



Synergy of Granular Activated Carbon and Anaerobic Mixed Culture in Phenol Bioremediation of Aqueous Solution

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A B S T R A C T

The present study focused on the synergistic effects bioremediation of phenol in aqueous solution using combination of anaerobic mixed culture and Granular Activated Carbon (GAC) as a biological GAC (BGAC). Meanwhile, the effect of contact time and various phenol concentrations on adsorption and biosorption process investigated. The phenol concentration was analyzed using UV/Vis spectrophotometer. The morphology and structure of two adsorbents (GAC and BGAC) were characterized by FESEM and BET specific surface area analysis. The batch experiments using mixed bacterial culture, isolated from wood and paper factory wastewater, were adapted to high concentrations of phenol and employed in order to evaluate the tolerance and biosorption capability of microorganisms for phenol biodegradation. The synergetic effect of phenol removal using combination of GAC with an anaerobic biofilm indicated that the removal efficiency for concentration of 700, 800, and 1000 mg/l at initial stages increased to 4, 10, and 12%, respectively and while by increment of the retention time did not shown significant impact on the removal efficiency. These results conducted both desorption of adsorbates due to biotransformation in the aqueous solution and direct assimilation of adsorbates on GAC by the microorganism's biofilm. The adsorption data were fitted with pseudo-first-order and pseudo-second-order models and it was found that the pseudo-second-order model explains the adsorption kinetics more efficiently. The compatibility of the Freundlich and Langmuir adsorption models to equilibrium data were investigated. In fact, the Langmuir isotherm was found to be the best fitting isotherm.

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INTRODUCTION

Phenolic compounds are priority pollutants with high toxicity even at low concentrations and beside the toxic effects, phenolic compounds create an oxygen demand in receiving waters, and impart taste and odor to water with minute concentration of their chlorinated compounds [1]. Phenols and phenolic compounds are abundantly found in many industrial effluents such as oil refining, petrochemicals, pharmaceuticals, coking operation, resin manufacturing, plastics, fiberglass manufacturing, herbicide production, paint, pulp, paper and wood products [2, 3]. The phenolic compounds from industrial wastewater (about 200- 2000 mg/L) were released into aquatic environment higher than the standard limits (mostly less than 0,5 mg/L) [4]. Phenol has hazardous health effects that can be both acute and chronic. Long-term exposure to phenol can cause a variety of adverse health impacts, including irregular breathing, muscles weakness, tremor, coma, and respiratory arrest if exposed to lethal doses in humans. Short-term exposure to phenol

can lead to irritation of the skin, burning eyes, head-aches and mucous membranes. Some of the chronic effects caused by phenol exposure are anorexia, weight loss, diarrhea, vertigo, salivation and a dark coloration of the urine. Thus, treatment of phenol is required before disposal of wastewater to the receiving environments [5, 6].

A wide variety of methods have been proposed for the treatment of wastewater contaminated with phenolic compounds like oxidation [7], precipitation [8], ion exchange, solvent extraction [9], evaporation [10, 11], electrocoagulation [12] and adsorption [13, 14]. Adsorption is one of the commonly used methods for the removal of pollutants due to easy application and economical saving potential. There are many adsorbents in use such as sawdust [4], rice husk [15], wood charcoal [16], wheat husk [17], chitosan beads [18, 19], red mud [20], bagasse fly ash [21], and peat and bentonite [22]. Activated carbon due to its high adsorption capacity and fast adsorption kinetics is used as an adsorbent to remove a wide range of contaminants from various matrices [23].

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Activated carbon not only functions as media for bacterial attachment, but can also work as adsorbent; therefore, combination of activated carbon adsorption with the biological process provides a possibility of enhancing the function of the biological process. Apart from adsorbing the micro-pollutants, GAC also functions as a carrier material for biofilm. As a result, micro-pollutants are concentrated on the GAC surface, which results in an increased contact time between the compounds and microorganisms [24]. Biodegradation is therefore an alternative pathway for removing micro-pollutants in GAC applications. A micro-pollutant will be adsorbed on GAC surface and eventually be biodegraded depends on the reversibility of adsorption and on the potential of the biofilm to biodegrade the micro-pollutant [25].

The aims of this research were to compare the adsorption and biosorption of high phenol concentrations using Granular Activated Carbon (GAC) and Biological GAC (BGAC) in anaerobic slurry batch reactor and their synergistic effects. Also, the effect of initial phenol concentration and contact time on the adsorption/biosorption process were evaluated. In order to demonstrate the sorption mechanism, the kinetic parameters were calculated using pseudo-first-order and pseudo-second-order kinetic models. In addition, by fitting the experimental data to Freundlich and Langmuir isotherms the adsorption equilibrium was evaluated. The morphology and structure of two adsorbent (GAC and BGAC) were characterized by FESEM and BET specific surface area analysis.

MATERIALS AND METHODS

Materials

For comparison of adsorption and biosorption of phenol degradation, two anaerobic slurry batch reactors with GAC and BGAC were operated. Synthetic contaminated wastewater mixed on magnetic stirrer and continuously fed to the reactor by the use of peristaltic pump at a constant flow rate of 30 mL/min. The synthetic contaminated wastewater using phenol as the contaminant was initially prepared by diluting the appropriate amount of phenol with distilled water. The influent concentration of phenol was varied at high initial concentrations, 700, 800, and 1000, mg /L.

An anaerobic mixed culture, isolated from wood and paper factory wastewater and adapted to high concentrations of phenol and employed in order to evaluate the capability of microorganisms for phenol biodegradation. The composition of synthetic wastewater summarized in Table 1. All chemicals were obtained from Merck (Darmstadt, Germany).

TABLE 1. Components of synthetic wastewater

Components	Value (g/L)	Components	Value (g/L)
NaCH ₃ COO	1.6	Yeast Extract	0.1
K ₂ HPO ₄	1.07	ZnSO ₄ .7H ₂ O	0.1
KH ₂ PO ₄	0.53	MnCl ₂ .4H ₂ O	0.03
NH ₄ Cl	0.15	CoCl ₂ .6H ₂ O	0.2
NaCl	0.5	NiCl ₂ .6H ₂ O	0.02
MgSO ₄	0.015	CuSO ₄ .5H ₂ O	0.01
CaCl ₂	0.02	H ₃ BO ₃	0.3

Characterization

The microstructure of used GAC was characterized before and after phenol adsorption and microbial attachment by using Field Emission Scanning Electron Microscope (FESEM). Furthermore, BET (Brunauer-Emmett-Teller) surface analysis was performed in order to determine the specific surface area and porosities of the granular activated carbon.

Batch bioremediation studies

To evaluate the capability of microorganism for phenol degradation, batch experiments were performed for initial concentrations of 700, 800, and 1000 mg/L using 250 mL Erlenmeyer flasks (containing 20 mL of sludge and 80 mL of aqueous phenol solution). All flasks were continuously stirred in an incubator at 110 rpm and temperature of 35±2 °C. For determining residual concentration of phenol, samples were centrifuged at 3000 rpm for 20 minutes and then the supernatant solution was analyzed for residual concentration of phenol with the use of direct photometric method. According to the standards in this method, a red coloration resulted from the rapid condensation of 4-aminoantipyrine followed by oxidation with alkaline potassium ferricyanide can be detected at wave-length of 500 nm by using spectrophotometer [26].

Adaptation of microorganisms

Stepwise increase in phenol concentration was implemented during adaptation period. For adaptation of microorganisms, 100 mL of artificial waste medium was inoculated with 20 mL of sludge in four different 250 mL Erlenmeyer flasks (the flasks were continuously stirred in an incubator at 110 rpm and temperature of 35 °C). Every 48 hours the sludge was sedimented for 15 minutes and 50 mL of the supernatant and then replaced with fresh medium. The phenol concentration during this procedure increased slightly by replacing 10 % of the carbon source of the medium with 10 % of phenol as the sole carbon source in 2 days intervals.

Adsorption procedure

Adsorption measurements were obtained in slurry batch reactor at $35 \text{ }^\circ\text{C} \pm 1$. In this process, 5.5 g of adsorbent was used in 250 mL of phenol solution with different initial concentrations 700 to 1000 mg L⁻¹. These flasks placed on the stirrer and incubated. Samples were withdrawn for determination of residual concentration of phenol at appropriate time intervals and centrifuged for 20 minutes at 3000 RPM, then the supernatant solution was analyzed for unadsorbed phenol concentration left in the solution using direct photometric method at wavelength of 500 nm. The amount of phenol adsorbed by 1 gram of the BGAC at time, t was calculated by Equation (1). Also, phenol removal efficiency i.e., the percentage of adsorption was calculated by the Equation (2).

$$q_t = \frac{(C_0 - C_t)V}{m} \quad (1)$$

$$R = \frac{(C_0 - C)}{C_0} \times 100 \quad (2)$$

where q_t is the amount of phenol adsorbed by BGAC (mg g⁻¹), C_0 is the initial phenol concentration (mg/L), C_t is the concentration of phenol at any time, V is the volume of solution (L) and m is the mass of activated carbon (g).

Adsorption kinetics

The reaction rate and the mechanism are two vital evaluation elements for an adsorption process. In this respect several kinetic models including the pseudo-first-order kinetic model and pseudo-second-order kinetic model were investigated [27, 28]. The kinetic model for pseudo-first-order is expressed as follows:

$$\log(q_e - q_t) = \log q_e - \frac{k}{2.303} t \quad (3)$$

where q_t is the amount of adsorbate adsorbed on the adsorbent at time t, q_e is the amount of adsorbate adsorbed on the adsorbent at equilibrium time, k is the pseudo-first-order rate constant and t is the contact time. The adsorption rate constant and the adsorption capacity at equilibrium can be obtained from the slope and intercept of the plot of $\log(q_e - q_t)$ against t, respectively.

Pseudo-second-order-kinetic model used for analysis of adsorption kinetic data is the pseudo- second-order kinetic model. Throughout the adsorption the model is compatible with the mechanism of rate-controlling step. The model is usually represented by its linear form as Equation (4):

$$\frac{t}{q_t} = \frac{1}{K q_e^2} + \frac{t}{q_e} \quad (4)$$

where k is the pseudo-second-order rate constant, q_e and k can be obtained from the slope and intercept of a plot between $\frac{t}{q_t}$ versus t, respectively.

Adsorption isotherm

Two commonly used adsorption models to describe experimental data of adsorption isotherms are the Freundlich [29] and Langmuir [30] models.

Freundlich isotherm

The Freundlich model is commonly used to describe the multi-layer adsorption characteristics for the heterogeneous surface. The Equation (5) is the empirical equation proposed by Freundlich. The linear form can be expressed as Equation (6):

$$q_e = K_f \cdot C_e^{(1/n)} \quad (5)$$

$$\ln q_e = 1/n \ln C_e + \ln K_f \quad (6)$$

where q_e is the amount of phenol adsorbed per unit of adsorbent at equilibrium (mg g⁻¹), C_e is equilibrium concentration of phenol, n and K_f are the Freundlich constants that show the adsorption capacity and intensity and can be obtained respectively from the slope and intercept of the linear plot of experimental data $\ln q_e$ versus $\ln C_e$.

Langmuir isotherm

The Langmuir model is described by the Equation (7).

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \quad (7)$$

The linear form can be expressed as Equation (8).

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m} \quad (8)$$

where q_e is the amount of phenol adsorbed per gram of the adsorbent at equilibrium (mg g⁻¹) and C_e is equilibrium concentration in solution. The Langmuir constants q_m and K_L can be determined from the slope and intercept of the linear plot of experimental data of $\frac{C_e}{q_e}$ versus C_e . The q_m gives the maximum single layer adsorption capacity of adsorbent. The Langmuir isotherm cannot clearly describe the equilibrium state for the heterogeneous adsorption system. Another essential characteristics of the Langmuir isotherm is the term " R_L " which is called separation factor or dimensionless equilibrium parameter [31] represented by the Equation (9).

$$R_L = \frac{1}{1 + K_L C_0} \quad (9)$$

where C_0 is the initial concentration. K_L is the Langmuir constant. The separation factor R_L gives valuable information about the nature of adsorption. There are four possibilities for the R_L value:

If the value of R_L is between 0 and 1 the adsorption is favourable, while $R_L > 1$ represents unfavourable adsorption, $R_L = 1$ indicates linearity of adsorption and adsorption is irreversible in case of $R_L = 0$.

RESULTS AND DISCUSSION

Characterization results

Field emission scanning electron microscope (FESEM) images of different magnifications are presented in Figure 1. The availability of various pore sizes and a large surface area is clearly displayed in the pure GAC image. As can be seen GAC has a porous structure and a crystalline nature. However, as shown in the figure pores have disappeared due to microbial attachment. Figure 1 shows that there was an accumulation of microorganisms inside the pores. The BET surface area was obtained from N₂ adsorption isotherms at 77k. The BET surface area, total pore volume and average pore diameter of granular activated carbon was defined to be 785.6 m²g⁻¹, 0.398 cm³g⁻¹, 2.026 nm, respectively. According to IUPAC classification GAC with average pore diameter of 2.026 nm has a mesoporous nature [32].

Effect of initial concentration of phenol

The performance of microorganism to biodegrade phenol

was evaluated by monitoring phenol removal in batch experiments at initial phenol concentrations of 700, 800, and 1000 mg/L by using 250 mL Erlenmeyer. Figure 2 indicates that in batch reactor, phenol concentration decreased with increasing time and the extent of phenol degradation and time required depends on initial phenol concentration. Also high initial phenol concentrations with equal amount of initial biomass need more time to be consumed.

The effect of contact time on the removal efficiency of phenol by BGAC is shown in Figure 3. The results indicated that 84 to 90% of phenol was adsorbed within a period of initial 2 hours, and that the removal efficiency increases with increasing contact time. As can be seen, equilibrium is reached within 12 hours for concentrations in range of 700 to 1000mg/L and the curves are smooth and continue leading to saturation. As can be seen, at the initial period of time the adsorption process can be considered very fast because of high phenol removal efficiency, this can be due to the fact that initially more adsorbent sites were vacant and the solute concentration gradient is high.

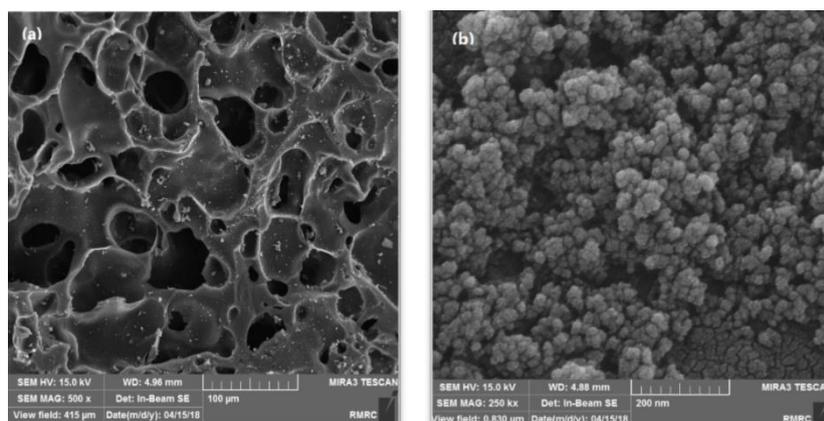


Figure 1. FESEM images of the GAC surface (a) Without biofilm, (b) After biofilm formation

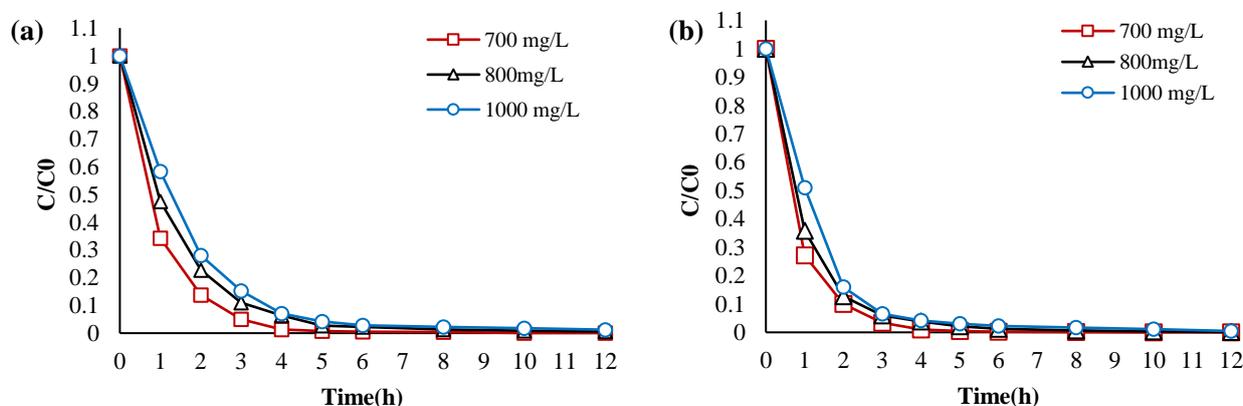


Figure 2. Variation of phenol adsorption and biosorption at different initial concentrations with a)GAC, and b)BGAC

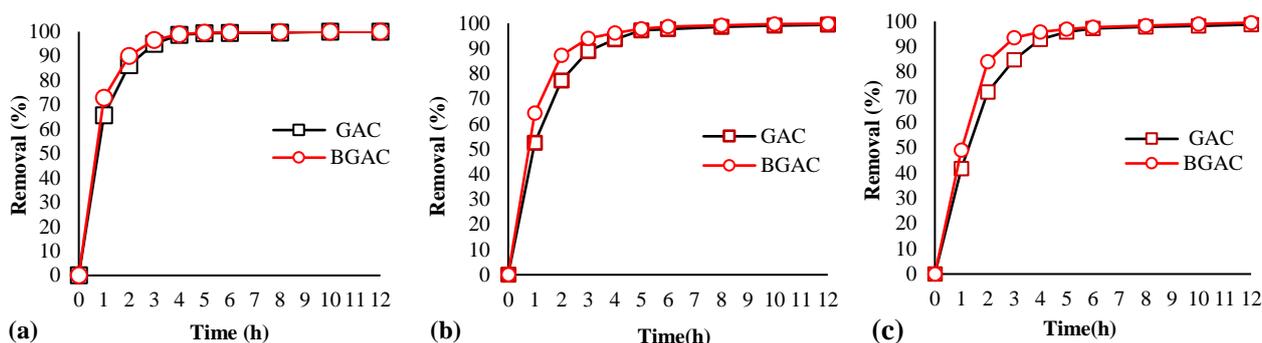


Figure 3. Removal efficiency of phenol biodegradation with GAC and BGAC at different initial concentrations (mg/l) a) 700, b) 800, and c) 1000

Kinetic adsorption experiments

Effect of contact time and initial phenol concentration on adsorption kinetics

The effect of contact time on the biosorption of phenol at three different ranges of initial concentrations onto BGAC was determined and the results are shown in Figure 4. As can be seen the amount of adsorption i.e., mg of adsorbate per gram of adsorbent increases with increasing contact time at all initial phenol concentrations, and by increasing initial concentrations, the time to reach equilibrium increases. It is also found from the figure that at the initial period of contact time the adsorption intensity is high but it decreases over time for all concentrations. Figure 4 Shows that for three different initial concentrations in range of 700 to 1000 mg /L the adsorption equilibrium was reached.

Adsorption kinetics

As different systems conform to different models, in this study the applicability of the pseudo- first-order model and pseudo-second-order model were investigated for the adsorption/biosorption of phenol onto BGAC. The correlation of $\log(q_e - q)$ versus time (t) for the first-order- kinetic model and $\frac{t}{q_t}$ versus t for the second-order-

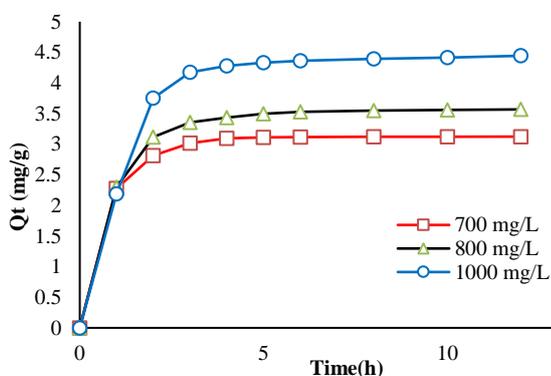


Figure 4. Effect of contact time on phenol adsorption by BGAC at different phenol concentrations

kinetic model were shown in Figure 5 and Figure 6. The results and data obtained for different phenol concentrations are presented in Table 2. As can be seen from the table both models describe the data well but the pseudo-second-order kinetic model with higher correlation coefficient R^2 close to unity is more applicable to phenol adsorption on BGAC, therefore the rate controlling step might be chemical reaction [28].

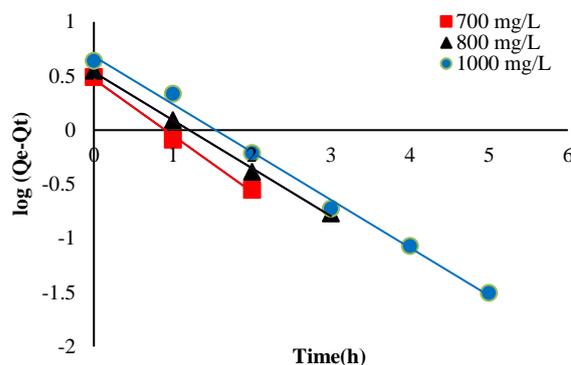


Figure 5. Pseudo-first-order-kinetic model of phenol adsorption at different phenol concentrations

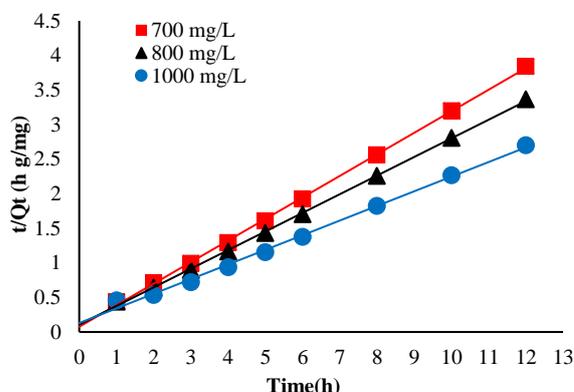


Figure 6. Pseudo-second-order kinetic model of phenol adsorption at different phenol concentrations

TABLE 2. Constants of kinetic models for phenol adsorption onto BGAC

C (mg/L)	Pseudo-first-order model			Pseudo-second-order model	
	q_e (mg g ⁻¹)	K_1 (h ⁻¹)	R ²	K_2 (g mg ⁻¹ h ⁻¹)	R ²
700	3.093	1.19	0.9959	1.393	0.9994
800	3.52	1.02	0.998	0.758	0.9992
1000	4.36	1.01	0.994	0.406	0.9958

Similar phenomena have also been observed in removal of Congo Red using activated carbon prepared from agricultural waste [33].

Evaluation of adsorption isotherms

Adsorption isotherms were used to demonstrate the relation between the equilibrium concentrations of adsorbate adsorbed per gram of adsorbent at equilibrium. In this study adsorption of phenol onto BGAC has been evaluated with the most frequently used models Langmuir and Freundlich (two ranges of phenol concentration has been considered). The most widely used linear forms of Langmuir and Freundlich models are presented in Table 3.

Linear isotherm

Values of Langmuir and Freundlich constants were shown in Table 4. As can be seen in this table, correlation coefficient values are high, showing a good linearity between experimental data and linearized form of isotherm. Comparing the correlation coefficient, it is clear that the Freundlich isotherm fits the experimental

data better than the Langmuir isotherm, because of the higher values of correlation coefficient whereas there is a good agreement between the experimental value and predicted value of Langmuir isotherm because of lower average related error, suggesting that this model is more suitable for describing the experimental equilibrium data.

Langmuir isotherm constant K_L , which is related to the adsorption energy has been calculated from the intercept of Langmuir plot, the calculated K_L values are 0.412 L mg⁻¹ for high phenol concentrations. Another significant Langmuir constant R_L which indicates the nature of adsorption was found to be less than 1 and greater than 0 representing a favourable adsorption.

K_f and $1/n$ are Freundlich constants indicated the adsorbent capacity and the surface heterogeneity that can be calculated from the slope and intercept of Freundlich plot respectively, the value of $1/n$ were found to be less than 1, suggesting a favourable adsorption behaviour of phenol onto BGAC.

TABLE 3. Isotherms and its linearized expressions

Isotherms	Linear expression	Plot	Parameters
Freundlich	$\ln q_e = 1/n \ln C_e + \ln K_f$	$\ln q_e$ vs. $\ln C_e$	$K_f, 1/n$
Langmuir	$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m}$	$\frac{C_e}{q_e}$ vs. C_e	K_L, q_m

TABLE 4. Parameters of the Langmuir and Freundlich isotherms

Isotherm	Langmuir			Freundlich			
	Parameter	q_m (mg/g)	K_L	R ²	n	K_f	R ²
Value		4.686	0.4122	0.9939	6.406	2.53	0.9581

CONCLUSION

The present study shows that phenolic contaminated wastewater was effectively treated in anaerobic slurry batch reactor with GAC and combined with anaerobic bacteria attached on GAC as BGAC. FESEM images revealed biofilm coverage of BGAC. The effect of contact time and various phenol concentrations on the

adsorption process has been determined and the results showed that the removal of phenol increases by increasing contact time and equilibrium is reached within 4 hours for concentration in 700 mg/L and in 6 hours for concentrations in 800 and 1000 mg/L. The kinetic studies showed that the phenol adsorption process followed pseudo-second-order model, indicating that the rate-limiting step of the process might be chemical reaction.

The equilibrium data were fitted to the most frequently forms of Freundlich and Langmuir models and the results indicate that the Langmuir model is more suitable for explaining the equilibrium data than the Freundlich model. The results also demonstrate that Langmuir and Freundlich constants R_L and n , both give an indication of favourable adsorption of phenol onto BGAC.

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Persian Abstract

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چکیده

مطالعه حاضر بر روی اثرات هم‌افزایی کربن فعال گرانولی (GAC) و کشت باکتری مخلوط بی‌هوازی به عنوان BGAC در زیست پالایی محلول آبی فنل متمرکز شده است. در همین حال، اثر زمان تماس و غلظت فنل بر روی فرآیند مختلف جذب و جذب زیستی بررسی شده است. غلظت فنل، قبل و بعد از تصفیه با استفاده از دستگاه اسپکتروفتومتر UV/VIS مورد تجزیه و تحلیل قرار گرفت. مورفولوژی و ساختار دو جاذب GAC و BGAC با تجزیه و تحلیل FESEM و سطح ویژه با استفاده از BET مشخص شد. آزمایش‌های سیستم بسته با استفاده از کشت باکتری مخلوط، جدا شده از فاضلاب کارخانه چوب و کاغذ، سازگار شده با غلظت‌های بالای فنول و به منظور ارزیابی میزان تحمل و قابلیت جذب بیولوژیکی میکروارگانیسم‌ها برای تجزیه بیولوژیکی فنل استفاده شد. اثر هم‌افزایی حذف فنل با استفاده از ترکیب GAC با بیوفیلیم بی‌هوازی نشان داد که راندمان حذف غلظت‌های ۷۰۰، ۸۰۰ و ۱۰۰۰ در مراحل اولیه به ترتیب به میزان ۴، ۱۰ و ۱۲ درصد افزایش می‌یابد در حالی که با افزایش زمان ماند تأثیر قابل توجهی بر راندمان حذف نشان نمی‌دهد. این نتیجه هم جذب جاذب ناشی از انتقال بیولوژیک به محلول آبی و هم جذب مستقیم روی جاذب GAC توسط بیوفیلیم میکروارگانیسم‌ها را نشان می‌دهد. داده‌های جذب با مدل مرتبه اول و مرتبه دوم برازش داده شدند و مشخص شد که مدل مرتبه اول سینتیک جذب را با کارایی بیشتری توضیح می‌دهد. مدل‌های سینتیک جذب فروندلیچ و لانگمویر با سازگاری داده‌ها با استفاده از روش‌های خطی بررسی شد. ایزوترم لانگمویر به عنوان ایزوترم مناسب تعیین گردید.