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Full Length Research Paper

Biodegradation of phenol by *Pseudomonas pictorum* on immobilized with chitin

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Biodegradation of phenol using *Pseudomonas pictorum* (ATCC 23328) a potential biodegradant of phenol was investigated under different operating conditions. Chitin was chosen as a support material and then partially characterized physically and chemically. The pH of the solution was varied over a range of 7 – 9. The maximum adsorption and degradation capacity of bacteria immobilized with chitin at 30°C when the phenol concentration was 0.200 mg/L is at pH 7.0. The results showed that the equilibrium data for all phenol-degradation sorbent systems fitted the Langmuir, Freundlich and Redlich-Peterson model best. Kinetic modeling of phenol degradation was done using the pseudo-first order and pseudo-second order rate expression. The biodegradation data generally fit the intraparticle diffusion rate equation from which biodegradation rate constant, diffusion rate constant were determined.

Key words: Adsorption, biodegradation, phenol, *Pseudomonas pictorum,* isotherm and kinetic studies.

INTRODUCTION

Phenol is one of the pollutants in waste water which is highly toxic. Its toxicity can be understood by the fact that it is fatal to fishes at 0.1 ppm and can create toxic problem to human beings and animals through food chain if it is consumed above a particular dosage continuously. Phenol is a toxic organic component often found in wastes from oil refinery, plastic, coke, petroleum refining, dyestuff, pharmaceuticals and petrochemical industries. Recent literature on the methods of removal of phenol and their compounds from waste water focuses on adsorption and microbial biodegradation process (Zhong-Cheng et al., 1994; Amellal et al., 2001; Xing, 2001; Battaglia-Brunet et al., 2002). Certain species like *Pseudomonas* sp. under very controlled conditions of pH, temperature and in the presence of some specific nutrients can degrade phenol. *Pseudomonas* strain, capable of degrading pentachlorophenol has been isolated around tannery soil and characterized as *Pseudomonas aeruginosa* (Suseela et al., 1991; Chitra et al., 1996; Rasmussen et al., 2002; Bogan and Sullivan, 2003).

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An immobilized cell is one of the approaches for 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 (Klein et al., 1979; Aharoni et al., 1979; Klein and Schara, 1981; Meggyes and Simon, 2000; Walter et al., 2000). Polyacrylamide silica gels have been the most extensively used immobilization materials for laboratory research studies.

The latter are very suitable for immobilization of microbial cells and can be manufacture at industrial scale. Such immobilized system integrates two processes in one structure-effective biocatalysts and separation. The temperature and pH were maintained at 30°C and 7, respectively, and the initial phenol concentration was fixed at 0.200 g/L. *P. pictorrm* immobilized with chitin was selected because it can effectively biodegrade phenol and pH 6.8–7.0 was reported to be optimal for the biodegradation of the substrates in the previous studies (Shreve and Vogel, 1993; Tutem et al., 1998; Garcia et al., 2000; Farrell and Quilty, 2002; Zimmerman et al., 2005). The present paper describes the possibility of *P.*

pictorrm cells immobilization on chitin and investigation of their phenol adsorption and degradation abilities.

MATERIAL AND METHODS

Media tested and sample preparation

Chitin to be used as the adsorbent was supplied by a local manufacturing industry. It was sieved into discrete particle sizes. Phenol (99% pure chemical grade), 4-amino antiphyrine were get from Sigma-Aldrich chemical company in USA. *P. pictorum* (ATCC 23328) species was obtained from culture collection in Bioresources Collection and Research Center, Food Industry Research and Development Institute, Taiwan. The organism was maintained in a standard nutrient agar medium. The basal salt solution (minimal medium) used in this work was composed of K_2HPO_4 1.5 g/l, KH_2PO_4 0.5 g/l, $(NH_4)_2$ SO_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.

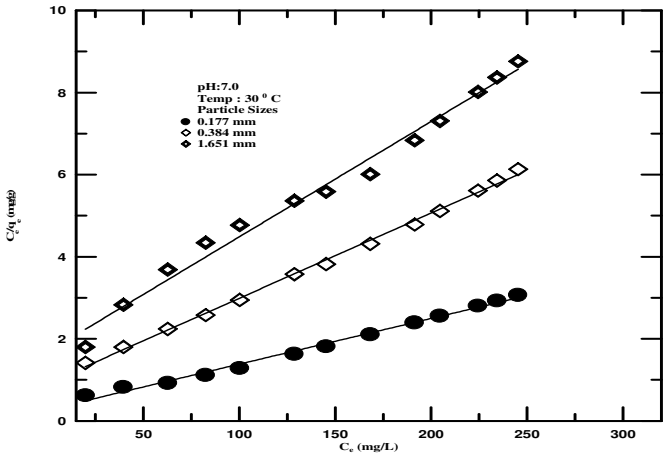


Figure 1. Langmuir plot at different particle sizes on immobilized chitin with bacteria.

Equilibrium tests

Equilibrium adsorption and degradation isotherm was determined from the batch studies done in the above minimal medium with a portion of adsorbent material namely, chitin, 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, 0.384 and 1.651 mm) with constant pH (7.0), and temperature (30°C) in conical flasks containing inoculated *P. pictorum* (ATCC 23328). The conical flasks were shaken at 180 rpm for 48 h to reach equilibrium.

Batch tests

Phenol solution of known concentration was treated with known weight of the adsorbent chitin, at different parameters like; (i) pH (7.0, 8.0 and 9.0) at constant phenol concentration (0.200 g/L) and temperature (30°C) (ii) temperature (30, 34, 38°C) at constant phenol concentration (0.200 g/L) and pH 7.0. Each of these experiments was carried out in conical flasks containing minimal medium and was inoculated with *P. pictorum* 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. Phenol was determined quantitatively by the spectrophotometric method (Beckmen DU40 model) using 4-amino antipyrine as the colour reagent (λ_{max}: 500 nm) according to standard methods of analysis (Clessceri et al., 1989).

SEM studies

Before and after the chitin immobilized with *P. pictorum* was allowed to adsorb the phenol the SEM images were taken and analyzed. Based on analysis of the images height heterogeneous pores with chitin particle were absorbed. After adsorption the pores were packed with phenol.

RESULTS

Langmuir isotherm

Adsorption and biodegradation equilibrium data are conveniently represented by adsorption isotherms which are helpful in determining the adsorption capacity of the immobilized matrix. In order to analyse an adsorption isotherm it is important to develop on equation which accurately represents the results and which may be used for design purposes. These isotherms, the Langmuir, Freundlich and Redlich-Peterson were considered for this purpose in the present study. Langmuir isotherm was used to analyse the equilibrium data for adsorption and biodegradation of phenol by chitin-immobilized *P. pictorum* (ATCC 23328). Traditionally, the Langmuir model is represented as in equation (1). One such equation considered is rearranged Langmuir isotherm model:

$$q_e = \frac{KbC_e}{(1+bC_e)} \quad (1)$$

$$\frac{C_e}{q_e} = \frac{C_e}{K} + \frac{1}{bK} \quad (2)$$

By the relationships: K = slope and b = intercept^{-1}. From the data of particle size obtained, the amount of phenol adsorbed was fitted with the function of equilibrium concentration in Langmuir in isotherm equation (2). K is the amount of phenol adsorbed per unit weight of adsorbent, and b is the Langmuir constant, determined from the linear plot of C_e/q_e versus C_e, as shown in Figure 1. The adsorption and degradation capacity due to monolayer coverage (K) was 0.0111 to 0.0280 (mg/g) for phenol at 30°C, pH -7.0. The Langmuir isotherm constants are given in Table 1. The essential characteristics of the Langmuir may be expressed in terms of a dimensionless equilibrium parameter 'R_L' (Weber and Morris, 1963; Weber and Chakaravortti, 1974; Macky et al., 1982, 1989; Stephen et al., 1989). Langmuir isotherms can be described by a separation factor:

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

Particle size (mm)	Langmuir				Freundlich			Redlich-Peterson		
	K (mg/g)	b (L/mg)	R_L	R^2	n	K_F (mg $^{-1}$L$^{1/n}$ g^{-1})	R^2	β	b_R (L/mg)	R^2
0.177	0.0111	0.271	0.9506	0.9923	2.35	1.085	0.9906	0.698	0.354	0.9528
0.384	0.0207	0.914	0.8454	0.9975	2.02	1.226	0.9763	0.612	2.256	0.9719
1.651	0.0208	1.677	0.7485	0.9866	2.11	2.131	0.9345	0.598	4.408	0.9907

$$R_L = 1/(1 + bC_e) \quad (3)$$

Where b is the Langmuir constant introduced in equation (1) and C_e is the initial concentration of the adsorbate in solution. This parameter indicates the isotherm shape according to the following adsorption characteristics: $R_L > 1$ unfavourable; $R_L = 1$ corresponds to linear; $0 < R_L < 1$ is favourable and $R_L = 0$ is irreversible are given in Table 1.

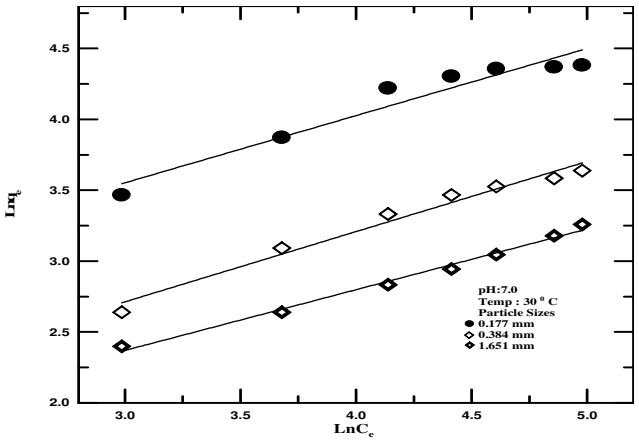


Figure 2. Freundlich plot at different particle sizes on immobilized chitin with bacteria.

Freundlich isotherm

Freundlich isotherm is used for heterogeneous surface energies system. The sorption isotherm is the most convenient form of representing the experimental data at different particle sizes. Figure 2 show the batch isothermal data fitted to the linear form of the Fruendlich isotherm (Freundlich, 1906):

$$q_e = K_F C_e^{1/n} \quad (4)$$

$$\ln q_e = \ln K_F /(1/n) \ln C_e \quad (5)$$

The intercept K_F is roughly an indicator of the sorption capacity and the slope (1/n) of sorption intensity and their values are given in Table 1.

Redlich – Peterson isotherm

The Redlich-Peterson isotherm contains three parameters and incorporated the features of the Langmuir and Freundlich isotherm (Redlich and Peterson, 1959; Mckay and Ho, 1999). The Redlich-Petersons has a linear dependence on concentration in the numerator and an exponential function in the denominator. It can be described as follows:

$$q_e = \frac{KC_e}{(1 + b_R C_e^{\beta})} \quad (6)$$

The equation reduces to a liner isotherm at low surface coverage, to the Freundlich isotherm at high adsorbate concentration and to the Langmuir isotherm when β = 1. However, the equation cannot be linearized for easy estimation of isotherm parameters. It has three isotherm constants, namely, K, b_R and β (0 < β < 1), which characterize the isotherm. The linearization of the expression gives

$$\ln(K\frac{C_e}{q_e} - 1) = \ln b_R + \beta \ln C_e \quad (7)$$

Plotting log [(K C_e/q_e) - 1] against ln C_e yields a straight line of slope = β and intercept = b_R as shown in Figure 3. A general trial and error procedure which is applicable to computer operation was developed to determine the coefficient of determination 'r^2' for a serious of values of K for the linear regression of [(K C_e/q_e) - 1] and to obtain the best value of K which yields a maximum optimized value 'r^2'. The quality of the fit of the experimental data to the isotherm equation is assessed by the magnitude of the correlation coefficient for the regression. In other words, the isotherm giving an 'r^2' value closest to unity provides the best fit. The solubility of a phenol is an essential property to enable the phenol to penetrate into the porous structure of the chitin. The Redlich-Peterson isotherm constant is shown in Table 1.

Adsorption kinetics

The kinetics of adsorption and biodegradation was studied for its possible importance in the treatment of phenol

Table 2. Pseudo-first order rate constant at different parameter like temperature and pH.

Temp (°C)	K_1 min^{-1}	q(mg/g)	R^2	pH	K_1 min^{-1}	q(mg/g)	R^2
30	0.0487	0.994	0.9728	7.0	0.0487	0.994	0.9728
34	0.0492	1.192	0.9946	8.0	0. 0349	1.414	0.9761
38	0.0457	1.422	0.9940	9.0	0.0300	1.405	0.9773

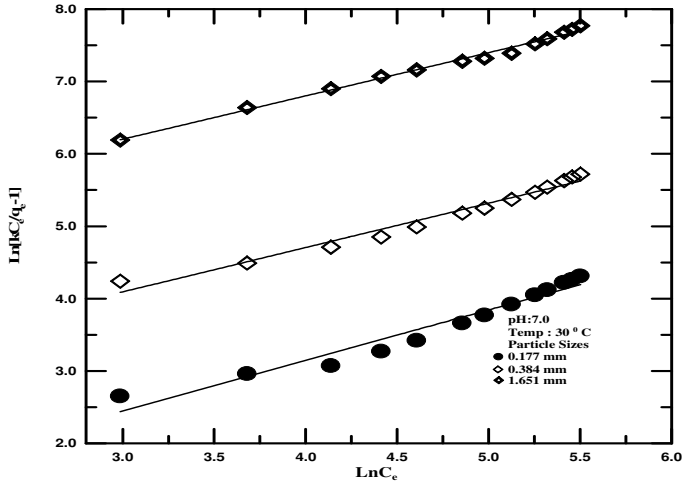


Figure 3. Redlich-Peterson plot at different particle sizes on immobilized chitin with bacteria.

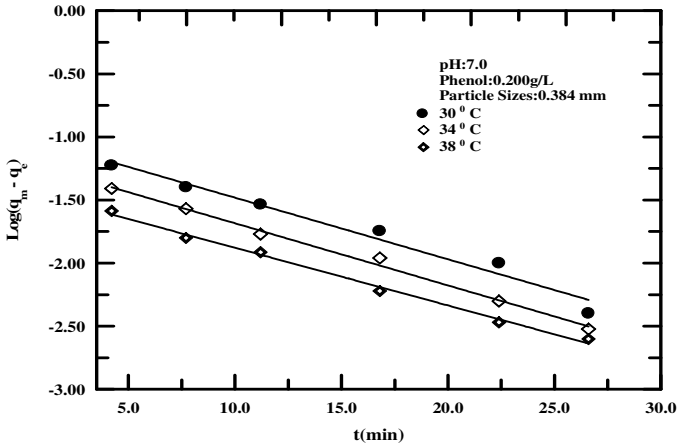


Figure 4. Adsorption and biodegradation kinetic parameters at different temperatures on immobilized chitin with bacteria.

containing industrial effluents. Numerous kinetic models have been proposed to elucidate the mechanism by which pollutants are adsorbed. The mechanism of adsorption depends on the physical and chemical characteristics of the adsorbent as well as on the mass-transport process. To investigate the mechanism of the phenol adsorption and biodegradation, kinetic models were considered.

The pseudo-first order kinetic model: Biodegradation of phenol at different initial phenol concentration, temperature and pH was studied using Lagergren's equation (Lagergren, 1898; Lakshmi and Narayana rao, 1994; Annadurai and Krishnan, 1996; Mckay and Ho, 1999). The integral from of the model is:

$$Log(q_m - q_t) = Logq_m - (K_1 / 2.303)t \qquad (8)$$

A plot of log (q_m - q_t) vs t is represented at different temperature (30, 34 and 38°C) and pH (7.0, 8.0, 9.0) as shown in Figures 4 and 5. A lower relation was observed indicating the applicability of the above equation and the first order nature of the process (Treybal, 1980; Mitra and Chattoraj, 1978). The average value of the rate constant (K_1) was calculated from the slope of the line representing different parameters as shown in Table 2. This model has been successfully applied to describe the kinetics of many adsorption systems.

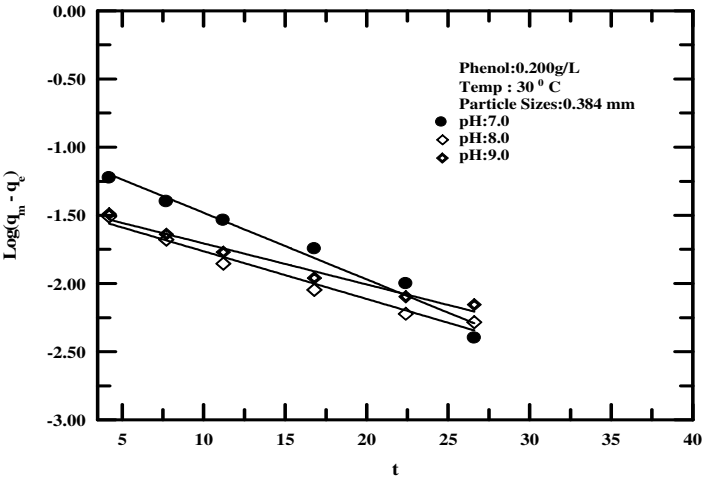


Figure 5. Adsorption and biodegradation kinetic parameters at different pH on immobilized chitin with bacteria.

The pseudo–second order kinetic model: The second-order kinetic model (Mckay and Ho, 1999) is expressed as:

$$t / q_t = 1 / K_2 q_m^{\ 2} + t / q_m \qquad (9)$$

Table 3. Pseudo-second order constants at different parameter like temperature and pH.

Temp (°C)	h (mg/g) (min)	K_2 g/(mg) (min)	R^2	pH	h (mg/g) (min)	K_2 g/(mg) (min)	R^2
30	34.974	7.723	0.9981	7.0	34.974	7.723	0.9981
34	48.706	11.62	0.9985	8.0	36.258	12.094	0.9997
38	48.958	15.37	0.9941	9.0	63.844	16.498	0.9987

The initial adsorption rate, *h* (mg/g min), as $t \rightarrow 0$ can be defined as:

$$h = K_2 q_m^2 \quad (10)$$

The initial adsorption rate (*h*), the equilibrium adsorption capacity (q_m), and the second-order constants k_2 (g/mg min) can be determined experimentally from the slope and intercept of plot t/q_t versus *t*. Calculated correlations are closer to unity for second-order kinetics model; therefore the adsorption kinetics could well be approximated more favourably by second-order kinetic model for phenol adsorption and degradation. The k_2 (g/mg min) and *h* (mg/g min) values as calculated are listed in Table 3.

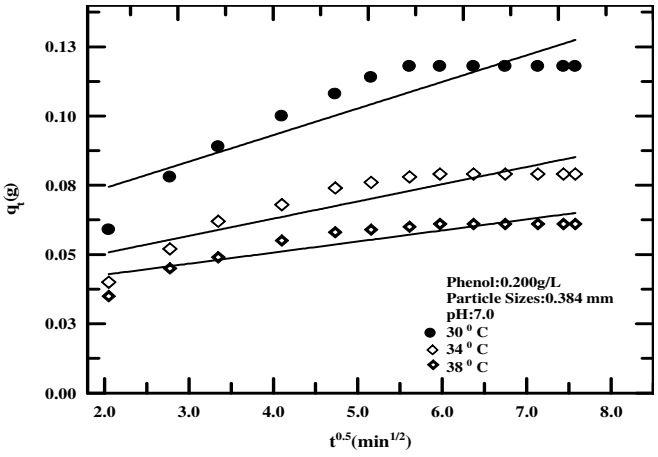


Figure 6. Intraparticle diffusion plot at different temperatures on immobilized chitin with bacteria.

Diffusion rate constant study: In the process of adsorption and biodegradation of phenol using chitin, there is the possibility of intraparticle diffusion. An empirically found functional relationship common to the most adsorption processes is that the uptake varies almost proportionally with $t^{1/2}$, Weber-Morris plot rather versus $t^{1/2}$ should be straight line with a slope K_p and intercept C, when adsorption mechanism follows the intraparticle diffusion processes. The overall rate of the than with the constant time, t as shown in Figures 6 and 7. According to Weber and Morris (1963) a plot of q sorption

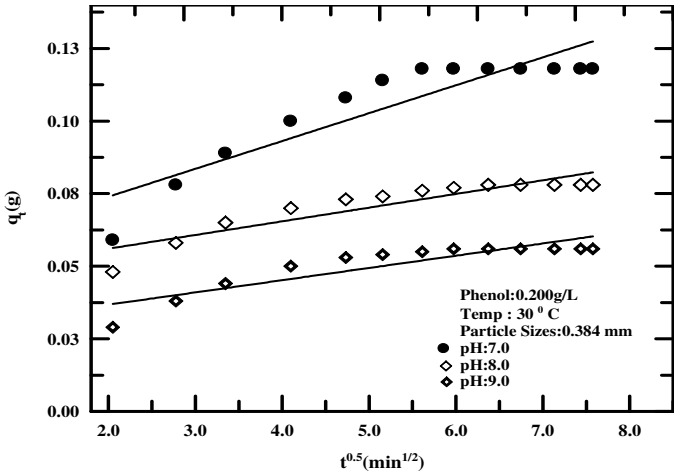


Figure 7. Intraparticle diffusion plot at different pH on immobilized chitin with bacteria.

process will be controlled by the slowest, the rate limiting step.

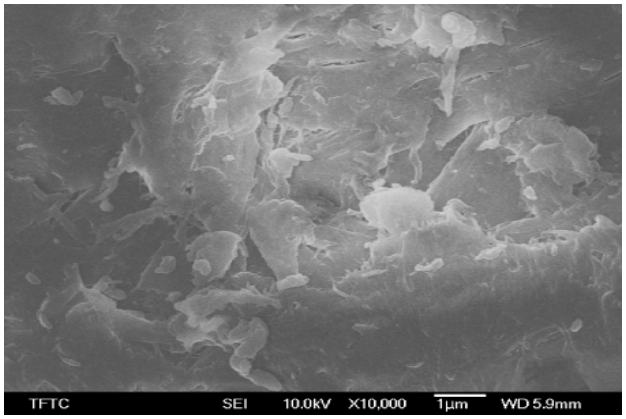
$$q = K_p t^{1/2} + C \quad (11)$$

The rate constants for intraparticle diffusion (K_p) and intercept C were calculated from slope of the linear plots of amounts of phenol adsorbed per unit mass versus half power of time at different parameters using equation by Weber and Morris (1963). K_p, under different conditions was calculated from the slope of the linear portions of these respective plots with units of (t) min$^{0.5}$ as shown in Table 4. Such plots may present multilinearity indicating that two (or) more steps take place. The first sharper, portion is the instantaneous adsorption stage (or) external surface adsorption. The second portion is the gradual adsorption stage, where intraparticle diffusion is rate limiting. The third portion is the final equilibrium stage where intraparticle diffusion stages to slow down due to extremely low adsorbate concentrations left in the solution. The double nature of these plots, as initial curve portions and final linear portions may be explained by thefact that the initial curved portions are boundary layer diffusion effects (Hall et al., 1966; Annadurai, 1997; Annadurai and Krishnan, 1997; Binary and Narendra, 1994). The final linear is the result of intraparticle diffusion effects. The linear plots are attributed to the macropore diffusion, which is the accessible site of adsorption.

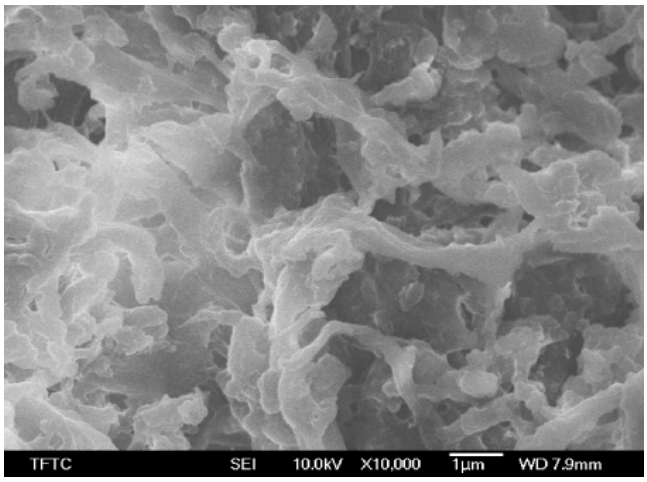
Table 4. Intra-particle diffusion constants at different parameter like temperature and pH.

Temp (^oC)	K_p (g/min)	C	R^2	pH	K_p (g/min)	C	R^2
30	0.0096	0.054	0.8276	7.0	0.0096	0.0547	0.8276
34	0.0062	0.038	0.8113	8.0	0.0047	0.0466	0.8347
38	0.0040	0.034	0.7982	9.0	0.0042	0.0283	0.7946

This is attributed to the instantaneous utilization of the most readily available adsorbing sites on the adsorbent surface.



(A) - Chitin



(**B)** – Chitin immobilized with *Pseudomonas pictorum*

Figure 8. Scanning electron micrograph of immobilized chitin with *Pseudomonas pictorum.* (A) Chitin. (B) Chitin Immobilized with *Pseudomonas pictorum.*

SEM Studies

Sample of fresh chitin and bacteria (*P. pictorum*) immobilized chitin were coated under vacuum with a thin layer of gold and examined by scanning electron microscopy. SEM is one of the most widely used surface diagnostic tools. Chitin has heterogeneous surface and macrospores as seen its SEM micrograph. The micrograph showing bacteria cells densely and homogeneously adhered to the surface of the carrier, as a result of either natural entrapment in to the porous chitin material (or) due to physical adsorption by electrostic forces (or) covalent binding between the cell chitin and the carrier. Its low BET surface area is confirming that chitin has macrospores. Chitin is a linear homopolymer of β-(1-4)-2 acetamido-2-deoxy-D-glucose and it is similar to cellulose in morphology. The SEM micrograph of the fresh chitin and *P. pictorum* immobilized with chitin from were presented in Figures 8 (A) and (B), respectively. The uniform distribution is an important criterion for the proper adsorption and degradation of phenol on the whole surface area of the bacteria immobilized chitin. Both pore diffusion and kinetic resistance are likely to affect the adsorption and degradation rate. It was determined at the end of 48 h cultivation period and no bacteria biomass increase was detected after this period.

DISCUSSION

Biological treatment using *Pseudomonas* sp. is the most effective method to degrade phenol from a variety of industrial effluents. 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 of cells initially; most bacteria are present as micro colonies in the porous 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 (Trulleyove and Rulik, 2004). 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. During the biodegradeation process, phenol molecules are assumed to be transported from bulk liquid, through a static sold. Liquid films on particle external surfaces are transported into the porous sold matrix sphere (Hall et al., 1966). The biodegradation process is isothermal and may be characterized by equilibrium; kinetic and diffusion rate constant have been investigated. It can be observed that as the

particle diameters, decrease the adsorption and biodegradation of phenol increases which is due to larger surface area that are associated with small particles (Poots et al., 1978; Mckay et al., 1982). For larger particles the diffusional resistance to mass transport is higher and most of the internal surface of the particle may not be utilized for adsorption and degradation consequently the amount of phenol adsorbed is less (Langmuir, 1918; Helby, 1952; Giles et al., 1974; Annadurai, 1997). The solute is adsorbed onto the surface due to the available monolayer sites taken up by them. It is proposed that some fresh internal surface can be created (Annadurai, 1997). The creation of the additional surface arises from the pressure of adsorbed molecule forcing into the macropore and micropore structures (Poots et al., 1978). Early work by Mackay et al. (1982) stated that the extended Langmuir isotherm could only be applied to solutes with similar adsorption characteristics, particularly affinities and equilibrium saturation capacities. Data from our equilibrium studies demonstrated that the percentage of adsorption and biodegradation of phenol by immobilized chitin with microorganism form 80-90% for an initial concentration of the range of 0.100 to 0.200 mg/L. For these experiments, a value of R_L less than one was observed indicating favourable adsorption.

The adsorption capacities were values for phenol adsorption and degradation particle size are obtained form the freundlich model were respectively 1.088 to 2.131 (mg/g). The Freundlich exponent 'n' between 2.11 to 2.35 indicates favourable adsorption, it has been stated by McKay and Ho (1999) and Treyball (1980) that magnitude of the exponent 1/n gives an indication of the capacity of the adsorbent/adsorbate system. In all cases the exponent $1 < n < 10$ shows beneficial adsorption.

Adsorption and biodegradation of phenol on chitin system for a fixed known weight of adsorbent and variable temperature and pH have been investigated. An increase in initial phenol concentration results in decrease in phenol adsorption. When temperature was increased from 30 - 38°C the intensity of adsorption was observed to decrease significantly. Adsorption process is usually physical in nature. Chitin may orient expand (or) contract laterally with an alteration in temperature (Mckay et al., 1982; Rayalu and Shrivastava, 1993; Annadurai and Krishnan, 1996). Increase in the rate, observed for decreasing pH values may be caused by an alteration in the adsorbent surface, particularly variation in its electro kinetic character with changing pH. The adsorption of cells on the surface of the chitin is through extracellular polymers which are monopolysaccharides in nature.

Conclusion

The adsorption and biodegradation of phenol form aqueous solution using chitin-immobilized with bacteria has been investigated, under different reaction conditions in batch and equilibrium mode. The monolayer adsorption capacity determined was reasonably high 0.118 (mg/g) at phenol concentration 0.200 (mg/L), temperature 30°C, pH - 7.0 and particle size (0.177 mm) for adsorption and biodegradation of phenol respectively. The kinetics of phenol adsorption and biodegradation nicely followed pseudo-first under rate expression and demonstrated that intraparticle diffusion plays a significant role in the adsorption and biodegradation mechanism. Langmuir, Freundlich and Redlich-Peterson models could be used to describe phenol sorption equilibrium and the kinetic data of chitin–immobilized *P. pictorum* also gave a better fit. The data reported here should be useful for the design and fabrication of an economically viable treatment process using batch or stirred tank reactors for adsorption and degradation phenol for dilute industrial effluents.

ACKNOWLEDGEMENT

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