

Removal of phenol from wastewater using activated waste tea leaves

Mohsin Kazmi*, Anwar R. Saleemi, Nadeem Feroze, Amir Yaqoob, Syed Waqas Ahmad

Department of Chemical Engineering, University of Engineering & Technology, Lahore, Pakistan, 54890

* Corresponding author: engr.smalikazmi@gmail.com

This investigation enumerates the treatment of phenol contaminated synthetic wastewater by Activated Waste Tea Leaves (AWTL). Phosphoric acid was used for the modification of waste tea leaves. The effects of initial pH, biosorbent dose, contact time, and initial phenol concentration were studied on the phenol uptake from the synthetic solution. Kinetic modelling was performed using pseudo 1st and 2nd order kinetics. The Langmuir and Freundlich's Models were employed to interpret the AWTL behaviour at various mass transfer gradients. The results show that the optimum values for pH, biosorbent dose and contact time were 2.2 g/L and 180 minutes, respectively. Pseudo 2nd order kinetic and the Langmuir's Models best described the kinetic and equilibrium behaviours, respectively.

Keywords: acid activation, batch study, biosorption, equilibrium study, kinetic study.

INTRODUCTION

Phenol and its compounds are produced to manufacture different compounds to fulfil various modern necessities. These compounds include a variety of resins (adhesives, epoxic, phenolic and polyamides etc), biocides, insecticides, pesticides, wood preservatives, glue, paints etc¹⁻⁴. These compounds act as importunate and fatal pollutants when come into contact with water without apposite treatment. In potable water they generate malicious odour when present in concentration $\geq 5 \mu\text{g/L}^2$. The fatality multiplies as the phenol is colourless and readily dissolves in water. Thus, it cannot be recognised by naked eyes. Phenol adverse effects involve harsh problems like cancer, nausea, paralysis, smoky coloured urine, vomiting etc³. Continuous use of phenol polluted water can cause stern pain which may lead to capillary damage and ultimately death⁵.

The prominence of biosorption is the utilization of waste biomass to treat polluted water⁶. Biosorption was originally identified for the economic removal of heavy metals from the dilute solution⁶. However, its success has been observed in the removal of organic compounds by many researchers⁷⁻¹³. Deceased biomasses represent the characteristic of pollutants removal from the solution due to the presence of various functional group in their structure^{6, 14}. These groups involve complex physicochemical removal mechanisms and cannot be described by a simple theory^{15, 16}.

Tea, made up of its plant leaf and bud, is the most famous non-alcoholic drink with the consumption of 18 to 20 billion cups worldwide^{17, 18}. It has been investigated as a beneficial beverage for cholesterol and hypertension reductions as well as an anti-cancer and anti-microbial drink. Tea has three races *Camellia sinensis*, *Camellia assamica* and *Cambodiensis*. Based on manufacturing, tea can be classified into four main categories viz, black tea, green tea, oolong tea and brick tea^{14, 18}. These valuable effects are with reference to the polyphenolic compounds present in tea¹⁴.

Pakistan trade tons of tea per annum. Once the tea has been brewed, it becomes a solid waste and is disposed of. Thus, waste tea leaves claims a huge figure among the agricultural wastes in Pakistan. Waste tea has been used by many researchers to remove organic and hazardous metals from the solution^{8, 11-13, 19}. Current investigation

explores the removal of phenol using H_3PO_4 activated waste tea leaves. A conventional study was performed to study the effects of initial pH, time, initial phenol concentration on phenol removal from wastewater.

MATERIALS AND METHODS

Preparation of Activated Waste Tea Leaves (AWTL)

Waste tea bags were picked from the cafeteria of University of Engineering and Technology Lahore, Pakistan. Waste tea was removed from the bags and thoroughly washed with tap water to remove the milk, sugar and dusts contents. Then, it was decolourised by repeated boiling, washing and filtering the residue to prevent any undesirable interferences in the experimentation or analytical procedure. Decolourised waste tea leaves were dried at 65°C for one day. Dried waste tea leaves were activated using Phosphoric acid (H_3PO_4). Hence, dried waste tea leaves were immersed in H_3PO_4 and vigorously agitated for a few minutes followed by washing with distilled water till the neutral pH of filtrate was observed. Activated Waste Tea Leaves (AWTL) were dried, grounded, sieved through 30 mesh screen. Undersize (below 30 Mesh) material was stored in plastic bottles for subsequent use.

Batch Study and Analytical Equipment

All chemicals were of analytical grade. Stock solution of 1000 mg/L phenol was prepared by dissolving 1 g of phenol in 1 L flask. Solutions of known concentrations were prepared by diluting this synthetic solution. The volume of analyte in all experiments was 200 mL. All the experiments were performed at room temperature ($26 \pm 1^\circ\text{C}$). The concentration of phenol was 100 mg/L unless otherwise stated. The pH was maintained using 0.1 M NaOH or 0.1 M HCl. Its effect was studied in the range of 2 to 9. During the kinetic study, flasks were removed at different time intervals between 5 to 300 minutes. The effect of initial concentration was noticed between 2 to 200 mg/L of phenol. The biosorbent dose was studied in the range of 1 g/L to 10 g/L. The residual concentration of phenol was determined using a Ultra Violet Spectrophotometer (Optima sp-3000). The experiments were performed in triplicate and average values with 5% variation were used in the calculations.

AWTL uptake capacity (q) was determined using the following expression:

$$q = \frac{(C_0 - C) \times V}{m}$$

Where C_0 (mg/L) is the initial phenol concentration, C (mg/L) is the residual phenol concentration, V (mL) is the volume of analyte and m (g) is mass of AWTL.

RESULTS AND DISCUSSIONS

Effect of initial pH

The initial pH of adsorbate plays a governing role in its uptake by the biosorbent. All the physico-chemical biosorption mechanisms are strongly affected by the solution pH as it strongly influences the charge of the functional groups present on the biosorbent. Thus, an investigation was conducted for the quantification of the initial solution pH on the % removal of phenol by AWTL as shown in Fig. 1. It shows that increasing pH from 2 to 12 resulted in a decrease in % removal of phenol from 74.61 to 11.05. Aksu and Akpinar (2001) observed the similar pattern while investigating the competitive biosorption of phenol and chromium (VI) onto dried anaerobic activated sludge¹. The trend may be explained as at lower pH the binding groups of AWTL carries positive charge due to excess of H^+ ions while Phenol exists in its anionic state in aqueous media. Thus maximum % removal was observed at pH 2 due to the strongest interaction of positive binding sites and negative phenolic ions. As the pH was increased the positive charge of the media tended to decrease. Thus, the % Removal of phenol declined by a very low % Removal i.e., 11.05% at pH 12. Since the % Removal was not 0 at pH 12, it showed that electrostatic interaction was not the only phenomenon responsible for phenol removal. Other mechanisms like ion-exchange, Van der Waal and H-binding may also play for the removal of phenol by AWTL¹. Thus, pH 2 was selected for all the experiments.

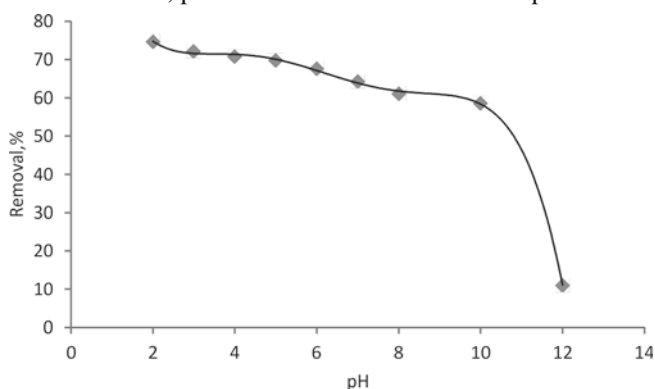


Figure 1. Effect of initial pH on the removal of phenol by AWTL (Dose = 2 g/L, C_0 = 100 mg/L, Time = 180 min)

Determination of Equilibrium Time

In order to establish the equilibrium time for the biosorption of phenol onto AWTL, a series of experiments were performed in which variation of phenol uptake capacity was observed with the passage of time as shown in Fig. 2. It is obvious from the Fig. 2 that removal of phenol by AWTL took place through multiple steps. The initial fast step, lasting between 0 – 10 minutes showed

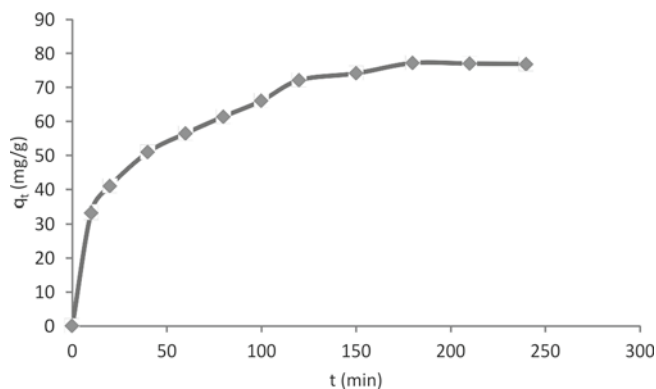


Figure 2. Effect of contact time on uptake of phenol (Dose = 2 g/L, C_0 = 100 mg/L, pH = 2)

an increase in AWTL uptake capacity from 0 to 33.39 mg/g. This may correspond to the uptake of phenol by vacant binding sites present on the surface of AWTL. The second step with mild speed lasted between 10 to 120 minutes in which AWTL uptake capacity increased from 33.39 mg/g to 72.14 mg/g. This part may be explained as with the passage of time competition among phenol molecules increased to occupy limited sites present on the surface. In this stage, partly uptake of phenol by AWTL may be attributed to the pore diffusion¹³. The last step with very slow speed was between 120 and 240 minutes showing the establishment of equilibrium between phenol and AWTL. In this step, a minor enhancement in uptake was observed i.e., 77.18 mg/g at 180 minutes. Thus, a contact time of 180 minute was taken as equilibrium time.

Kinetic Modelling

Kinetic modelling is an indispensable part of a conventional biosorption study. It helps to inspect the data from the view point of reaction order, mechanism and a suitable mathematical description. Thus, three kinetic models were investigated for the phenol biosorption onto AWTL as given in Table 1 along with the details of the models. Figure 3 shows the fitting of pseudo 1st order model on the removal of phenol by AWTL. It has been derived based on the assumption that rate of uptake of a component by a biosorbent is directly proportional to the vacant adsorption sites. A good R^2 value was observed for pseudo first order model. The value of q_e estimated from the model was 63.13 mg/g representing an error of 18.2% based on the experimentally observed uptake.

Figure 4 depicts the application of Pseudo second order kinetic model for removal of phenol by AWTL. It has

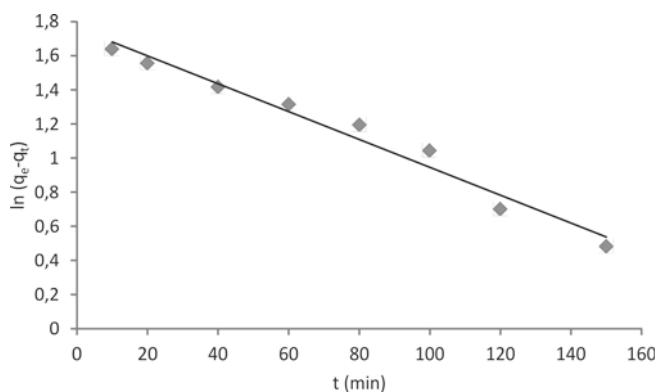
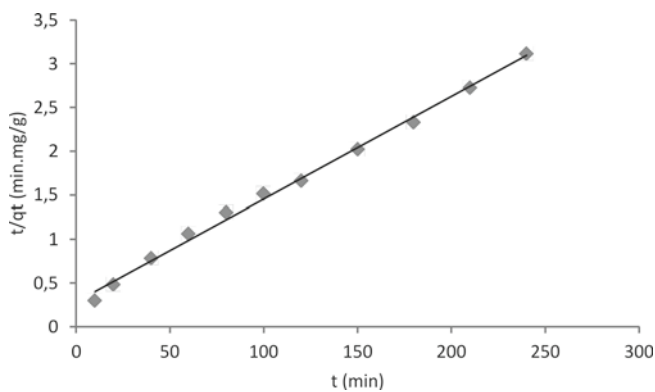


Figure 3. Fitting of pseudo-1st order kinetic model on biosorption of phenol onto AWTL

Table 1. Details of different kinetic models

Kinetic model	Differential	Integral	Plot	Reference
Pseudo 1 st Order	$\frac{dq_t}{dt} = k_1(q_e - q_t)$	$\ln(q_e - q_t) = \ln q_e - k_1 t$	$\ln(q_e - q_t)$ vs t	[20]
Pseudo-2 nd Order	$\frac{dq_t}{dt} = k_2(q_e - q_t)^2$	$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2}$	$\frac{t}{q_t}$ vs t	[20]
Weber-Morris	-----	$q_t = kt^{0.5}$	q_t vs $t^{0.5}$	[13]
Details of kinetic model parameters				
Pseudo 1 st Order	$k_1(\text{min}^{-1})$	-0.0197	$R^2 = 0.9733$	
	$q_{e(\text{model})}(\text{mg/g})$	63.13		
	$q_{e(\text{exp})}(\text{mg/g})$	77.18		
Pseudo 2 nd Order	$k_2(\text{g mg}^{-1} \cdot \text{min}^{-1})$	4.8×10^{-4}	$R^2 = 0.9960$	
	$q_{e(\text{model})}(\text{mg/g})$	85.34		
	$q_{e(\text{exp})}(\text{mg/g})$	77.18		
Weber-Morris	$k(\text{g}^{-1} \text{mg} \cdot \text{min}^{-0.5})$	4.62	$R^2 = 0.9068$	

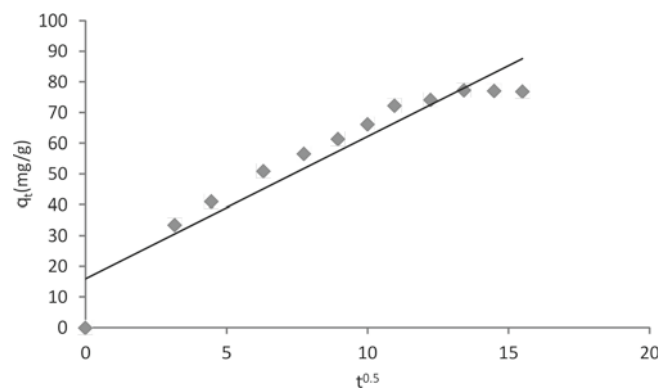
**Figure 4.** Fitting of pseudo second order kinetic model for the biosorption of phenol on AWTL

been derived based on the assumption that the rate of an uptake of a solute species is directly proportional to the square of the empty binding sites. The value of correlation coefficient observed was excellent i.e., $R^2 = 0.9960$. The equilibrium uptake determined based on the pseudo second order kinetic was 85.34 mg/g indicating a % error of 10.57. Thus, it may be concluded that biosorption of phenol onto AWTL followed the pseudo second order model.

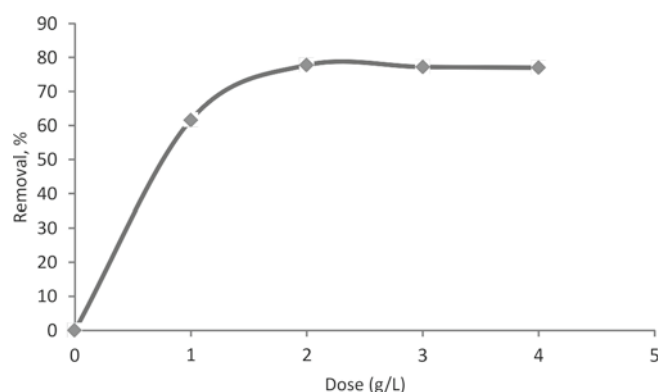
To identify the removal mechanism, Weber-Morris model was also used as shown in Figure 5. This equation has been derived on the supposition that the rate controlling step is the pore diffusion¹³. This assumption may only be justified if the trend between $t^{0.5}$ and q is a straight line and passes through the origin. Fig. 5 indicates a linear graph with an intercept. Thus, it may be concluded that pore diffusion was not rate controlling step. However, a fairly higher value of R^2 might suggest that pore diffusion have played an important role in the biosorption of phenol on AWTL. A similar trend has been observed by Hameed (2009) while studying removal of basic dye from aqueous solutions by spent tea leaves¹³.

Effect of Biosorbent Dose

It is important to study the effect of biosorbent dose as this factor is directly linked with the number of binding sites available for the adsorbate. In order to compute the effect of biosorbent dose, a sequence of four experiments were performed in which AWTL concentration in

**Figure 5.** Fitting of Weber-Morris pore diffusion model on the biosorption of phenol onto AWTL

the solution was varied from 1 g/L to 4 g/L as shown in Fig. 6. It can be seen that on increasing the biosorbent dose from 1 g/L to 2 g/L, steeply increase the % Removal improved from 61.69% to 77.74%. However, further improvement in dose did not result in any increment of % Removal. This trend can be explained as on increasing the solid to liquid ratio from 1 to 2 g/L, provided the more sites for phenol binding. Nevertheless, due to an establishment of equilibrium between the phenolic ions and AWTL binding sites, on increasing the biosorbent dose beyond 2 g/L no further increment in % Removal was noticed. Hence, 2 g/L of AWTL may be taken as optimum dose.

**Figure 6.** Effect of AWTL dose on the removal of phenol (Time = 180 min, $C_0 = 100$ mg/L, pH = 2)

Effect of initial Phenol Concentration

Evaluation of AWTL at various mass transfer driving forces was studied by contacting its fixed quantity with solutions of varying phenol concentrations as shown in Fig. 7. It shows the uptake of phenol was increased from 20.6 mg/g to 93.48 mg/g when initial concentration was varied from 50 to 300 mg/L. This may be interpreted as the occupation of more and more binding sites when concentration of phenol was increased. However, another interesting trend regarding decrease in % Removal was noticed as indicated in Fig. 7. It shows that with the increase in initial concentration of phenol, the %Removal was decreased from 82.4% to 62.34%. It may be interpreted as the increase in phenol concentration enhanced the competition among the phenolic ions resulting in lower %removal. Establishment of equilibrium might be the reason of the same trend.

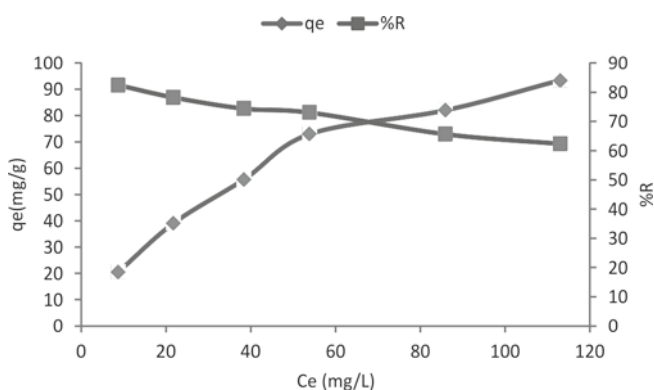


Figure 7. Effect initial concentration on the uptake (mg/g) and removal of phenol by AWTL. (Dose = 2 g/L, Time = 180 min, pH = 2)

Equilibrium Modelling

The importance of equilibrium modelling is vital for the design of adsorption systems. Generally, the empirical models are employed to interpret the data consisting of equilibrium concentration and uptake at equilibrium. Application of these models on an adsorbate-adsorbent system provides quick familiarization of underlying mechanisms. Each model is characterized by a set of parameters which correspond to the surface properties of biosorbent and affinity of adsorbate to the binding sites and may act as the basis for comparison with other adsorbate-biosorbent systems.

Freundlich and Langmuir are two widely used models in this aspect as given in Table 2. The Freundlich model has been characterised by the incidence of heterogeneous binding sites on the biosorbent and is employed by plotting $\log q_e$ versus $\log C_e$ as shown in Fig. 8. It showed a good fitting of the Freundlich's model as the R^2 for this system was 0.9816. The values of K_f and n were obtained by the slope and intercept of the Trend in Fig. 8 and

Table 2. Details of adsorption isotherms

	Isotherm	Linearized form	Parameters	Plot	References
Langmuir	$q_e = q_{max} \frac{bC_e}{1 + bC_e}$	$\frac{C_e}{q_e} = \frac{1}{bq_{max}} + \frac{C_e}{q_{max}}$	q_{max}, b	$\frac{C_e}{q_e}$ vs C_e	[21]
Freundlich	$q_e = K_f C_e^{(1/n)}$	$\log(q_e) = \log(K_f) + (1/n)\log(C_e)$	K_f, n	$\log(C_e)$ vs $\log(q_e)$	[22]
Details of adsorption isotherm parameters					
Freundlich	K_f (mg/g)	6.02		$R^2 = 0.9816$	
	n	1.68			
Langmuir	q_{max} (mg/g)	134.01		$R^2 = 0.9913$	
	b	0.0197			

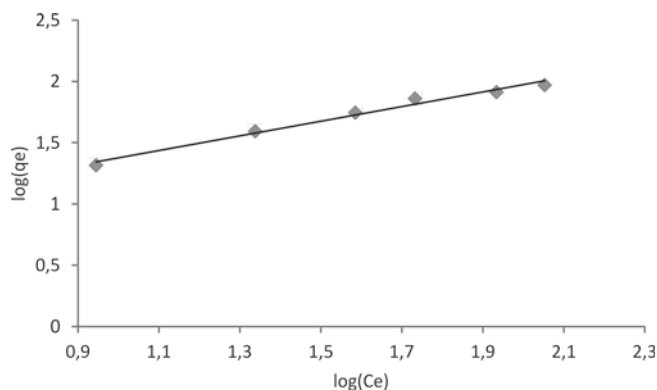


Figure 8. Fitting of Freundlich's model for biosorption of phenol by AWTL

were 6.02 mg/g and 1.68, respectively. The greater the value of K_f , called adsorption capacity, the better is the biosorption of a component. On the other hand the value of n , called adsorption intensity, greater than 1 shows the favourable adsorption. Also, the more the value of n is closer to 0, the greater is the system heterogeneity. As, the value of n was greater than 1, therefore, there was a possibility of better fitting by other adsorption model like the Langmuir's Model.

Figure 9 demonstrates the fitting of the Langmuir's model by plotting a graph between C_e/q_e versus C_e . This model postulates the biosorption of adsorbate through the formation of monolayer on the biosorbent. The details of model parameters are given in Table 2. R^2 value for this model was 0.9913 which showed that Langmuir's model enumerated better the biosorption of phenol by AWTL than the Freundlich's model. q_{max} and b were deduced from the determination of slope and intercept of Fig. 9 and were 134.01 mg/g and 0.0197. The greater the value of q_{max} , called maximum uptake, the better is the performance of the biosorbent. Table 3 shows the comparison of AWTL with other biosorbents used for the phenol removal. It can be seen that AWTL showed better uptake by microorganism and dried activated slud-

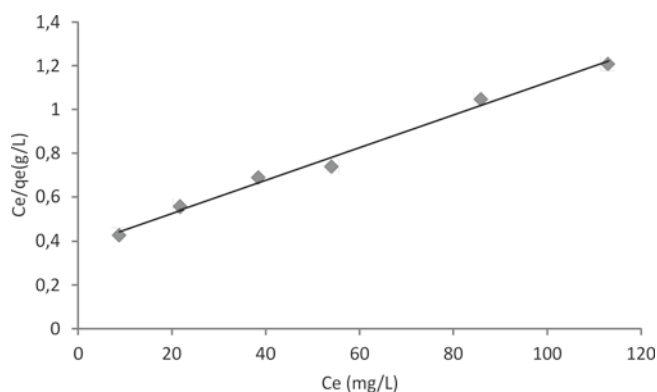


Figure 9. Fitting of Langmuir's model for uptake of phenol by AWTL

Table 3. Comparison of AWTL with other biosorbents for phenol removal

Sr#	Biosorbent	Uptake (mg/g)	Reference
1.	Dried anaerobic activated sludge	460.8	[1]
2.	Dried Aerobic Activated Sludge	194.2	[7]
3.	Activated waste tea leaves	134.01	This study
4.	Fungus	120	[3]
5.	Dried activated sludge	86.1	[4]
6.	Aspergillus Niger	0.328	[5]

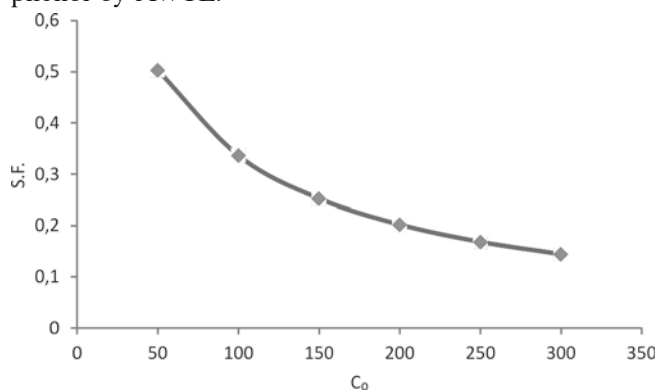
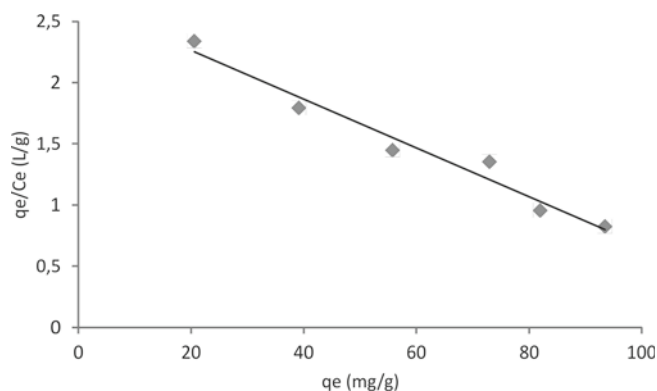
ge. The parameter, b , denotes the affinity of the phenol towards AWTL and is used to determine an important parameter called separation factor (S.F.) given by the following equation⁵:

$$SF = \frac{1}{1 + bC_0}$$

Figure 10 shows the trend between S.F. and initial concentration of phenol. The trend shows that the value of S.F. was in the range of 0 and 1 showing that biosorption of phenol by AWTL was favourable⁵.

q_{\max} is another important

As the correlation coefficient for both models for biosorption of phenol by AWTL was close, Scatchard plot was drawn to identify the model governing the biosorption of phenol by AWTL. Figure 11 indicates an almost linear graph between q_e/C_e versus q_e . According to Scatchard's plot a linear trend shows the involvement of uniform binding sites^{15, 23}. Thus the Scatchard plot supported the Langmuir's model for the biosorption of phenol by AWTL.

**Figure 10.** Effect of initial concentration on separation factor**Figure 11.** Scatchard plot for the biosorption of phenol by AWTL

CONCLUSIONS

AWTL may be used to effectively remove phenol from the wastewater. To ensure the optimum removal of phenol from single component wastewater system, pH and AWTL concentration should be ensured at 2 and 2 g/L, respectively. The contact time of 3 hr (180 minutes) was found to be sufficient to establish equilibrium between phenol in aqueous phase and on the biomass and was well explained by pseudo 2nd order kinetics. Application of the Langmuir and Freundlich's models indicated good fitting of the equilibrium data. However, Scatchard plot helped to identify the Langmuir's model as a more suitable isotherm for the biosorption of phenol onto AWTL.

NOMENCLATURE

k_1	Pseudo 1 st order rate constant (1/min)
q_e	Equilibrium uptake (mg/g)
q_t	Uptake at any time (mg/g)
k_2	Pseudo 2 nd order rate constant (g/mg · min)
K	Pore diffusion rate constant (mg/g · min ^{0.5})
C_e	Equilibrium concentration (mg/L)
q_{\max}	Langmuir constant (mg/g)
B	Langmuir constant (L/mg)
K_F	Freundlich constant (mg/g)
n	Freundlich constant
C_0	Initial concentration (mg/L)
C	Residual concentration (mg/L)

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LITERATURE CITED

- Aksu, Z. & Akpınar, D. (2001). Competitive biosorption of phenol and chromium(VI) from binary mixtures onto dried anaerobic activated sludge, *Biochem. Eng. J.* 7, 183–193. DOI: 10.1016/S1369-703X(00)00126-1.
- Navarro, A.E., Portales, R.F., Sun-Kou, M.R. & Llanos, B.P. (2008). Effect of pH on phenol biosorption by marine seaweeds, *J. Hazard. Mater.* 156, 405–411. DOI: 10.1016/j.jhazmat.2007.12.039.
- Kumar, N.S. & Min, K. (2011). Phenolic compounds biosorption onto Schizophyllum commune fungus: FTIR analysis, kinetics and adsorption isotherms modelling, *Chem. Eng. J.* 168, 562–571. DOI: 10.1016/j.cej.2011.01.023.
- Aksu, Z. & Yener, J. (1998). Investigation of the biosorption of phenol and monochlorinated phenols on the dried activated sludge, *Process. Biochem.* 33 (6), 649–655. DOI: 10.1016/S0032-9592(98)00029-6.
- Rao, J.R. & Viraraghavan, T. (2002). Biosorption of phenol from an aqueous solution by Aspergillus niger biomass, *Bioresour. Technol.* 85, 165–171. DOI: 10.1016/S0960-8524(02)00079-2.
- Volesky, B. (2003). Sorption and Biosorption, Bv sorbex Inc., Montreal, 35–50.
- Aksu, Z. & Akpınar, D. (2000). Modelling of simultaneous biosorption of phenol and nickel(II) onto dried aerobic activated sludge, *Sep. Purif. Technol.* 21, 87–99. DOI:10.1016/S1383-5866(00)00194-5.
- Auta, M. & Hameed, B.H. (2011). Preparation of waste tea activated carbon using potassium acetate as an activating

agent for adsorption of Acid Blue 25 dye, *Chem. Eng. J.* 171, 502–509. DOI: 10.1016/j.cej.2011.04.017.

9. Wu, J. & Yu, H.Q.(2006). Biosorption of phenol and chlorophenols from aqueous solutions by fungal mycelia, *Proces. Biochem.* 41, 44–49. DOI: 10.1016/j.procbio.2005.03.065.

10. Aksu, Z. & Yener, J. (2001). A comparative adsorption/biosorption study of mono-chlorinated phenols onto various sorbents, *Waste Manage.* 21, 695–702. DOI: 10.1016/S0956-053X(01)00006-X.

11. Giahi, M., Rakhshae, R. & Bagherinia, M.A. (2011). Removal of methylene blue by tea wastages from the synthesis waste waters, *Chinese Chem. Lett.* 22, 225–228. DOI: 10.1016/j.ccl.2010.07.030.

12. Murugesan, G.S., Sathishkumar, M. & Swaminathan, K. (2006). Arsenic removal from groundwater by pretreated waste tea fungal biomass, *Bioresour. Technol.* 97, 483–487. DOI: 10.1016/j.biortech.2005.03.008.

13. Hameed, B.H. (2009). Spent tea leaves: A new non-conventional and low-cost adsorbent for removal of basic dye from aqueous solutions, *J. Hazard. Mater.* 161, 753–759. DOI: 10.1016/j.jhazmat.2008.04.019.

14. Sanga, S., Lambert, J.D., Hoc, C.T. & Yang, C.S. (2011). The chemistry and biotransformation of tea constituents, *Pharmacol. Res.* 64, 87–99. DOI: 10.1016/j.phrs.2011.02.007.

15. Kazmi, M., Feroze, N., Naveed, S. & Javed, S.H. (2011). Biosorption of copper(II) on prunus amygdalus shell: Characterization, biosorption size analysis, kinetics, equilibrium and mechanistic studies. *Korean J. Chem. Eng.*, 28 (10), 2033–2040. DOI: 10.1007/s11814-011-0072-y.

16. Feroze, N., Kazmi, M. & Ramzan, N. (2013). Dead immobilized *Rhizopus Arrhizus* as potential biosorbent for copper removal. *Korean J. Chem. Eng.*, 30 (1), 95–100. DOI: 10.1007/s11814-012-0088-y.

17. Nie, S.P. & Xie, M.Y. (2011). A review on the isolation and structure of tea polysaccharides and their bioactivities, *Food Hydrocolloid.* 25, 144–149. DOI: 10.1016/j.foodhyd.2010.04.010.

18. Karak, T. & Bhagat, R.M. (2010). Trace elements in tea leaves, made tea and tea infusion: A review, *Food Res. Int.* 43, 2234–2252. DOI: 10.1016/j.foodres.2010.08.010.

19. Malkoc, E. & Nuhoglu, Y. (2006). Fixed bed studies for the sorption of chromium(VI) onto tea factory waste, *Chem. Eng. Sci.* 61, 4363–4372. DOI:10.1016/j.ces.2006.02.005.

20. Ho, Y.S. (2006). Review of second – order models for adsorption systems. *J. Hazard. Mater.*, B136, 681–689. DOI: 10.1016/j.jhazmat.2005.12.043.

21. Langmuir, I. (1918). The adsorption of gases on plane surfaces of glass, mica and platinum, *J. Am. Chem. Soc.* 40, 1361–1403. DOI: 10.1021/ja02242a004.

22. Freundlich, H. (1906). Über die adsorption in lösungen (Adsorption in solution), *Z. Phys. Chem.* 57, 384–470 [in German].

23. Dahlquist, E.W. (1978). The meaning of Scatchard and Hill plots, in: CHW Hirs, SN Timmaseff (Eds.), *Methods of Enzymology*, Academic press, New York, 270 – 299.