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Bioremediation of Acid blue 25 dye by anthracene degrading *Pseudomonas pseudoalcaligenes* ASU-016

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ABSTRACT

Presence of synthetic dyes in water bodies, even at very low concentrations can be highly toxic to living organisms, reducing the growth of different microorganisms and preventing the photosynthesis in aqueous flora. *Pseudomonas pseudoalcaligenes* and biofilm supported on activated carbon were used for adsorption of acid blue 25 (AB 25). The effect of initial dye concentration (0-300 mg/L), contact time (0- 240 min), and pH values (3.0- 8.0) were studied. The optimum pH value for biosorption of AB 25 was pH 3.0 for the two biosorbents, while contact time was180 min for *P. pseudoalcaligenes* cells and 210 min for the biofilm. The biosorption isotherm study revealed that both Langmuir and Freundlich models were suitable for biosorption process. The maximum adsorption capacities for AB 25 by *P. pseudoalcaligenes* and biofilm were 98 and 113.4 (mg/g), respectively. On the other hand, the pseudo-second order was a good model for description the biosorption kinetics. Fourier Transform Infrared FT-IR spectrum analysis showed that the presence of amines plays an important role in biosorption process. According to experimental data, the presence of *P. pseudoalcaligenes* could be enhanced the capacity of activated carbon to adsorb the dye from aqueous solutions.

Keywords: Acid blue 25; Biosorption; Kinetics; Isotherm; *Pseudomonas pseudoalcaligenes*.

INTRODUCTION

Municipal and manufacture wastewater consist of different types of pollutants as dyes, heavy metals, insecticides and pesticides which represented great environmental concerns. Due to complex aromatic structures, resistant to light and moderate oxidizing agents, dyes are biologically non-degradable and highly stable in the environment. Textile dyes present in water body due to different human activities like dyes manufacturing industries, electroplating factories, distillers and Foods Company. They have many toxic impacts on organisms depending upon the time of exposure and concentration of dye. As an anthraquinone compound, acid blue (AB25) is considered mutagenic and carcinogenic (Brown 1980, Sendelbach 1989). Therefore, many strategies have been used for removal of such compounds from wastewater.

Physicochemical methods like photo-catalytic degradation (Tanaka *et al.,* 2000), membrane filtration (Tanaka *et al.,* 2000, Kim *et al.,* 2005), electrochemical oxidation (Panizza and Cerisola 2007) were used for removal of different dye staff. These methods are quietly undesirable due to high costs, energy waste and generating secondary pollution during the treatment process (Wu *et al.,* 2006). Microbiological deco-mposition (Saratale et al., 2009, Al-Garni et al., 2013), microbial biosorption (Chen *et al.,* 2003) are particularly competitive, economically cost effective and efficient process for the removal of dyes, heavy metals and other organic and inorganic hazardous impurities from aqueous solutions (Vijayaraghavan and Yun 2008).

Textile dyes are different in their chemistry, and their interactions with microorganisms depend on the chemistry of a particular dye and the specific chemistry of microbial biomass. Using of bacteria as biosorbents is a fast growing field in remediation due to their small size, ubiquity, ability to grow under specific conditions and resistance to a wide range of environmental conditions (Vijayaraghavan and Yun 2008).

Free cells of *Bacillus subtilis* is good biosorbent of reactive blue 4 (Binupriya *et al.,* 2010) and the consortium of *Citrobacter freundii*, *Moraxella osloensis*, *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa* CLRI and BL22 could degrade 90% of acid blue 113 dye by 22 h in 80% diluted textile effluent supplemented with glucose and ammonium nitrate (Nachiyae *et al*., 2012).

Another promising technology used in removal of dyes is bacterial biofilm supported on granular activated carbon (Ong *et al.,* 2008).

It has been demonstrated that immobilized microbial systems greatly improve bioreactor efficiency. For instance, increasing process stability and tolerance to shock loadings, allowing higher treatment capacity per unit biomass and generating relatively less biological sludge (Ong *et al.,* 2008) *Pseudomonas pseudo-alcaligenes* is a bacterial strain known by its great ability to degrade different phenolic compounds (Nishino and Spain 1993). Besides that, some research articles mentioned that this strain capable of heavy metal biosorption from aqueous solutions (Sheng *et al.,* 2011).

The main objectives of this study are to i) use of *Pseudomonas pseudoalcaligenes* ASU-016 and biofilm as biosorbents for anionic dye AB 25, ii) study the adsorption isotherms of these biosorbents and iii) determination of adsorption kinetics .

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MATERIALS AND METHODS

Textile dye

Acid blue 25 (AB 25) dye is purchased from Egyptian Local Company. Its chemical formula and molecular weight are $C_{20}H_{13}N_2NaO_5S$ and 416.38 g/mol, respectively. The calibration curve was performed using different concentration (5-300mg/L) and measured by UV-spectrophotometer at λ_{max} 600 nm.

Biosorbents

Activated carbon

Activated carbon was purchased from ADWIC Company, Egypt.

Pseudomonas pseudoalcaligenes

P. pseudoalcaligenes ASU-016 was originally isolated from oil contaminated sludge in Wadi El-Sahl Petroleum company in Egypt, identified based on 16S rRNA gene and lied in Gene Bank under accession number of KC342252. The bacterium was inoculated in 50 mL / 250 mL conical flasks of LB medium at 30°C, pH 7.0 ± 0.2 and incubated under shake at 150 rpm for 24 h; the growing cells were autoclaved; harvested by centrifugation at 5600 xg for 15 min. The pellets were washed three times with sterile deionized water; centrifuged again and finally the *P. pseudoalcaligenes* ASU-016 cells were dried at 50°C overnight and stored for further use.

Biofilm

The biofilm was prepared by shaking method. Granulated activated carbon (1g) and *P. pseudoalcaligenes* ASU-016 plain cells (1g) were mixed in deionized water (500 ml) and incubated at 37°C for 72 hours under shaking followed by centrifugation, the pellets were dried in an oven at 50°C over night to ensure that the sample was completely dry (Rivera-Utrilla *et al.*, 2001).

Effect of pH on biosorption process

The optimum pH value for biosorption of AB25 was determined by analyzing the amount adsorbed (mg/g) of 20 ml AB25 (100mg/L) on 20 mg of each biosorbent over a range of pH 3.0 to 8.5. The pH was adjusted by 1M of KOH and/or 1M of HCl.

Biosorption studies

Effect of contact time on dye uptake and biosorption kinetics

Twenty mg of the bacterium *P. pseudoalcaligenes* ASU-016 or biofilm was suspended in 20 mL deionized water amended with 100 mg/L of AB25. Adsorption was carried out at 30ºC; pH 3.0±0.2; 150 rpm shaking and for 2 hr. Deionized water containing (100mg/L) dye served as negative control. Samples were withdrawn each 30 minutes intervals under aseptic condition. The samples were centrifuged at 5600 xg for 15 min and dye concentration of supernatant was determined at λmax 600 nm by UV-Vis spectrophotometer. The amount of AB25 adsobred at equilibrium q_e (mg/g) was analyzed by the following equation:

$$(1) qe = (C_o - C_t)\ V/m$$

Where C_o is the initial concentration of AB25 (mg/L), C_t is the concentration of AB25 at time t (mg/L), V is the total volume of the suspension (L), and m is the mass of adsorbent (g). Pseudo-first order and Pseudo-second order models were used for calculation of $q_{ecalc.}$ (mg/g).

Biosorption isotherm

Adsorption isotherm was carried out by using different concentrations of AB 25 (25, 50, 100,150, 200 and 300 mg/L) under the same conditions that was previously mentioned. The Langmuir and Freundlich models were applied to determine the most suitable one for AB25 biosorption.

FTIR analysis

FTIR analysis for the samples under investigation was performed to give a qualitative and preliminary characterization of the main chemical groups present on *P. pseudoalcaligenes* and biofilm which responsible for biosorption of AB25. A 0.01g of each dried (overnight at 60°C) sample was mixed with 0.1g KBr and pressed by bench press to form transparent pellets. The pellets are ready for analyzing by FT-IR Model 470 Shimadzu Corporation adopting KBr disk technique.

RESULTS AND DISCUSSIONS

Decolorization activity

Hesham *et al.*, 2014 mentioned that *P. pseudoalcaligenes* ASU-016 had a great ability to degrade low and high molecular weight polycyclic aromatic hydrocarbons (PAHs) like anthracene and pyrene. At this study the strain was used to determine its ability to remove another type of a toxic pollutant which is AB 25. It showed the great ability to decolorize an anionic toxic dye (AB 25) from water body. P.pseudoal-caligenes could remove 55.3 % of 100 mg/L of dye concentration when the strain used as a biosorbent. This percentage jumped to 85.6 % when it supported on activated carbon in a form of biofilm.

Effect of pH on dye uptake

pH is a critical parameter in biosorption of different dyes as it affects the uptake capacity of dye on the surface adsorbent (Binupriya et al., 2010). It may affect the surface charge and degree of ionization of adsorbent during reaction. Results in Fig.1 demonstrated that the optimum pH value required for highest adsorption of AB25 was pH 3.0. The highest values of adsorbed of dye qe (mg/g) were 55.3 and 85.6, for *P. pseudoalcaligenes* and biofilm, respectively. Amount adsorbed of AB25 at equilibrium qe (mg/g) was gradually decreased by increasing pH. This may be occurred due to attraction force increased between the negative charge of anionic sulfonic group on acid blue 25 (AB25) and positive charged that present at low pH value (Azlan *et al.*, 2009). In acidic solutions the protonation of amine functions allows the electrostatic

attraction of dye molecules that are negatively charged (sulfonic groups). This electrostatic attraction mechanism between anionic dyes and cationic surface of the biomass in acidic solutions may explain the higher efficiency of dye biosorption at pH below 4.

Hydrogen ion acts as a bridging ligand between the biosorbents and the dye molecule (Vijayaraghavan and Yun 2008). This force gradually became more weak by increasing pH due to repulsion and competition between negative charged dye and OH^- group of medium and subsequently decrease the number of adsorption site on the anionic dye (Azlan *et al.*, 2009, Binupriya *et al.*, 2010). The adsorption may be carried out by formation of chemical bonds or by ion exchange between molecules.

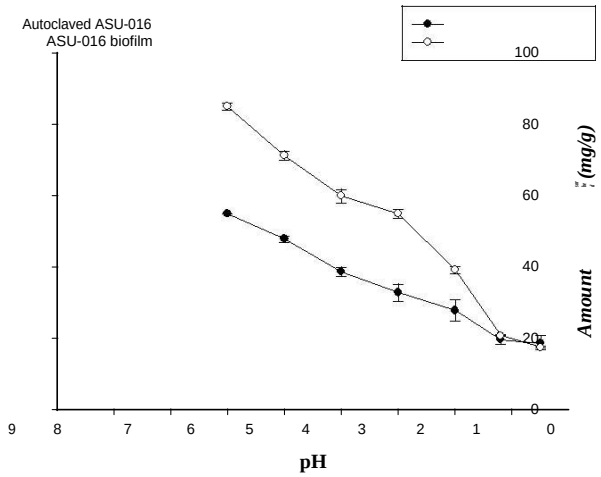

Figure (1): Effect of pH on the biosorption of AB25 (100 mg/L) by *P. pseudoalcaligenes* ASU-016 and biofilm, at 30°C.

Effect of contact time on biosorption

The fast biosorbent is more favorable to use in the biosorption treatment process. The results in Fig. 2a illustrated that biosorption rapidly increased at the first 90 min and then it became slightly slow to reach the equilibrium at 180 min for *P. pseudoalcaligenes* and 210 min for biofilm. This dynamic changes in biosorption explained that there were difference between charge density and topography and/or surface area of used biosorbant (Daneshvar *et al.*, 2012, Gupta and Rastogi 2009), or by the resistance to intraparticle diffusion (Aksu and Tezer 2005). 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 (Gupta and Rastogi 2009, Daneshvar et al., 2012). There were many researches discussed the time of equilibrium of many biosorbents on different types of dye. (Çolak *et al.*, 2009) documented that *Paenibacillus macerans* could adsorb acidic dyes and reach the equilibrium at 90 min contact time. Removal of AB1 with brown macroalga *Stoechospermum marginatum* takes 90min contact time (Daneshvar *et al.*, 2012).

Adsorption kinetics

Adsorption kinetics is mainly depending upon the interaction between the adsorbate, biosorbents and experimental conditions. Experimental data of amount adsorbed dye (Fig. 2a.) illustrated that qe (mg/g) of *P. pseudoalcaligenes* and biofilm were 50 and 76.3 mg/g, respectively. Adsorption kinetics was analyzed by using Pseudo-first and Pseudo-second order models to estimate the biosorption rate. The linear form of Pseudo-first order equation is as the following:

$$\text{Log}(qe - qt) = \text{Log } qe - K1t/\ 2.303 \quad (2)$$

Where k_1 is the Pseudo first-order rate constant and q_e and q_t is the adsorption capacity of dye molecules on to biosorbent at equilibrium and at time t, respectively. The values of k_1 and q_e was evaluated by plotting log (q_e -q_t) versus the time and the calculated qe are demonstrated in Table 1. The amount adsorbed of AB25 on the surface of biofilm, ASU-016 cells and activated carbon is 2.87, 57.7 and 0.96 (mg/g), respectively, on the other hand the r^2 value was relatively low (0.97, 0.78 and 0.83, respectively.

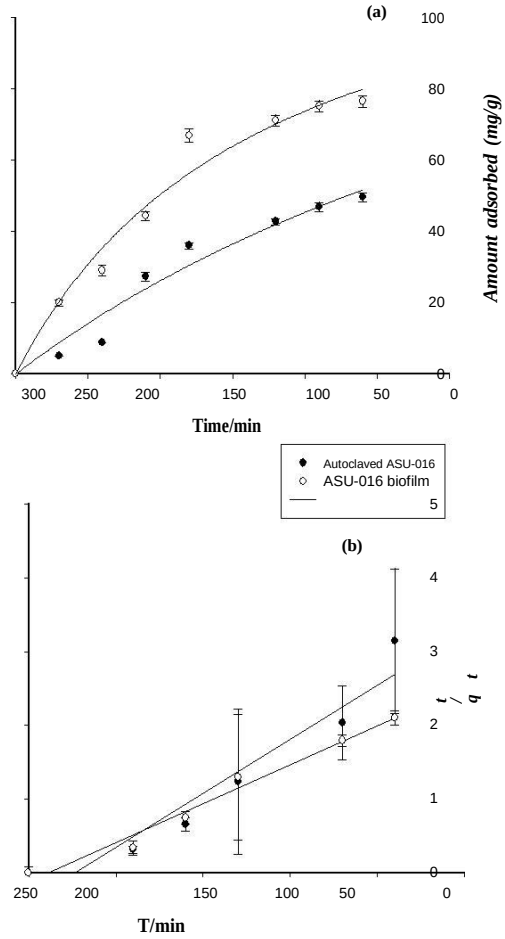

Figure (2): Data showing the contact time (a) and kinetics by Pseudo-second order model on/for biosorption capacity of AB25 (100mg/L) using *P. pseudoalcaligenes* ASU-016 and biofilm (b), at 30°C and pH 3.0.

AS the experimental values of q_e (Fig. 2a) are not equal the amount adsorbed (q_e) calculated from plot as illustrated in (Table 1), the Pseudo-first order model is not fitted to the adsorption process.

Linear form of Pseudo-second order model is described at the following equation:

$$t/q = 1/K2qe\text{^}2+1/qet \quad (3)$$

By plotting t/q_t versus t the values of K_2 and q_e was calculated (Fig.2b).

Table (1) showed that there were identical similarities between the experimental q_e (mg/g) and theoretical one (66.7; (r^2 0. 97) and 76.2 (r^2 0. 97)) for *P. Pseudo-alcaligenes* cells and biofilm, respectively. So, it can be concluded that Pseudo-second order (Ho and McKay 1999) was the suitable model for the adsorption process of AB25 dye by used biodorbent.

Table (1): Biosorption kinetics using Pseudo-first order and Pseudo-second order models and Langmuir and Freundlich isotherm parameters for adsorption of AB25 on biofilm and *P. pseudoalcaligenes* ASU-016.

Biosorption Isotherm						**Biosorption kinetics**							
Freundlich			Langmuir			Pseudo-second order			Pseudo-first order				
r^2	n	K_F	r^2	b(1/mg)	Q_{max} (mg/g)	r^2	K_2	$q_{e(cal)}$ (mg/g)	r^2	K_1	$q_{e(cal)}$ (mg/g)	$qe_{(exp)}$ mg/g	**Adsorbent**
0.96	1.22 ± 0.85	0.03± 0.001	0.98	1.23 ± 0.02	113.4± 0.25	0.97	0.008 ± 0	76.9 ± 0.81	0.97	0.017 ± 0.02	3.9 ± 0.25	76.2	**Biofilm**
0.999	1.1 ± 0.42	0.001 ±0	0.95	0.1 ± 0.012	98 ± 0.5	0.98	0.002±0	52.6 ± 2.5	0.78	0.017± 0.004	57.7 ± 1.8	50.3	**ASU-016**

The adsorption isotherm

The linear forms of Langmuir (Volesky 2001) and Freundlich equation (Kratochvil et al., 1995) (Table 1) were used to detect the maximum adsorption capacity based on homogenous layer of biosorbent was covered by monolayer of AB25. Applicability of these equations was compared by judging the correlation coefficients (R^2).

Langmuir model assumed that one adsorbate molecule interacts with only one binding site of the adsorbent to form monolayer adsorption. The Langmuir equation was expressed as the following:

$$qc = (qmax\ bCc)/(1 + bCe) \quad (4)$$

While the linear form is:

$$Ceq/qe = 1/qmaxb + Ceq/qmax \quad (5)$$

Where b is the Langmuir constant related to affinity between adsobate and adsorbent and qmax is the maximum monolayer adsorption capacity. The values of qmax 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. 4a). The Langmuir parameters are shown in (Table 1). The correlation coefficient of the isotherm is relatively high (0.98 and 0.95) and the maximum absorption capacity (113.5 and 98mg/g) (Fig. 3) biofilm and *P. pseudoalcaligenes*, respectively.

The affinity of dye for binging the biofilm was the highest value (b= 1.23/mg) and *P. pseudoalcaligenes* cells was 0.04/mg. The results showed that the Langmuir model was suitable for used adsorbents. This indicated that the monolayer of dye was adsorbed on homogeneous surface of adsorbent without interaction between molecules as reported by (Joo *et al.*, 2010).

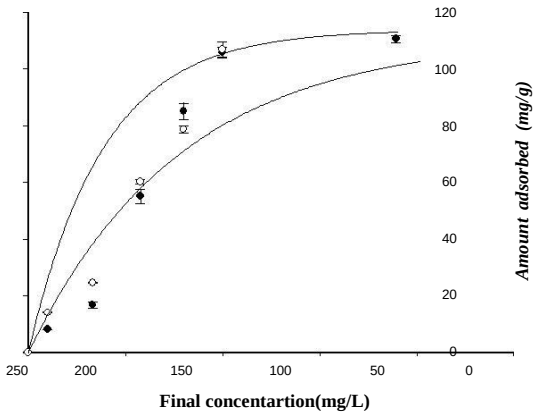

Figure (3): Biosorption isotherm of AB25 by *P. Pseudo-oalcaligenes* ASU-016 and biofilm, at 30°C and pH 3.0.

Fruendlich was more complicated adsorption model which deals with adsorption of monolayer dye on heterogeneous surface. This model is expressed by the following equation:

$$= KF\ Ceq^{1/n}$$

And the linear form is:

$$\ln qeq = (1/n)\ln Ceq + \ln Kf$$

Where, K_f 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 lnqe versus lnCe, the slope is the value of 1/n and the intercept is equal to $\ln K_f$.

The linear form of the Freundlich equation for the biosorption of AB 25 on used biosorbents is shown in Fig. 4b. Freundlich adsorption parameters are illustrated in Table 1. The highest n (1.22) and K_f (0.03) values were observed with biofilm. *P.pseudoalcaligenes* autoclaved cells represented the lowest values of n (0.001) and K_f (1.1). This means that the biofilm is the most desirable AB25 biosorbent rather than *P.pseudoalcaligenes* cells.

These data depicted that both Langmuir and Freundlich isotherm models were suitable for biosorption of AB25 by *P.pseudoalcaligenes* and biofilm. By increasing the concentration of AB25 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 (Joo *et al.,* 2010).

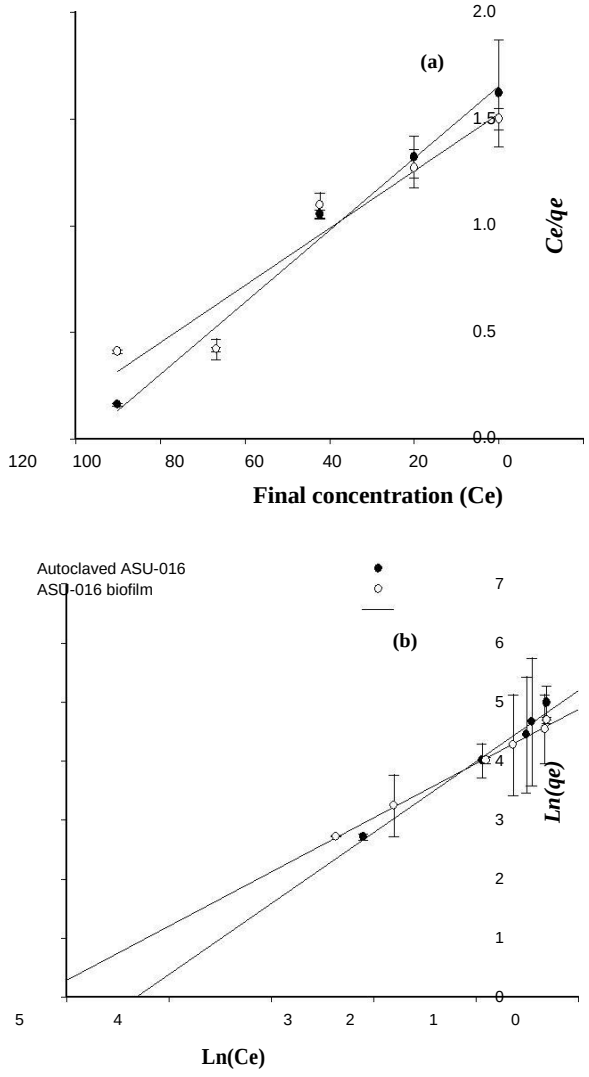

Figure (4): The linear form of Langmuir (a) and Freundlich (b) adsorption isotherm of biofilm and *P. pseudoalcaligenes* ASU-016.

FT-IR

FT-IR spectra is carried out in order to determine the main functional groups present at the surface of biosorbents which responsible for adsorption process. It was performed from 500 to 4000 cm^{-1} before (Figure 5a) and after (Figure 5b) biosorption of AB 25. (Figure 5a) showed that the strong peaks at 1616.27 cm^{-1} assigned for amides group (Filip and Hermann 2001). This peak became broad with biofilm and shifted to 1651.9 cm^{-1} with *P. pseudoalcaligenes* ASU-016. Strong peak at 1315.48 cm^{-1} assigned to -C-N stretch of aromatic amines that are attributing to the vibrations of the heterocyclic skeleton of the dye molecule (Sharma and Das 2012).

This peak shifted to 1114.7 cm-1 with *P. pseudoalcaligenes* ASU-016 and 1032.7 cm-1 with biofilm to be assigned $C\text{-}H_3$ bending vibration (Ovchinnikov *et al.,* 2007). The peaks from 2900 to 2999 cm^{-1} and from 3400 to 3450 cm^{-1} assigned aliphatic C-H stretching (Peng *et al.,* 2010), O-H stretching of phenolic groups (Aguayo-Villarreal *et al.,* 2013), respectively. The results of FT-IR spectrum indicated that the main functional groups for biosorption of AB25 dye are amides. These agreed with (Gao *et al.,* 2010) whose reported that amine I-III groups responsible for uptake of Acid yellow 17 in bacteria and Protozoa. (Binupriya *et al.,* 2010) mentioned that the main functional group present on *Bacillus subtilis* cells for adsorption of reactive blue 4 are amine group. The results depicted that using of biofilm as biosorbent was significantly enhanced the level of decolorization along the time. The degree of freedom was $p < 0.001$. All the results were determined by the SD of three replicates.

Comparison with other studies

Many studies have discussed the biosorption of AB 25 by using different biosorbents. Some of these have been mentioned in (Table 2).

CONCLUSION

Activated carbon has a large surface area and limited active site but bacteria have the opposite thing; a small surface area and wide varieties of active sites for binding AB25. Therefore, compatibility between bacteria and activated carbon in the form of biofilm may enhance the capacity of AB25 adsorption. Beside that the using of dead bacteria such *P. pseudoalcaligenes* ASU-016 may be safer than using of viable one to avoid toxicity and secondary metabolites that may be produced. Langmuir and Freundlich isotherm models are suitable for description of AB25 biosorption of by biofilm and autoclaved *P. pseudoalcaligenes* ASU-016. Attraction between anionic dye and hydrogen ion at high acidic pH values paved the way for perfect uptake capacity on the surface of used adsorbents. Finally, the main functional group responsible for adsorption was amine groups. So, *P. pseudoalcaligenes* ASU-016 is highly recommended strain for application in field of bioremediation due to its ability to remove two types of

organic pollutants; anthracene as one of polycyclic aromatic hydrocarbons and Acid blue 25 as one of carcinogenic dye.

ACKNOWLEDGMENT

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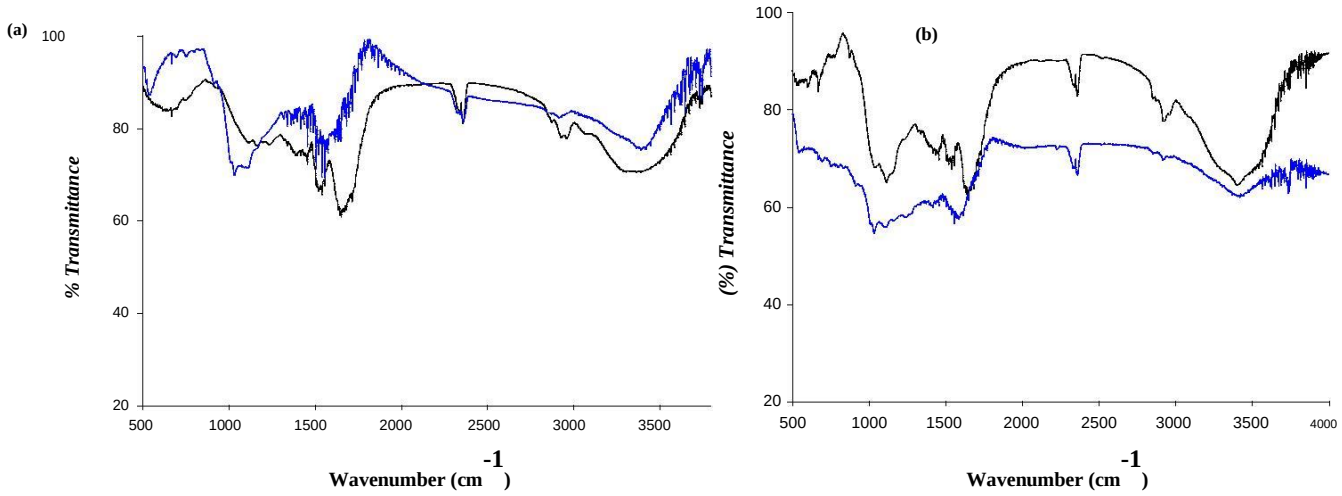

Figure (5): FTIR spectra of used biosorbent at room temperature of Acid blue (AB 25) 100 mg/L by *P. pseudoalcaligenes* ASU-0106 and biofilm before (a) and after biosorption (b). The blue line for *P. pseudoalcaligenes* ASU-016, while the black one was for biofilm.

Table (2): The comparision studies of biosorption of AB 25 using different biosorbents at different conditions.

	Adsorption parameters				Initial Conc. (mg/L)	Biosorbent
Ref.	**qe(exp) mg/g**	**C.T(min)**	**Temp(°C)**	***pH***		
(Hernández-Montoya *et al.*,2011)	6	---	30	5	100	**Pericarp of Pecan**
(Hernández-Montoya *et al.*,2011)	79	---	30	9	100	**Activated carbon**
(Badii *et al.*, 2010)	6	---	25	2	50	**Diatoms**
(Hanafiah *et al.*, 2012)	24.39	60	30	2	100	**(BTSD)**
(Daneshvar *et al.*, 2012)	22.2	90	27	2	30	***Stoechospermum marginatum***
Current study	50	180	30	3	100	***Pseudomonas pseudoalcaligenes***
Current study	76.2	210	30	3	100	**Biofim**

Base Treated Shorea dasyphylla (BTSD) sawdust, C.T. contact time per min, q_e (exp) experimentally calculated maximum adsorption cabacity (mg/g).

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المعالجة البيولوجية لصبغ الحمض الازرق 25 باستخدام بكتريا *Pseudomonas pseudoalcaligenes* المحللة للانثراسين

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الملخص العربي

وجود الاصباغ الاصطناعية في المسطحات المائية ، ولو بتركيزات منخفضة جدا من الممكن ان يكون له سمية عالية للكائنات الحية والحد من نموها، وكذلك أعاقة عملية التمثيل الضوئى للكائنات المائية .استخدمت في هذه الدراسة سلالة *Pseudomonas pseudoalcaligenes* وبيوفيلم مدعم علي الكربون النشط وذلك لامتزاز صبغ الحمض الازرق 25 .ولبيان الظروف المثلي لعملية الامتزاز، استخدمت الصبغة بتركيزات (صفر الي300ملجرام/لتر) في زمن اتصال قدره من (صفر الى 240 دقيقة) ورقم هيدروجينى من (3 الي 8). وقد سجلت النتائج ان اعلي نسبة امتزاز حيوى كانت عند رقم هيدروجيني 3 وعند زمن اتصال قدره 180دقيقة لسلالة *P . pseudoalcaligenes* و 210دقيقة في حالة البيوفيلم. وقد اوضحت الدراسة الايزوثيرمية ان نموذج لانجمير وفرويندلش كلاهما يناسبان عملية الامتزاز. حيث كانت أعلي سعة امتزاز للصبغ 98ملجرام/جم في حالة *P . pseudoalcaligenes* بينما كانت 113.4 ملجرام/جم في حالة استخدام البيوفيلم. من ناحية اخري فقد كان نموذج pseudo-second order ملائم جدا لوصف حركية الامتزاز . وقد اوضحت نتائج التحليل باستخدام الأشعة التحت حمراء FTIR ان وجود المجموعات الأمينية قد لعب دور كبير و اساسي في اتمام عملية الامتزاز. وفي النهاية فان وجود بكتريا *P . pseudoalcaligenes* مع الكربون النشط في صورة بيوفيلم يزيد من كفاءة امتزاز الصبغة علي سطحه في الاوساط المائية.