in a linear graphical relation indicating the application of the above model as shown in Fig. 2. The values are calculated from the slopes and intercepts of different straight lines representing the different particle sizes. K and a are isotherm constants. The values of Langmuir isotherm constants are given in Table 1. It can be observed that as the particle diameter decreases, the removal of phenol increases which is due to larger surface area associated with small particles. For larger particles the diffusion resistance to mass transport is higher and most of the internal surface of the particle may not be utilized for adsorption and consequently the amount of phenol adsorbed is small [47]. Small particles are better for phenol removal from the effluents. However, one cannot use small particles in a continuous packed bed adsorber because of higher pressure drops encountered [41, 48].

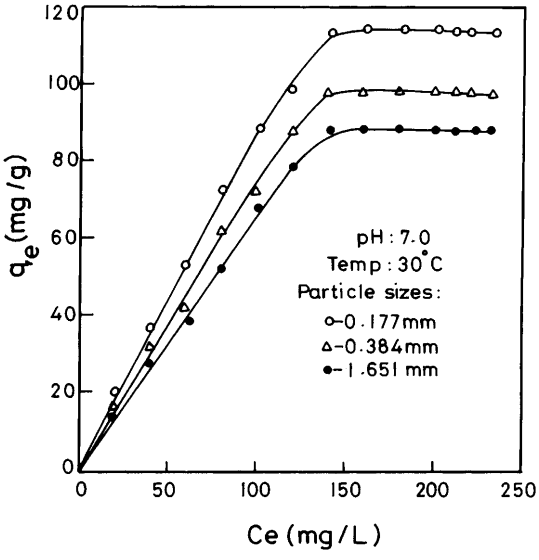

Fig. 1. Effect of particle size on biodegradation of phenol

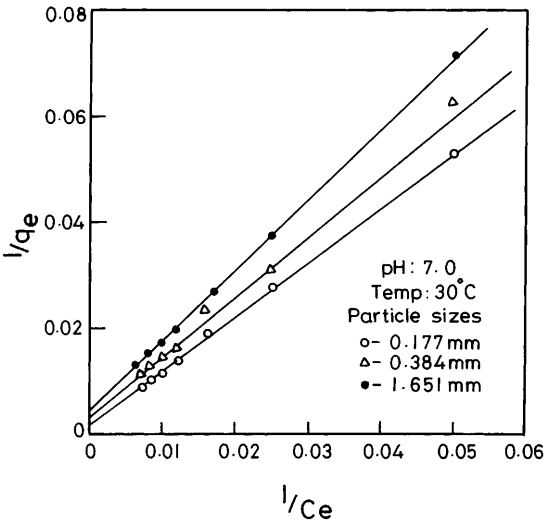

Fig. 2. Langmuir plot at different particle size on chitosan

The Langmuir adsorption isotherm assumes that the adsorbed layer is one molecule in thickness. The Langmuir constant (k) in Eq. (1) is a measure of the amount of phenol adsorbed, when saturation is attained. The solute is adsorbed onto the surface due to the available monolayer sites which are then taken up by them. It is proposed by [45] that some fresh internal surface can be created. The creation of the additional surfaces arises from the pressure of adsorbed molecule forcing into the macropore and micropore structures. It is also clear from the shape of the adsorption isotherm, that it belongs to the L_2 category of isotherm, which indicates the normal (or) Langmuir type of adsorption [41, 45]. Such isotherms are often encountered when the adsorbate has a strong intermolecular attraction for the surface of the adsorbent. The L_2 shape of isotherm observed in the present case clearly implies that phenol molecules must be strongly attached to chitosan. The adsorbate molecules can be linked to ranges of molecules, creating access to new surfaces and effectively clearing blocked pores.

The essential characteristics of Langmuir isotherms can be described by a separation factor [41–44, 48] which is defined by

$$R_L = \frac{1}{(1 + aC_e)} \quad , \tag{2}$$

where C_e is the initial phenol concentration. The separation factor R_L indicates the isotherm shape as follows: $R_L < 1$ unfavourable, $R_L > 1$ unfavourable, $R_L = 1$ linear, $0 < R_L < 1$ favourable and $R_L = 0$ irreversible. One and greater than one were reported for favourable and unfavourable adsorption, respectively. For this experiment values of R_L less than one, indicating favourable adsorption are given in Table 2.

Table 2. Langmuir isotherm with separation factor (R_L) at different particle sizes

Initial phenol concentration (mg/l)	Particle sizes (mm)		
	0.177	0.384	1.651
20	0.9968	0.9952	0.9934
40	0.9936	0.9904	0.9869
60	0.9905	0.9858	0.9806
80	0.9874	0.9811	0.9743
100	0.9842	0.9765	0.9680
120	0.9811	0.9720	0.9619
140	0.9762	0.9674	0.9558
160	0.9750	0.9630	0.9498
180	0.9720	0.9585	0.9439
200	0.9690	0.9541	0.9381

3.2 Freundlich isotherm

The Freundlich isotherm is used for heterogeneous surface energies system. The linearised sorption isotherm is the most convenient form of representing the experimental data at different particle sizes. Figure 3 shows the batch isothermal data fitted to the linear form of the Freundlich isotherm [41, 44, 49]:

$$q = K_f C_e^{1/n} \quad . \tag{3}$$

The fit of the data to Freundlich isotherm indicates the heterogeneity [49] of the sorbent surface and the Freun-

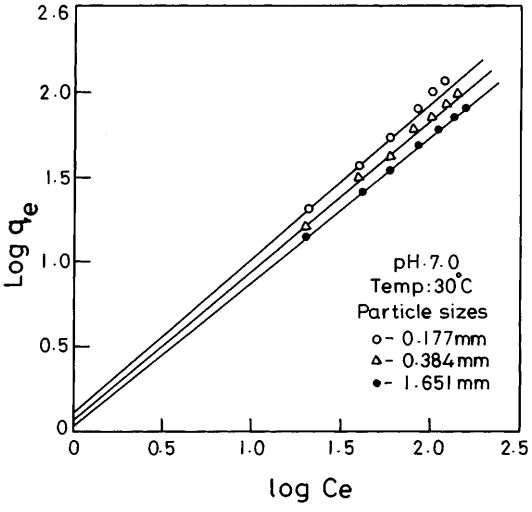

Fig. 3. Freundlich plot at different particle size on chitosan

Table 1. Langmuir, Freundlich and Redlich–Peterson isotherm constants at different particles sizes

Particle sizes (mm)	Langmuir		Freundlich		Redlich–Peterson	
	K (mg/g)	a (mg/l)	$1/n$	K_f	a_R	b
0.177	5867.33	0.00165	1.0362	0.084	5.7852	0.1814
0.384	3306.96	0.00024	0.9365	0.011	7.7085	0.1734
1.651	2213.52	0.00018	0.9276	0.007	8.1244	0.1498

dlich equation for isothermal sorption is a special case for heterogeneous surface energy in which the energy term in the Langmuir equation varies as function of surface coverage strictly due to variation of the sorption. The intercept K_f is roughly an indicator of the sorption capacity and the slope ($1/n$) of the sorption intensity and their values are given in Table 1. It has been stated by [41–44, 50] that magnitude of the exponent $1/n$ gives an indication of the favourability and capacity of the adsorbent/adsorbate system. Values $n > 1$ represent favourable adsorption conditions according to Treybal [51]. In most cases, the exponent between $1 < n < 10$ shows beneficial adsorption.

3.3
Redlich–Peterson isotherm

Redlich and Peterson isotherms present a general isothermic equation in agreement with that of Langmuir and Freundlich [44, 45, 60].

$$q = \frac{KC_e}{1 + a_R C_e^b} \quad . \tag{4}$$

From the plot $\ln\left(K\frac{C_e}{q_e} - 1\right)$ vs $\ln C_e$ is represented by Redlich-Peterson isotherm as shown in Fig. 4. The solubility of phenol is an essential property to enable the phenol to penetrate into the porous structure of the chitosan. The process will be assisted if the phenol ion with the adsorbent carries an opposite charge. The isotherm constants are given in Table 1. The isothermal data were plotted linearly because the Langmuir, Freundlich, Redlich–Peterson isotherm model consistently gave a better fit to experimental data.

3.4
Kinetics of adsorption

Kinetic study of the adsorption process using chitosan at different initial phenol concentration (0.100, 0.200, 0.300 g/l), temperature (30 °C, 34 °C, 38 °C) and pH (7, 8, 9) have been undertaken.

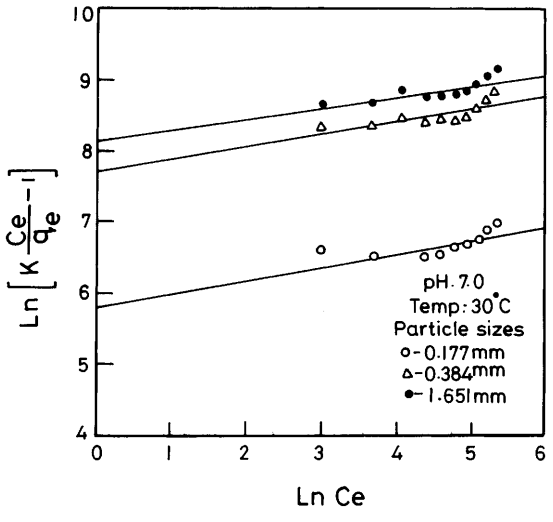

Fig. 4. Redlich–Peterson plot at different particle size on chitosan

3.4.1
Effect of initial phenol concentrations

The kinetics of phenol on chitosan system for a fixed known weight of adsorbent and variable initial phenol concentration is depicted in Fig. 5. An increase in initial phenol concentration results in decrease in phenol adsorption. The latter indicates reduction in immediate solute adsorption due to the lack of available active sites on the chitosan surface compared to the relatively large required number of sites for large phenol concentration.

3.4.2
Effect of temperature

Effect of temperature on the adsorption of phenol onto chitosan can be explained in accordance with batch adsorption findings. In Fig. 6 the biodegradation of phenol by *Pseudomonas putida* (NICM 2174) onto chitosan at

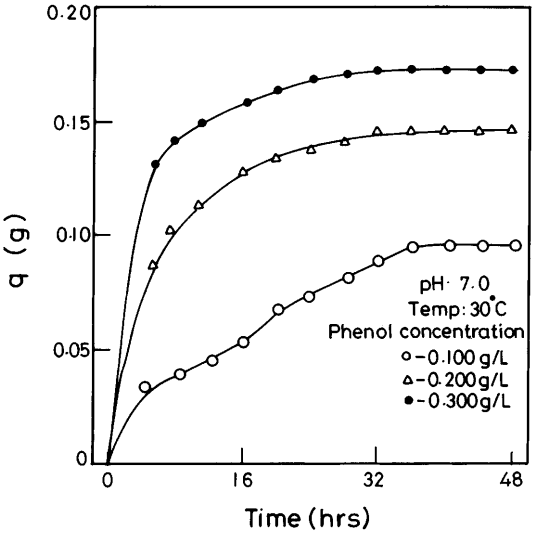

Fig. 5. Variation of specific phenol degradation with time (h) at different phenol concentration

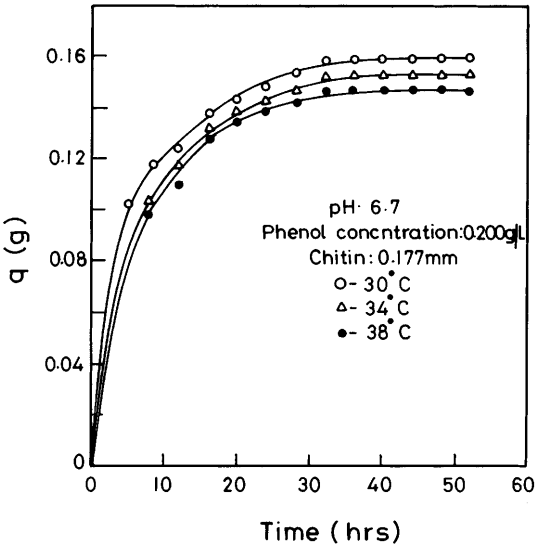

Fig. 6. Variation of specific phenol degradation with time (h) at different temperature concentration

different temperature have been compared with each other. When temperature was increased from 30–38 °C the intensity of adsorption was observed to decrease significantly. Adsorption process is unusually physical in nature. Chitosan may orient, expand (or) contract laterally with an [52] alteration in temperature. The temperature affects the solubility and pH chemical potential of the adsorbate, the latter being a controlling factor for the adsorption. It has been reported earlier that if the solubility of the adsorbate increases with increase in temperature [45] the chemical potential decreases and both the effects, work in the same direction, causing a decrease in the adsorption of phenol. Adsorption decreases with rise in temperature indicating that it is an exothermic adsorption process.

3.4.3
Effect of pH

Figure 7 shows the effect of pH on the biodegradation of phenol. The maximum degradation capacity occurred at pH 7.0. The hydrogen ion concentration (pH) of primary sorbate and the surface properties of the sorbent, lead to alterations in the kinetic behaviour of the sorption process. From Fig. 7, it is clear that the removal rate of phenols varies with pH. At lower pH level, the lone-pair of electrons of the oxygen atom of the undissociated –OH group present in the benzene ring co-ordinates with the highly positively charged surface. Increase in the rate observed at decreasing pH values may be caused by an alteration in the adsorbent surface, particularly variation in its electrokinetic character with changing pH. The adsorption of cells on the surface of the chitosan is through extracellular polymers which are monopolysaccharides in nature. The extracellular polymers exhibit zwitter ionic characters, that are highly influenced by the pH of the medium [53]. The monolayer coverage of biopolymer on the chitosan surface is controlled by available free vacant sites. This has been verified in the experiment relating magnitude of adsorption and amount of chitosan [41–44].

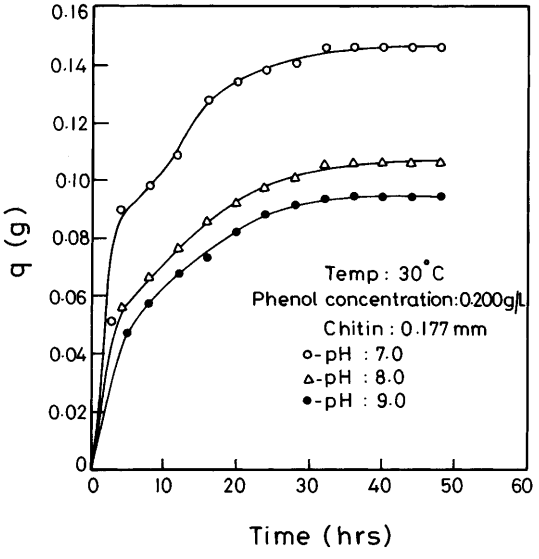

Fig. 7. Variation of specific phenol degradation with time (h) at different pH

3.5
Rate constant studies

The rate constant for the adsorption for solution of different amount of initial concentration, temperature and pH was studied using Lagergren's equation [41–43, 54]:

$$\log(q_e - q) = \log q_e - (K_{ad}/2.303)t \quad . \tag{4}$$

A plot of log $(q_e - q)$ vs 't' is represented at different parameters like initial phenol concentration (0.100, 0.200, 0.300 g/l), temperature (30 °C, 34 °C, 38 °C) and pH (7.0, 8.0, 9.0) as shown in Figs. 8, 9 and 10. A linear relation was observed indicating the applicability of the above equation and the first order of the process [1, 41–43]. The average value of the rate constant (K_{ad}) was calculated from the slope of the curves representing different conditions as recorded in Table 3.

3.5.1
Diffusion rate constant study

In adsorption of phenol using chitosan there is the possibility of intraparticle diffusion. It can be described by

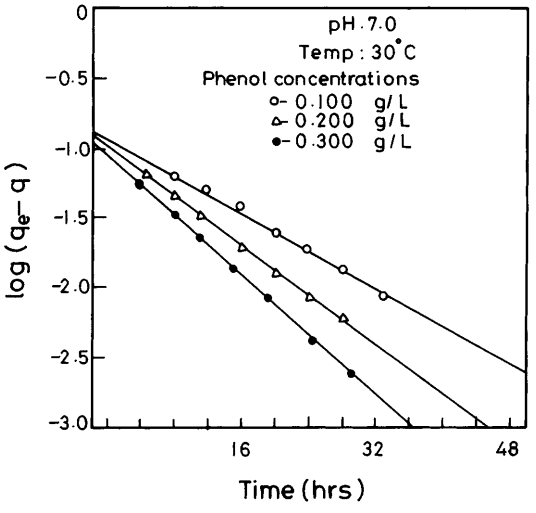

Fig. 8. Biodegradation kinetic parameters at different phenol concentration

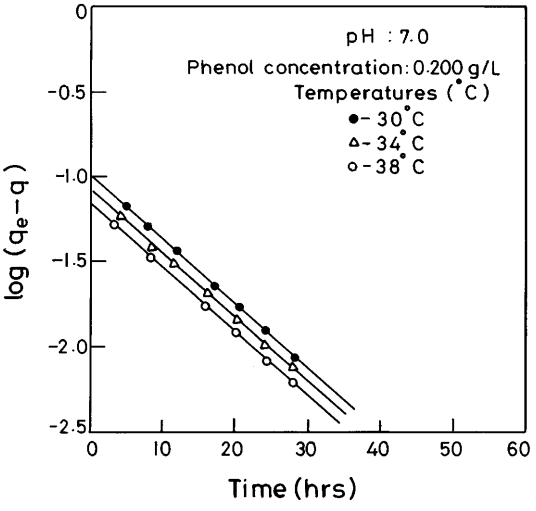

Fig. 9. Biodegradation kinetic parameters at different temperature

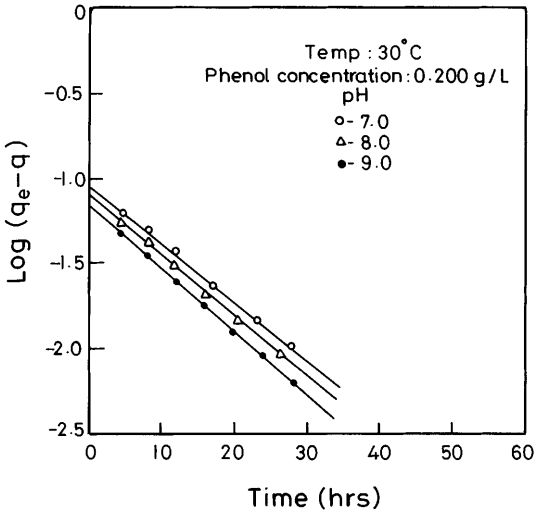

Fig. 10. Biodegradation kinetic parameters at different pH

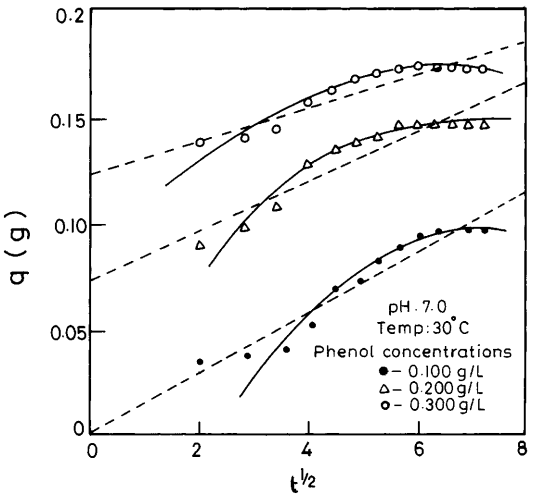

Fig. 11. Intraparticle diffusion plot for biodegradation of phenol at different phenol concentration

three consecutive steps. (i) The transport of sorbate from bulk solution to outer surface of the sorbent by molecular diffusion, known as external (or) film diffusion; (ii) internal diffusion, the transport of sorbate from the particles surface into interior sites; (iii) the sorption of the solute particles from the active sites into the interior surface of the pores [1, 41–43, 55]. Pore diffusion being the rate limiting mechanism has been drawn from McKay's plot [56]. The overall rate of the sorption process will be controlled by the slowest, the rate limiting step. The nature of the rate limiting step in a batch system can be determined from the properties of the solute and sorbent. The rate constants, for intraparticle diffusion (K_p) at different parameters are determined using equation given by Waber and Morris [57].

$$q = K_p t^{1/2} \quad .$$

K_p, under different conditions of phenol concentration, pH and temperature were calculated from the slope of the linear portions to the respective plots (Figures 11, 12 and 13) as shown in Table 3. The double nature of these plots, initial curve portions and final linear portions may be explained by the fact that the initial curved portions are boundary layer diffusion effects [1, 41–44, 53]. The final linear portions are a result of intraparticle diffusion effects. An extrapolation of the linear portions of the plots back to the axis yield intercepts which are proportional to

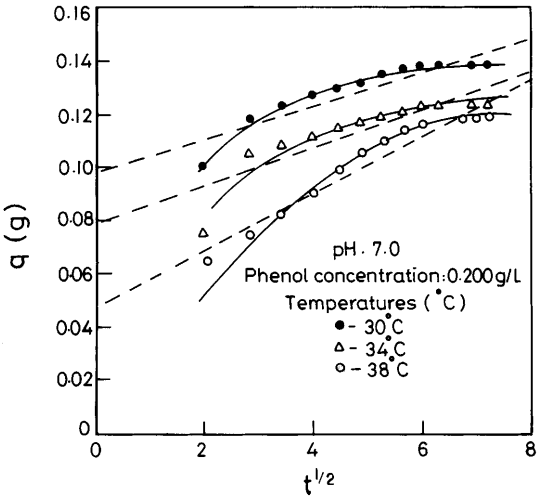

Fig. 12. Intraparticle diffusion plot for biodegradation of phenol at different temperature

Table 3. Rate constant, intraparticle diffusion, and pore diffusion constant at different parameter like initial phenol concentration, temperature and pH

Parameters	Rate constant K_{ad} min^{-1}	Intraparticle diffusion K_p g $min^{-0.5}$	$t^{1/2}$	Pore diffusion D cm^2 s^{-1}
Initial concentration (g/l)				
0.100	0.0792	0.0139	8.75	3.0×10^{-8}
0.200	0.1021	0.0114	6.87	3.9×10^{-8}
0.300	0.1222	0.0076	5.67	4.7×10^{-8}
Temperature (°C)				
30	0.1021	0.0114	6.87	3.9×10^{-8}
34	0.0955	0.0069	7.25	3.7×10^{-8}
38	0.0807	0.0060	8.59	3.1×10^{-8}
pH				
7.0	0.1021	0.0114	6.87	3.9×10^{-8}
8.0	0.0810	0.0099	8.55	3.1×10^{-8}
9.0	0.1041	0.0091	6.66	4.0×10^{-8}

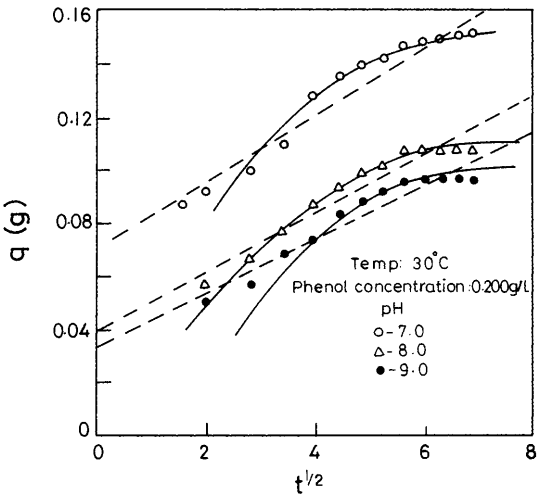

Fig. 13. Intraparticle diffusion plot for biodegradation of phenol at different pH

the extent of boundary layer thickness. Figs. 11, 12, and 13 at high concentration of phenol, temperature, and pH justify the assumption that particle diffusion becomes the rate limiting step, as McKay's plots at lower concentrations are reported to be linear and it has also been observed in this case as a purely film diffusion at lower concentrations (0.100, 0.200, 0.300 g/l), (30 °C, 34 °C, 38 °C) and pH (7, 8, 9). It is apparent from the above approaches that intraparticle diffusion is expected to be mainly the rate limiting step. The pore diffusion coefficients within the pores of adsorbents at various concentrations were determined using [1, 55–58, 59]:

$$D = \frac{0.03 r_0^2}{t^{1/2}} \quad . \tag{6}$$

The values of the pore diffusion coefficients were calculated using Eq. (6). The values of D were found in the order of above equation as shown in Table 3 indicating that the process is governed by diffusion.

4 Conclusion

Chitosan used in this study has a large surface area and desirable micropores. *Pseudomonas putida* (NICM 2174) appears to have been immobilized strongly inside the pores, thereby avoiding it from attrition. The optimization of initial phenol concentration, temperature and pH were found to be 0.200 g/l, 7.0, and 30 °C respectively. The values of rate constant (K_{ad}), Intraparticle diffusion (K_p), and pore diffusion (D) varied with initial phenol concentration, temperature and pH. Chitosan showed competitive properties for bacterial biomass immobilization and may have potential in process applications because of its relatively high biomass loading and good chemical and physical stabilities. Langmuir, Freundlich, Redlich–Peterson, intraparticle diffusion and pore diffusion models could be used to describe phenol sorption equilibrium and the kinetic data of chitosan – immobilized *Pseudomonas putida* (NICM 2174) also gave a better fit. The treatment is simple and economic. The reaction kinetics data thus generated may be used for designing a treatment plant for phenolic effluents wherein continuous removal (or) collection can be achieved on large scale.

References

1. **Binary, K.S.; Narendra, S.R.:** Comparative sorption kinetic studies of phenolic compounds on fly ash and impregnated fly ash. J. Chem. Tech. Biotechnol. 61 (1994) 57–65
2. **Smith, R.L.:** Human metabolic risk factors determining susceptibility to environmental chemicals. International seminar on Environmental Toxicology, Bangkok, Thailand, Part I (1988) 99–118
3. **Mahadew Swamy, M.; Mall, I.D.; Prasad, B.; Mishra, I.M.:** Removal of phenol by adsorption of coal fly ash and activated carbon. Pull. Res. 16(3) (1997) 107–175
4. **Suseela, G.R.; Basu, S.K.; Nandy, S.C.:** Degradation of pentachlorophenol by *Pseudomonas aeruginosa*. Indian J. Environ. Hlth. 33(4) (1991) 425–432
5. **Chitra, S.:** Studies on biodegradation of phenolic compound by *Pseudomonas pictorum*. Ph.D. thesis CLRI. Madras University. Chennai-25. India (1997)
6. **Shashirekha, S.; Uma, L.; Subramanian, G.:** Phenol degradation by the marine *Cyanobacterium phormidium valderianum* BDU 30501. J. Indus. Microb. Biotechnol. 30 (1997) 130–133
7. **Furukawa, K.; Matsumura, F.; Tonomura, K.:** Alkaligenes and acinetobactor capable of degrading polychlorinated biphenyls. Agri. Biol. Chem. 42 (1978) 543–548
8. **Gardon, A.H.; Camptell, W.R.:** Substrate inhibition kinetics: phenol degradation by *Pseudomonas putida*. Biotechnol and Bioeng. XVII (1975) 1599–1615
9. **Allsop, P.J.; Chisti, Y.; Mou-Young, M.; Sullivan, G.R.:** Dynamics ecosystem. A selected review of recent literature. Marine. Env. Res. 2 (1993) 87–181
10. **Palleroni, N.J.:** General properties taxonomy of the genius *Pseudomonas* 1–36, In. Clarke, P.H.; Richmond, M.H.: (Eds) Genetics and Biochemistry of *Pseudomonas*, Wiley, Toronto (1975)
11. **Loung, J.H.T.:** Generalization of monod kinetics for analysis of growth data with substrate inhibition. Biotechnol. Bioeng. 29 (1987) 242–248
12. **Sokol, W.; Howell, J.A.:** Kinetics of phenol oxidation by washed cells. Biotechnol. Bioeng. 23 (1981) 2039–2049
13. **Skol, W.:** Oxidation of an inhibitory substrate by washed cells oxidation of phenol by *Pseudomonas putida*. Biotechnol. Bioeng. 30 (1987) 921–927
14. **Sokol, W.:** Dynamics of an continuous stirred-tank biotechnical reactor utilizing inhibitory substrate. Biotechnol. Bioeng. 31 (1988a) 198–203
15. **Sokol, W.:** Uptake rate of phenol by *Pseudomonas putida* grown in unsteady state. Biotechnol. Bioeng. 3 (1988b) 1097–1103
16. **Ren, D.Y.; Aithur, F.H.:** Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures. Biotechnol. Bioeng. XVII (1975) 1211–1235
17. **Zhong-Cheng, H.U.; Roger, A.K.; William, E.L.; Ron, L.C.:** Adsorption and biodegradation of pentachlorophenol by polyurethane immobilized flavobacterium. Environ. Sci. Technol. 28 (1994) 491–496
18. **Bocke, C.; Inwebb, C.; Black, C.; Atkinson, B.:** Process Engineering aspects of immobilized cell systems. J. Chem. Eng. 14 (1986) 602–614
19. **Baoshan, X.; Willian, B.M.; Mortin, J.D.:** Sorption of phenol by selected biopolymers, isotherm, energetics and polarity. Environ. Sci. Technol. 28 (1994) 466–473
20. **Scolt, C.D.:** Immobilized cells a review of recent literature. Enzyme. Microb. Technol. 9 (1997) 66–73
21. **Begin, A.; Castaigne, F.; Goulet, J.:** Production of alginate beads by a rotative atomizer. Biotechnol. Lett. 5(6) (1991) 459–464

22. **Bhatia, S.K.:** Effective diffusivity of phenol in activated carbon. Chem. Eng. Common. 98 (1990) 139–154
23. **Brady, D.; Duncan, J.R.:** Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 41 (1994) 149–154
24. **Huang, T.C.; Cao, L.T.:** Batch adsorption of p-nitrophenol and P-chlorophenol on anion exchange resin. J. Chem. Eng. Japan 21 (1988) 498–503
25. **Price, D.M.; Varma, A.:** Effective diffusivity measurement through on adsorbing porous solid. AICHE. Symp. Ser. 266 (1988) 88–96
26. **Tsezos, M.:** Adsorption by microbial biomass as a process for removal of ions from process or waste solutions. In: Eccles, H.; Hunt, S.: (Eds) Immobilization of ions by biosorption. Ellis Horwood, Chichester, UK (1986) 201–218
27. **Wang, X.; Rukenstein, E.:** Preparation of porous polyurethane particles and their use in enzyme immobilization. Biotechnol. Prog. 9 (1993) 661–664
28. **Whits, C.; Gadd, G.M.:** Bisorption of radionuclides by fungal biomass. J. Chem. Technol. Biotechnol. 49 (1990) 331–343
29. **Macaskie, L.E.:** An immobilized cell bioprocess for the removal of heavy metal from aqueous solution. J. Chem. Technol. Biotechnol. 49 (1990) 357–379
30. **Macaskie, L.E.:** The application of biotechnology to the treatment of wastes produced from the nuclear fuel cycle: Biodegradation and bioaccumulation as a means of treating radionuclide containing streams. Crit. Rev. Biotechnol. 11 (1991) 41–112
31. **Macoskie, L.E.; Dean, A.C.R.:** Use of immobilized biofilm of *citrobactor* sp. for the removal of uranium and lead from aqueous flows. Enzyme Microb. Technol. 9 (1987) 2–4
32. **Nakajima, A.; Sakaguchi, T.:** Selective accumulation of heavy metals by microorganisms. Appl. Microbiol. Biotechnol. 24 (1986) 59–64
33. **Nakajima, A.; Horikoshi, T.; Sakaguchi, T.:** Recovery of uranium by immobilized microorganisms. Eur. J. Appl. Microbiol. Biotechnol. 16 (1982) 88–91
34. **Klein, J.; Schara, P.:** Entrapment of living microbial cells in covalent polymeric networks. II. A quantitative study on the kinetics of oxidative phenol degradation by entrapped cadida tropicalis cells. Appl. Biochem. Biotechnol. 6 (1981) 91–107
35. **Beltmen, H.; Rehm, H.J.:** Degradation of phenol by polymer entrapped microorganisms. Appl. Microbiol. Biotechnol. 20 (1984) 285–290
36. **Klein, J.; Hackel, U.; Wagner, F.:** Phenol degradation by candida tropicalis whole cells entrapped in polymeric ionic networks ACS. Symp. Ser. 106 (1979) 101–118
37. **Ehrhardt, H.M.; Rehm, H.J.:** Phenol degradation by microorganisms adsorbed on activated carbon. Appl. Microbiol. Biotechnol. 21 (1985) 23–36
38. **Morsen, A.; Rehm, H.J.:** Degradation of phenol by a defined mixed culture immobilised by adsorption on activated carbon and sintered glass. Appl. Microbiol. Biotechnol. 33 (1990) 206–212
39. **Clessceri, L.S.; Greenberg, A.E.; Trussel, R.R.:** Standard methods for the determination of water and wastewater. American Public Health Association, Washington, DC 2005 (1989) 5.48–5.53
40. **McKay, G.; Otterburn, M.S.; Aga, J.:** Fullers earth for dyestuff – equilibrium rate studies. W.A.S.P. 24 (1985) 307–322
41. **Annadurai, G.:** Colour removal from aqueous environ by waste derived Biopolymer. Ph.D. thesis. Anna University, Chennai-600 025, India (1997)
42. **Annadurai, G.; Krishnan, M.R.V.:** Batch-kinetic studies of adsorption reactive dye using chitosan. Indian. J. Env. Prot. 17 (15) (1997a) 328–333
43. **Annadurai, G.; Krishnan, M.R.V.:** Adsorption of acid dye from aqueous solution by chitin: Bacth kinetic studies. Indian J. Chem. Technol. 4 (1997b) 213–222
44. **Annadurai, G.; Krishnan, M.R.V.:** Batch equlibrium adsorption of reactive dye on to Natural Biopolymer. Iranian Polymor. J. 16 (1997c) 169–172
45. **Stephen, J.A.; Mckay, G.; Khafer, K.Y.H.:** Equilibrium adsorption isotehrm for basic dyes onto lignite. J. Chem. Tech. Biotechnol. 45 (1989) 291–302
46. **Langmuir, I.:** Adsorption of gases on plain surfaces of glass mica platinum. J. Am. Chem. Soc. 40 (1918) 1361–1403
47. **Lakshmi Narayana Rao, K.c.; Krishnaiah, K.; Ashutosh, A.:** Colour removal from a dyestuff industry effluent using activated carbon. Indian J. Chem. Technol. 1 (1994) 13–19
48. **Hall, K.R.; Eagleton, L.C.; Axerivos, A.; Vermeulen, T.:** Pore and solid diffusion kinetics in fixed bed adsorption under constant pattern conditions. Ind. Eng. Chem. Funfam. 5 (1966) 212–216
49. **Freundlich, H.:** Adsorption solution. Z. Phys. Chemie. 57 (1906) 384–470
50. **McKay, G.; Blair, H.S.; Gardner, J.R.:** Adsorption of dyes chitin-I: Equilibrium studies. J. App. Poly. Sci. 27 (1982) 3043–3057
51. **Treybal, R.E.:** Mass transfer operations. McGrew-Hill, New York, 10th edition (1988)
52. **Mitra, S.P.; Chattoraj, D.K.:** Some thermodynamic aspects of expand and condensed films of BSA adsorbed at the alumina-water interface. J. Biochem Biophys. 15 (1978) 147–152
53. **Chitra, S.; Sekaran, G.; Gowri, C.:** Adsorption of mutant strian of *Pseudomonas pictorum* on rice bran based activated carbon. J. Haz. mat. 48 (1996) 239–250
54. **Lagergren, S.; Bill, K.:** Srenskar Vatens Kapsakad Handl. (1898) 24
55. **Rayalu, S.S.; Shrivastava, A.:** Adsorption of phenol on new adsorbent-activated carbon cloth. Indian J. Env. Prot. 13(6) (1993) 407–414
56. **Poots, V.J.P.; McKay, G.; Healy, J.J.:** Removal of basic dye from effluent using wood as an adsorbent. Water Poll. Cont. Fed. 50 (1978) 926–979
57. **Weber, W.J.; Morris, J.C.:** Kinetics of adsorption on carbon from solution. J. San. Eng. Div. A.S.C.E. 89SA2 (1963) 31–59
58. **Bhattacharya, A.K.; Venkobachar, C.:** Removal of cadmium (II) by low cost adsorbents. J. Env. Eng. 110(1) (1994) 110–122
59. **Michelson, L.d.; Gideon, E.G.P.; Kutal, L.H.:** Removal of soluble mercury from wastewater by complexing techniques. U.S.D.I. office of water research and technology. Bull. No. 74 (1975)
60. **Redlich, O.; Peterson, D.L.A.:** Useful adsorption isotherm. J. Phys. Chem. 63 (1959) 1024–1029