
Application of Fungal Biofilm Supported on Activated Carbon for Adsorption of Two Azodyes: Adsorption Kinetics and Isotherms

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Abstract: Waste effluents from textile industries, newspaper printing and photography contain residues of dyes and chemicals. Synthetic dyes present in water bodies, even at very low concentrations, can be extremely toxic to the living organisms due to cutting the development of bacteria and preventing the photosynthesis in aqueous flora. Acid Blue 25 (AB25) and Pigment Yellow 101 (PY101) are two types of azodyes which are toxic organic pollutants that consist of both N=N and C=C chromophoric groups. They cause considerable health effects being irritating to skin, eye and respiratory system and in some cases they may cause cancers. In this study, a biofilm of *Aspergillus terreus* supported on activated carbon was used as a biological tool for removal of these dyes from water body. The adsorption kinetics and contact time were determined along 240 minutes (30 minute interval). The initial concentration of dyes was 100 mg/L for each one. The biomass dosage was 1g / L. The optimum pH value for adsorption process was 3.0. The results depicted that the contact time for biosorption of both dyes was 180 min while adsorption kinetics of was quietly fitted with Pseudo second-order kinetics equation models. The maximum adsorption capacity was 82.3 and 78.2 mg/g for AB 25 and PY 101, respectively. Freundlich model was a good model for adsorption isotherm of two dyes on biofilm.

Keywords: Adsorption, *Aspergillus terreus*, Acid Blue 25, Pigment Yellow 101, Kinetics

1. Introduction

The huge number of azodyes are widely produced annually. Dyestuff have variable application in the paper industry, textile, printing and dyehouses. Acid Blue 25 (AB 25) is one of the most applicable anthraquinone azodye in the field of wool and leather dyeing [1]. Pigment Yellow 101 is considered the major fluorescent dye that have limited usage in printing inks viscose dying [2].

The pollution of water resources with industrial effluents containing organic compounds and toxic substances is a matter of great concerns. Industrial dyes are one of aromatic toxic compounds presents in water streams. They cause huge harms to all biota and human due to their complex aromatic structures, resistant to the light and moderate oxidizing agents, high stability and they are usually biologically non-degradable. The industrial dyes may cause skin irritation, mutagenicity and carcinogenicity [3, 4]. Thus due to high toxicity, it is necessary to get rid of organic pollutants by

proper treatment techniques.

Adsorption has been shown to be the most promising option for all these non-biodegradable organics for the removal from aqueous streams, activated carbons (AC) being the most common adsorbent for this process due to its effectiveness and versatility [5]. By an adsorption process the dye species are transferred to a solid phase (adsorbent) and subsequently can be recovered by a desorption process and stored in a dry place without direct contact with the environment [6]

Activated carbon is the most widely used adsorbent for the removal of color and treatment of textile effluents but due to its high price it is not used on a great scale [7-11]. This has led many workers to search for the use of cheap and efficient alternative materials such as bagasse pith, carbonized bark, peat, soil, tree and eucalyptus barks, chitosan, rice husk, wood and fly ash [12-14].

The use of microorganisms as biosorbents is an attractive and alternative methods for toxicity reduction and removal of dyes from industrial effluents because of their good performance and low cost. Dye adsorption studies using microorganisms have been carried out by many researchers. Some microorganisms that have been used as dye adsorbents are *Trametes versicolor* [15], *Rhizopus arrhizus* [16], *Penicillium* sp [17] *Aspergillus fumigatus* [5], and bacterial consortium [18].

The main objective of this study was (i) determine the capacity of *Aspergillus terreus* biofilm supported on activated carbon for adsorption of azodyes, (ii) detect the adsorption kinetics, and the isotherms

2. Materials and Methods

2.1. Adsorbate Preparation

AB 25 and PY 101 were prepared by dissolving 0.1% of each one in deionized water separately to detect the maximum absorbance of dyes using UV-Vis Spectrophotometer. Then a serial dilution of each dye from (5-500 mg/L) were prepared to construct calibration curve at λ max.

2.2. Fungal Strain and Biosorbent Preparation

AUMC-050 is a fungal isolate that was originally isolated from the textile dye effluent sample. The isolate was identified by Assiut University Mycological Center (AUMC) as *Aspergillus terreus* based on the color of isolate on solid medium and some biochemical tests. This isolate was listed in the AUMC culture collection as AUMC-050.

The strain AUMC-050 was cultivated on MBS liquid medium amended with 0.2 % glucose under static condition at 28°C 7-days. The fungal biomass was collected by filtration, washed, oven-dried at 80° C for 24 h, powdered and used as a biosorbent. A 20 mg dry weight of the fungal biomass was suspended in 20 mL of deionized water containing either (100 mg/L) AB 25 or PY 101.

2.3. Biofilm Preparation

The biofilm was prepared by shaking method. Granulated activated carbon (AC) (0.5g) and AUMC-050 cells (0.5g) were mixed in deionized water (500 ml) and incubated at 37 °C for 72 hours under shaking followed by centrifugation, the pellet was dried in an oven at 50 °C, overnight to ensure that the sample was complete drying [19, 20].

2.4. Effect of Contact Time and pH on the Biosorption

A 20 mg of *A. terreus* biofilm was suspended in 20 ml deionized water consists of AB 25 or PY 101 (100 mg/L). Adsorption was carried out at 30°C; pH 7.0 ± 0.2; 150 rpm shaking and for 4 hr. Deionized water containing (100mg/L)

dye served as negative control. Samples were withdrawn each 30 minute intervals under aseptic condition. The samples were centrifuged at 10000 rpm for 15 min and dye concentration of supernatant was determined at λ max of each dye by UV-Vis spectrophotometer

The optimum pH values for biosorption of dyed were determined by analyzing the amount adsorbed (mg/g) of 20 ml AB 25 or PY 101 (100 mg/L for each) on 20 mg of biosorbent over a range of pH 3.0 to 8.5. The pH was adjusted by 1M of KOH and/or 1M of HCl.

The amount of dye adsorbed at equilibrium q_e (mg/g) was analyzed by the following equation:

$$q_e = (C_0 - C_t)V/m \quad (1)$$

Where C_0 is the initial concentration of dye (mg/L), C_t is the concentration of dye at time t (mg/L), V is the total volume of the suspension (L), and m is the mass of adsorbent (g).

2.5. Biosorption Kinetics

In order to investigate the mechanism of biosorption and potential rate controlling steps such as mass transport and chemical reaction processes, kinetic models have been used to test experimental data at constant pH, temperature, dye concentration and contact time. The pseudo first-order and pseudo second-order equations were used in this case, assuming that measured concentrations are equal to cell surface concentrations.

2.6. Biosorption Isotherm

Adsorption isotherm was carried out by using different concentration of either AB 25 or PY 101 (25, 50, 100,150, 200 and 300 mg/L) at optimal pH and at 30 °C. The Langmuir and Freundlich models were applied for determine the most suitable one for describe biosorption isotherm of two used dyes at constant temperature.

3. Results

3.1. Removal of Different Azodyes by Different Adsorbent

The strain AUMC-050, AC and Biofilm of AUMC-050 supported on AC were separately tested for biosorption of either AB 25 or PY 101 (100 mg /L).

The percentage of AB 25 adsorption capacity on AUMC-050, AC and Biofilm after 210 min were 52, 70, 82 % (Fig.1), while the percentage of PY 101 removal were 42.1, 56, 78.1%, respectively (Fig. 2).

These results depicted that, the biofilm of *A. terreus* AUMC-050 supported on AC was the most efficient biosorbent for biosorption of AB 25 and PY 101. So, this biofilm was selected for detailed biosorption studies.

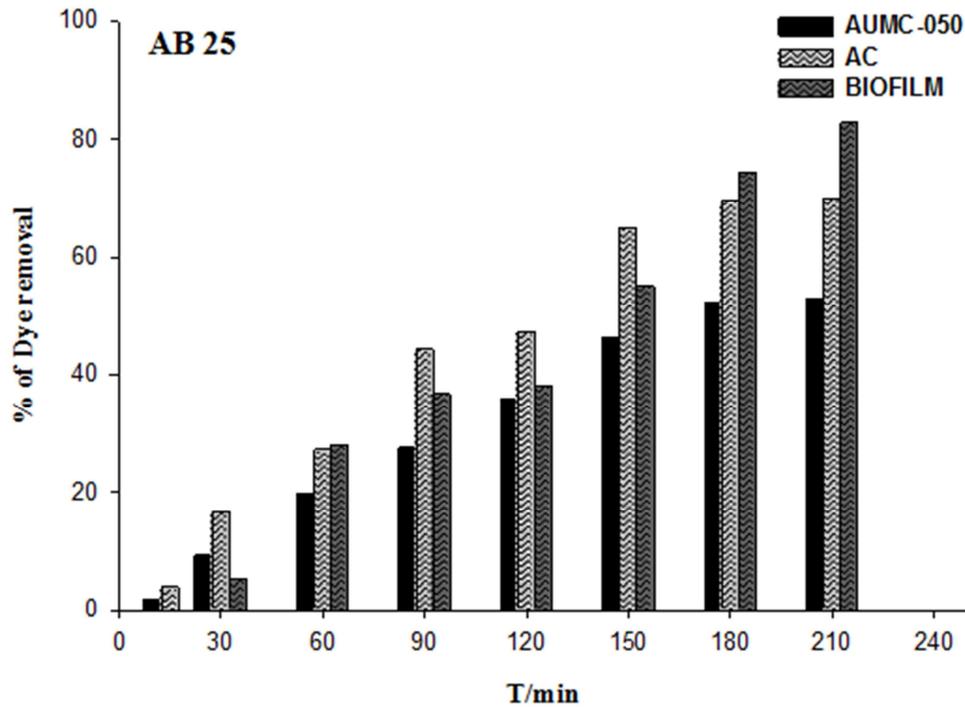


Fig. 1. The percentage of AB 25 removal by AUMC-050, AC and a biofilm at initial dye concentration (100mg/L) and temperature 30°C.

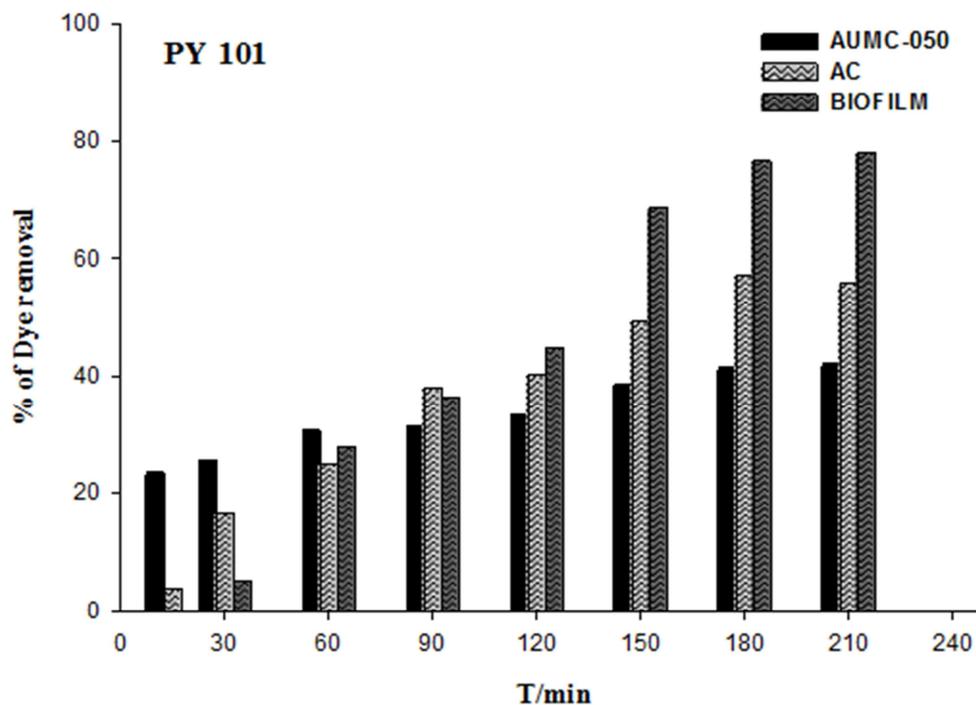


Fig. 2. The percentage of PY 101 removal by AUMC-050, AC and a biofilm at initial dye concentration (100mg/L) and temperature 30°C.

3.2. Effect of Initial pH on Biosorption Capacity

The interaction between pH of the solution, biosorbent surface properties and adsorbate charges controlled the biosorption process. The amount of adsorbed dye (mg/g) was plotted versus the pH values from (3.0 to 9). It was observed

that, at low pH value (3.0), the maximum adsorption capacity of AB25 and PY101 was achieved (75.8 and 71.3 mg dye /g), respectively. This level dropped gradually by increasing the pH value. The minimum value of adsorption capacity was recorded at alkaline pH values (9.0) (Fig. 3).

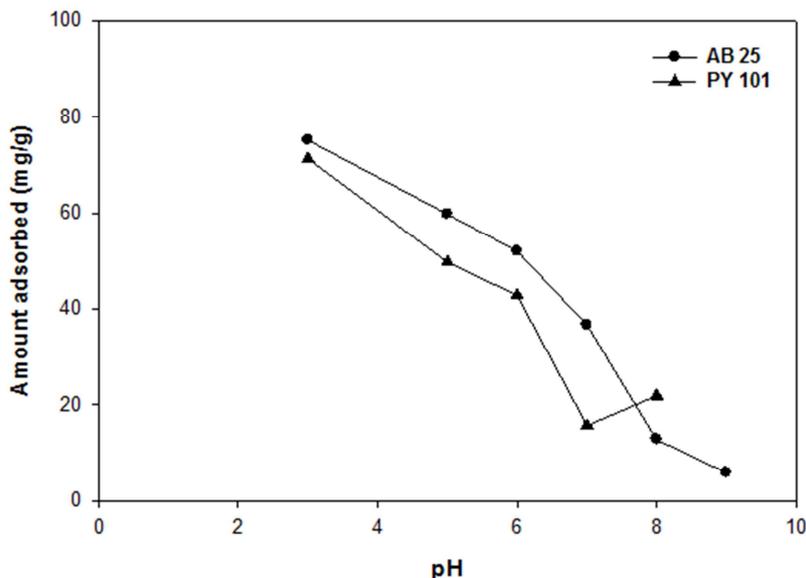


Fig. 3. Effect of pH on the biosorption of AB 25 and PY 101 on biofilm of *A. terreus* supported on AC. Initial dye concentration (100mg/L); Temperature 30 °C.

3.3. Effect of Temperature

As various textile dye effluents are discharged at relatively high temperatures (50–60 °C), so temperature is an important design parameter affecting the biosorption capacity in the real application of biosorption by biomass [21]. To investigate the effect of temperature on the maximum biosorption capacity of AB25 and PY101 onto the dried biofilm, five constant temperatures of 10, 20, 30, 40, 50 and 60 °C were studied. As shown in Fig.5, the ability of biofilm

to adsorb the AB 25 was exceeded its ability to PY101 adsorption at different temperatures. The low temperature (10-20 °C) may decrease the adsorption capacity of dyes. However, the adsorption capacity became at the maximum value 80.2 and 75.4 mg/g for AB 25 and PY 101 at 40 °C, respectively. The high temperature 50 and 60 °C significantly decreased the biosorption capacity of AB 25 and completely inhibited the biosorption of PY 101.

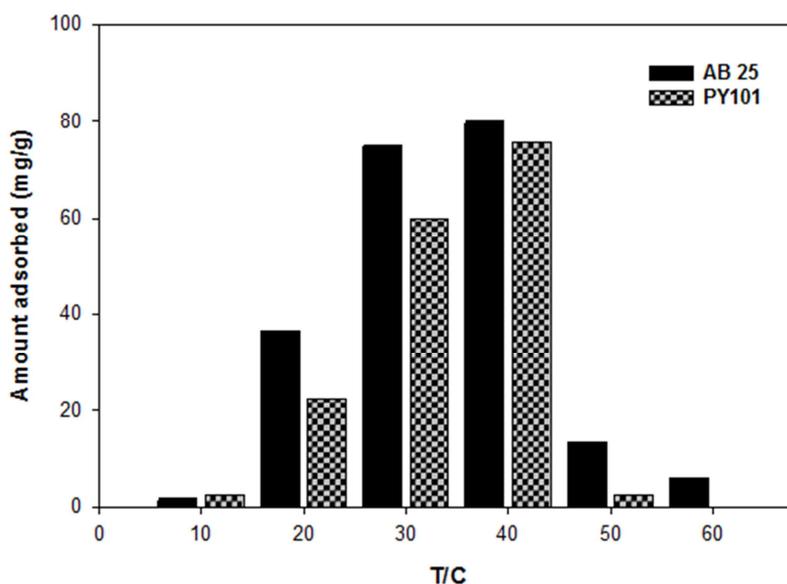


Fig. 4. Effect of temperature on the biosorption of AB 25 and PY 101 on biofilm of *A. terreus* supported on AC. Initial dye concentration (100mg/L) and pH 3.0.

3.4. Effect Contact Time on Biosorption of Dyes

Time is the major parameter which controls any adsorption process. The amount of adsorbent was measured for 210 min (10 min intervals). The results in Fig. 6 showed that, the figure could be classified into two main regions. The first one,

from 0-180 min, the amount adsorption increased gradually by increasing the time to achieve equilibrium at contact time of 150 min for biosorption of AB 25 (82.3 mg/g). On the other hand, the maximum adsorption capacity for PY 101 was 78.2 mg/g at contact time 180 min. The second region were from 180-210 min, this region could be called static

adsorption region at which there were any increase in the amount adsorption along the time.

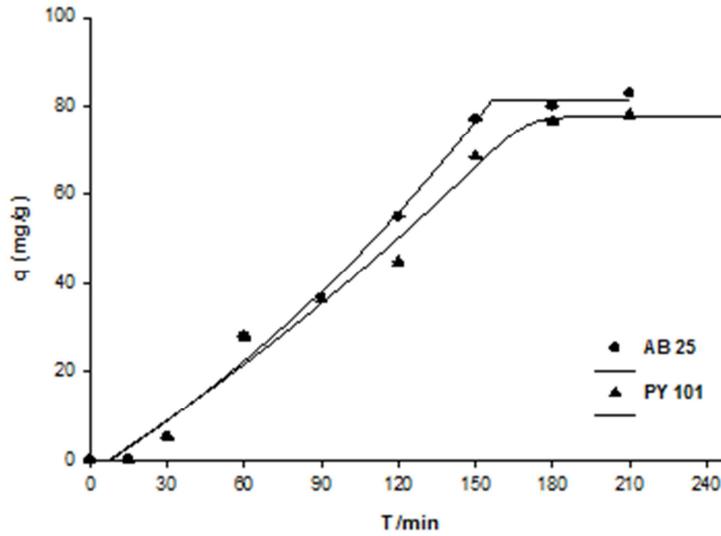


Fig. 5. The effect of contact time on biosorption of AB 25 and PY 101 using biofilm Initial concentration (100mg/L); Temperature 30 °C; pH 3.

3.5. Biosorption Kinetics

It is very important to determine the biosorption kinetics to investigate which model is more suitable for biosorption process. The kinetics parameter of adsorption is determined by Pseudo first-order [22] which expressed as the following

$$\text{Log}(q_e - q_t) = \text{log } q_e - k_1 t / 2.303$$

Where k_1 is the pseudo first-order rate constant and q_e and q_t are the adsorption capacity of the dye molecules on adsorbent at equilibrium and at time t , respectively. The values of k_1 and q_e were evaluated by plotting $\text{log}(q_e - q_t)$ versus the time and the calculated q_e were demonstrated in Fig.6. The results showed that, the calculated q_e were 13.7 and 6.8 g/mg for biosorption of AB 25 and PY 101 on the biofilm. The value of K_1 and correlation co-efficient were summarized in Table 1. This result was not consistent with the experimental results so, the pseudo first-order was unconvincing model to describe the biosorption kinetics.

The linear pattern of pseudo second-order kinetics is presented by the equation

$$t/q_t = 1/k_2 q_e^2 + t/q_e$$

Where k_2 is the pseudo second-order adsorption rate constant. The values of k_2 and q_e were estimated from the slope and intercept of plots of t/q_e versus t Fig. 7 and exhibited in Table 1.

It is observed that, although the r^2 value of biosorption of AB 25 was less than the pseudo first-order kinetics model, the estimated q_e (90.2 mg/g) was highly closed to the experimental one (82.3 mg/g). Also, the estimated q_e (81.6 mg/g) value for PY 101 biosorption was much closed to the experimental one (78.2 mg/g). The calculated K_2 and r^2 were listed in Table1.

The results showed that the Pseudo second-order model was the most suitable model for description of adsorption kinetics of two used dyes.

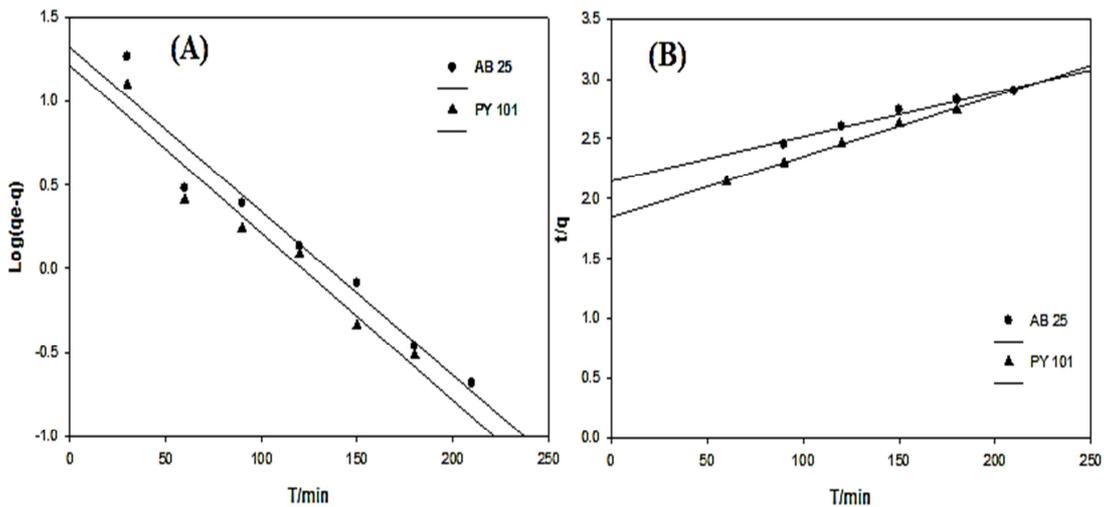


Fig. 6. Pseudo first- order (A) and Pseudo-second order (B) models for adsorption kinetics of AB 25 and PY 101 on the Biofilm.

Table 1. Biosorption kinetics parameters by Pseudo first-order and Pseudo second-order models for adsorption of AB25 and PY 101 on the biofilm.

Adsorbate	$q_{e(\text{exp})}$ (mg/g)	Pseudo first-order			Pseudo second-order		
		$q_{e(\text{cal})}$ (mg/g)	K_1	r^2	$q_{e(\text{cal})}$ (mg/g)	K_2	r^2
AB 25	81.2	22.4 ± 1.8	0.022 ± 0.014	0.94	76.9 ± 0.81	0.008 ± 0.05	0.97
PY 101	77.3	15.8 ± 0.25	0.023 ± 0.02	0.95	$70.1.6 \pm 2.5$	0.002 ± 0.042	0.98

3.6. Biosorption Isotherm

Different isotherm models were used to determine the biosorption characteristics of the biofilm. In this study, Langmuir and Freundlich models were applied.

The fundamental assumption of the Langmuir isotherm model is that biosorption takes place at specific sites within the biosorbent. Once a biosorbate occupies a binding site, no further biosorption occurs at this site. Meaning that the process is monolayer biosorption. The linear form of Langmuir is expressed as the following [23]

$$C_e/q_e = 1/q_{\text{max}} + C_e/q_{\text{max}}b$$

Where b is the Langmuir constant (dm^3/mg) related to affinity between adsorbate and adsorbent and q_{max} is the maximum monolayer adsorption capacity (mg/g). The values of q_{max} and b also give an indication of the affinity of the dye for binding sites on the biosorbent. These values are calculated from the slope and intercept of the linear plot of C_e/q_e against C_e as shown in (Fig. 8A). The Langmuir parameters are presented in Table 2. The calculated q_{max} were 100 and 88 mg/g for for biosorption of AB 25 and PY 101.

It was observed that the calculated values of q_{max} were closed to the experimental value of q_e (Fig. 7). The correlation co-efficient of AB 25 and PY 101 were 0.88 and 0.998 meaning that , the Langmuir model was a more favorable model for the biosorption of PY 101 but it was unsuitable for AB 25.

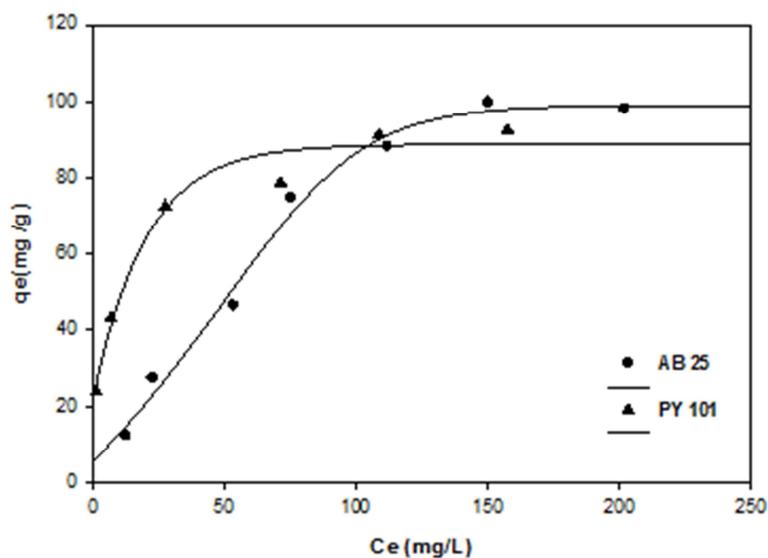
The Freundlich isotherm is an empirical equation based on biosorption on heterogeneous surface and also possibly in multi- layer biosorption. Linear form of the Freundlich equation [24]

$$\ln q_{\text{eq}} = (1/n)\ln C_{\text{eq}} + \ln K_f$$

Where, K_f (dm^3/g) and n are the adsorption capacity and the intensity of adsorption, respectively. Freundlich parameters can be determined from the linear form of the equation. By plotting the $\ln q_e$ versus $\ln C_e$, the slope is the value of $1/n$ and the intercept is equal to $\ln K_f$. The linear form of the Freundlich equation was shown in Fig. 9B. Freundlich adsorption parameters are illustrated in Table 2. The correlation co-efficient of AB 25 and PY 101 were 0.95 and 0.97. Thus, it could be interpreted that the model of Freundlich was efficient to describe the biosorption of the used dyes on the surface of the biofilm.

Table 2. Langmuir and Freundlich isotherm parameters for adsorption of AB25 and PY 101 on the biofilm.

Adsorbate	Q_e experimental	Langmuir			Freundlich		
		Q_{max} (mg/g)	B (dm^3/mg)	r^2	K_f (dm^3/mg)	N	r^2
AB 25	102 ± 0.25	100 ± 1.8	0.035 ± 0.001	0.88	3.98	1.25 ± 0.85	0.95
PY 101	85 ± 0.5	88 ± 5.1	0.121 ± 0.012	0.99	1513.6	3.6 ± 0.42	0.97

**Fig. 7.** The experimental plot of biosorption isotherm of AB 25 and PY 101 on the Biofilm at different concentration of dyes, at $T = 30^\circ\text{C}$ and $\text{pH} = 3.0$.

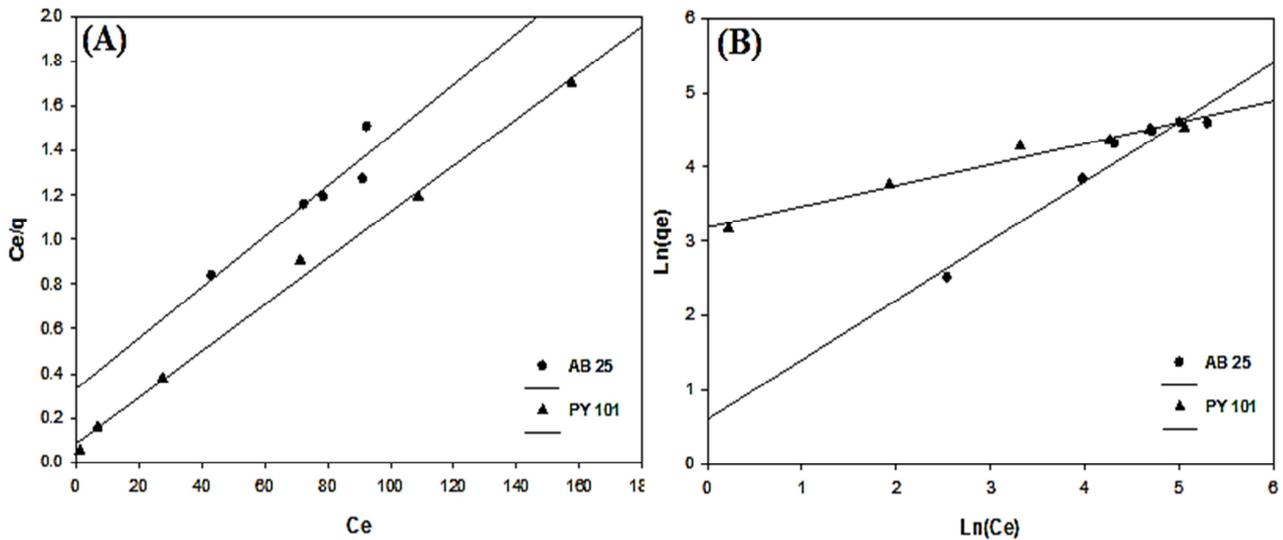


Fig. 8. Linear form of Langmuir (A) and Freundlich (B) models for AB 25 and PY 101 biosorption on the Biofilm.

4. Discussion

Adsorption has been shown to be the most promising option for removal of different non-biodegradable compounds from aqueous streams. Activated carbons are the most common adsorbent for this procedure due to its potency and versatility. Activated carbons are usually obtained from materials with a high carbon content and possess a great adsorption capacity, which is mainly determined by their porous structure [8].

Another promising technology used in removal of dyes is microbial biofilm supported on granular activated carbon. The role of biomass supported on carbonaceous material like the activated carbon has a great advantages, especially, if the dye-containing effluent is very toxic [25]. Biomass adsorption is effective when conditions are not always favorable for the growth and maintenance of the microbial population [26].

A. terreus cell wall is mainly constituted of functional groups like carboxyl, sulfate and amine. In acidic solutions the protonation of amine functions allows the electrostatic attraction of dye molecules that are negatively charged (anionic groups). This attraction mechanism between anionic dyes and cationic surface of the biomass in acidic solutions may explain the higher efficiency of dye biosorption at pH 2.0 [27]. Because hydrogen ion acts as a bridging ligand between the fungal cell wall and the dye molecule. By increasing the pH value this mechanism become weak due to the deprotonation of amine group which reduce the affinity of *A. terreus* to dyes. After pH 6.0 the carboxylic became negatively charged which consequently increase the electrostatic repulsion between anionic dye and the anionic reactive groups [28]. So, it could be said that adsorption by biomass occurs by ion exchange. This result agreed with the studies of biosorption behaviors of different anionic dyes by *Aspergillus fumigatus* and *Penicillium restrictum* [5, 29].

The temperature has two main effects on the adsorption

processes. Increasing temperature is known to increase the diffusion rate of the adsorbate molecules within the pores as a result of decreasing solution viscosity and will also modify the equilibrium capacity of the adsorbent for a particular adsorbate [30]. High temperature may increase the diffusion rate of adsorbate through the biosorbent's pore due to decrease the viscosity of solution. Increasing temperature caused an increase in the number molecules acquiring sufficient energy to undergo chemical reaction which shows chemisorption [31]. As various textile dye effluents are discharged at relatively high temperatures (50–60 °C), so temperature is an important design parameter affecting the biosorption capacity in the real application of biosorption by biomass [21].

Chu and Chen [32, 33] studied the effect of temperature on biosorption of Basic Violet 3 and Basic Yellow 24 dyes using dried activated sludge biomass and they observed that, adsorption capacity decreased from 113.6 to 109.8 mg/g for Basic Violet 3 and decreased from 57.0 to 51.3 mg/g for Basic Yellow 24 with increasing temperature from 20 to 40 °C. These results indicated that both biosorption processes are exothermic in nature.

Regarding to the contact (equilibrium) time, the dynamic changes in biosorption along the time explained that there were difference between charge density and topography and/or surface area of used biosorbant [34, 35], or by the resistance to intraparticle diffusion [36]. The first rapid increase may involve physical adsorption or ion exchange at cell surface and the subsequent slower phase may involve other mechanisms such as complexation, micro-precipitation or saturation of binding sites [34, 35]. *Pseudomonas pseudoalcaligenes* could remove 50 mg/g of initial concentration of AB 25 (100 mg/L) within 180 min equilibrium time [37].

Information on the kinetics of dye adsorption is required for selecting optimum operating conditions for full-scale batch dye removal processes. Mathematical models that can

describe the behavior of a batch biosorption process operated under different experimental conditions are very useful for scale-up studies or process optimization [38]. Adsorption kinetics explains how fast the sorption process occurs and also the factors affecting the reaction rate. The nature of sorption will depend on the physical or chemical characteristics of the adsorbent system and also on the system conditions [17].

Many kinetic models have been used to explain the reaction order of the system. In the present investigation the sorption data were analyzed using simplest kinetic models such as Langmuir, Freundlich, pseudo first and second order models. The data depicted that Freundlich isotherm models were suitable for biosorption of AB25 and PY 101. By increasing the concentration of dye the adsorption capacity is increasing. Because at low concentration of dye the fast saturation of surface due to fast uptake of dye on the other hand at high concentration dyes molecules need to diffuse on the surface of biosorbent and highly hydrolyzed molecules will be diffused with slow rate [39].

It could be concluded that, the biofilm of *A. terreus* supported on activated carbon (AC) enhance the biosorption of two different azodyes AB 25 and PY 101 rather than *A. terreus* or AC alone. The model of Pseudo second-order was the most suitable model for description the adsorption kinetics while the Freundlich model was the best one for adsorption isotherm. Also, the ability of the biofilm for biosorption of AB 25 was higher than its ability for biosorption of PY 101. This may be due to the chemical structure of PY 101 is more complicated than AB 25. Compatibility between microorganism and activated carbon in the form of biofilm may enhance the capacity of dye biosorption.

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