

Full Length Research Paper

# Use of *Aspergillus wentii* for biosorption of methylene blue from aqueous solution

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In this study, *Aspergillus wentii* was used as a biosorbent for the adsorption of methylene blue from aqueous solution. The effects of contact time, initial dye concentration, solution pH and temperature on biosorption were investigated. The contact time required (that is, the equilibrium time) for maximum dye biosorption was found to be 120 min. The amount of the dye biosorbed increased with increasing initial dye concentrations and solution pH, while it was decreasing with an increase in temperature. Percent biosorption was changed between 14.86 and 85.04 under all conditions studied. Desorption studies were performed by changing of the value pH among 3 - 10. Desorption was considerably affected by lower pH. The maximum percentage of desorption was found to be 29.51 at pH 3. Biosorption isotherm from equilibrium values followed Freundlich model.

**Key words:** *Aspergillus wentii*, dye, methylene blue, biosorption, desorption, isotherm.

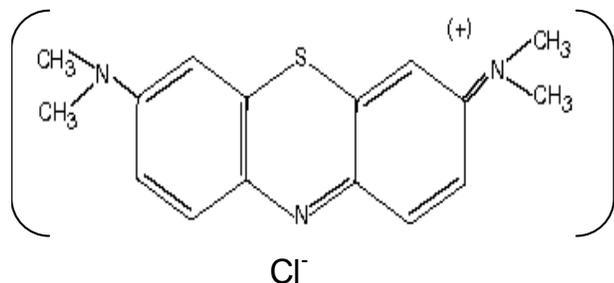
## INTRODUCTION

Wastewaters from textile, cosmetics, printing, dyeing, food coloring, paper-making industries are polluted by dyes. The dye pollution caused by the industrial wastewater is an important problem faced by many countries. Dye pollution from industrial effluents disturbs human's health and ecological equilibrium. Dyes even in low concentrations affect aquatic and terrestrial life. Some dyes show carcinogenic and mutagenic effect when inhaled and when in contact with the skin. Therefore, the removal of the dyes from wastewater is required. For this purpose, many methods such as activated carbon adsorption, chemical oxidation, coagulation, ultra-filtration, electro-chemical etc. as mentioned in the work of Dogan and Alkan have been developed for treating dye-containing wastewaters (Dogan and Alkan, 2003a). Of those methods, activated carbon adsorption is highly effective for the removal of different pollution from wastewater and industrial effluents. However, the use of activated carbon is not suitable for developing countries because of its

high cost. Therefore, many efforts have been made to find cheaper control methods and materials recently. For example, low-cost sorbents such as peat (Ho and McKay, 1998), fly ash (Acemioğlu, 2004), perlite (Acemioğlu, 2005; Dogan and Alkan, 2003b), sawdust (Asfour et al., 1985), etc. have been utilized for dye removal. On the other hand, biosorption process has also been commonly used for the removal of dyes as well as heavy metal and organic pollution. Some researchers have also extensively studied the removal of dyes by various biological biomasses such as *Aspergillus niger* (Fu and Viraraghavan, 2001), *Aspergillus foetidus* (Sumathi and Manju, 2000), *Phanerochaete chrysosporium* (Tatarko and Bumpus, 1998), *Rhizopus arrhizus* (O'Mohany, 2002) and *Spirodela polyrrhiza* (Waranusantigul et al., 2003), recently. Biomass species such as *A. niger* and *A. foetidus* have various functional groups such as carboxyl, amino, phosphate and sulfonate. These groups act as excellent binding sites in the biosorption of heavy metals and various dyes (Kapoor and Viraraghavan, 1998).

In this work, *Aspergillus wentii* which is a fungus was produced in laboratory and then it was killed by drying at 80°C in an oven. The fungal biomass thus prepared was used as a biosorbent for the removal of methylene blue

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**Figure 1.** Structure of methylene blue.

(MB) from aqueous solution. The effects of contact time, initial dye concentration, solution pH and temperature on the biosorption of MB were investigated. Moreover, the capacity of *A. wentii* for the biosorption of MB was compared to the capacities of another sorbents used for the sorption of MB. The isotherm of the biosorption process was studied, also.

## MATERIALS AND METHODS

### Preparation of biomass

*A. wentii* (ATCC #10584) was maintained on Potato Dextro Agar slants and grown on a Potato Dextro Agar solid medium for 7 days. Then, the fungus was cultivated in a liquid growth medium composed of 30 g of glucose, 2.0 g of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 1.0 g of K<sub>2</sub>HPO<sub>4</sub>, 0.5 g of KCl, 3 g of CaCO<sub>3</sub>, 0.5 g of MgSO<sub>4</sub>·7H<sub>2</sub>O and 0.01 g of FeSO<sub>4</sub>·7H<sub>2</sub>O in a liter of distilled water (Sukhodolskaya et al., 2000). The pH of the growth medium was adjusted to 5.30 using 1 N HCl before autoclaving. Then the 100 ml of the liquid medium was transferred to Erlenmeyer flasks of 250 mL and it was autoclaved at 121°C for 15 min. Subsequently, the test fungus was inoculated to each flask, which was then grown on a biologic incubator at 25°C for 14 days. After incubation, the biomass was harvested from the growth medium and thoroughly washed with distilled water. Living fungal biomass (200 g wet weight) was dried at 80°C at an oven for 6 h for the preparation of the dead fungus. The dead fungal biomass pellicles obtained was grinded by using a mortar and a pestle. The powdered biomass was sieved through a molecular sieve of 80-mesh (Retsch AS-200). And then it was utilized as a biosorbent for batch biosorption studies.

### Preparation of dye solutions

Methylene Blue (MB), a cationic dye (C.I. 52015), was purchased from Merck and it was used as received without further purification. The structure of the dye containing a secondary amine group is presented in Figure 1. Physicochemical characteristics of dye are presented in Table 1. The stock solutions at the desired concentrations were prepared with distilled water. The pH values of dye solutions were adjusted with 0.1 N NaOH or HCl solutions using a pH meter (WTW pH Meter 320, Germany).

### Methods

Batch biosorption experiments were performed in 150-mL Erlenmeyer flasks using 0.10 g of the biomass with 50 mL of dye

**Table 1.** Physicochemical characteristics of methylene blue.

Common name	Methylene blue
Other name	Swiss blue
C.I. name	Basic blue 9
C.I. number	52015
Class	Thiazin
Ionization	Basic
Solubility aqueous(%)	3.55
Color	Blue
Absorption maximum (nm)	664
Chemical formula	C <sub>16</sub> H <sub>18</sub> N <sub>3</sub> SCl
Formula weight (g/mol)	319.9

solutions, whose concentrations, pH and temperature was previously known. The samples were shaken at a constant speed of 130 rpm in a temperature-controlled shaking incubator. After the desired treatment time, the samples were taken from incubator and then they were centrifuged. The supernatant was analyzed to determine the concentrations of remained dye in solution using a Shimadzu UV-vis 160 A Spectrophotometer set at a wavelength of 664 nm, maximum absorbance. The concentrations of the samples in supernatant were determined by using a standard curve. The amounts of MB biosorbed onto biomass were calculated using the equation

$$q_e = (C_0 - C_e)V/W, \quad (1)$$

where C<sub>0</sub> is initial concentration (in mg/l), C<sub>e</sub> is concentration of dye in solution at any time (in mg/l), W is the weight of biomass (g) and V is the volume of dye solution (l).

### Desorption studies

The biomass utilized for the biosorption of initial dye concentrations of 8.00 mg/l were separated from the dye solution. The dye-loaded biomass was washed gently with pure water for the removal of any unadsorbed dye. And then, dye-loaded biomass were stirred using a magnetic stirrer with 50 ml of the distilled water of eight different pHs among 3 - 10, one by one. The amount of dye desorbed was determined as mentioned before.

### FT-IR measurements

*A. wentii* was first dried to the constant weight in an oven at 60°C for 12 h. Afterward, 1.0 mg of the dried samples was mixed with 100 mg of KBr to make pellet. The infrared spectra of the pellets were recorded in the wave number range of 4000 - 650 cm<sup>-1</sup> using a Perkin Elmer FT-IR spectrometer.

## RESULTS AND DISCUSSION

### Effect of contact time on biosorption

The effects of contact time on the amount of MB biosorbed per unit biomass were investigated under all experiment conditions, that is, concentration, temperature and pH. It is seen that a rapid biosorption take places at 5

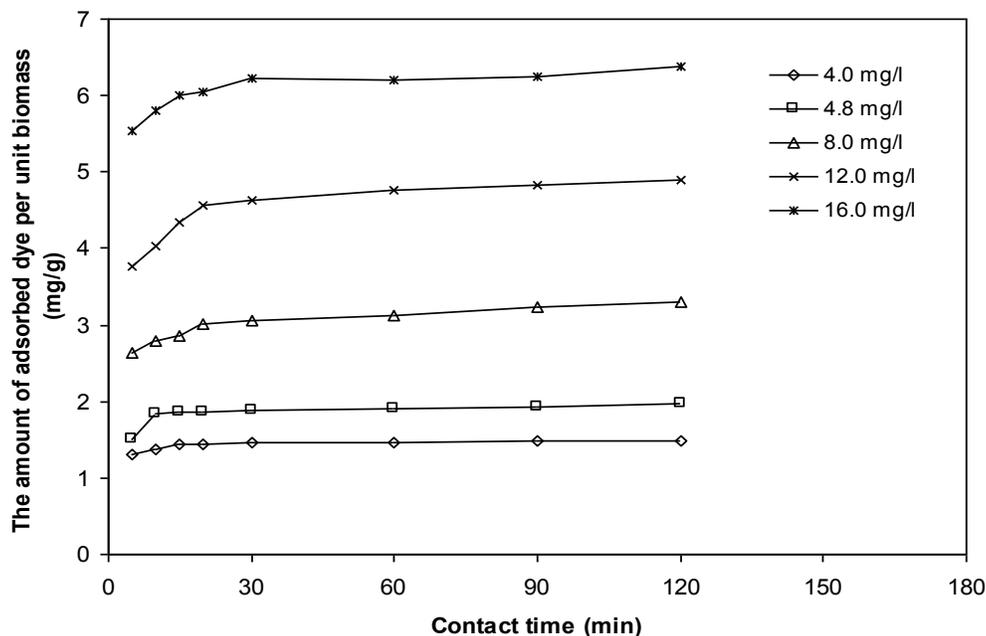


Figure 2. Effect of concentration on biosorption of methylene blue onto *A. wentii*.

min and thereafter the gradual increase in biosorption occurs with increasing contact time up to 120 min. After this time, the amount of dye biosorbed was not significant and therefore the time of 120 min was fixed as the optimum contact time. A similar result has been recorded for the removal of methylene blue by biosolid in a work done by Sarioglu and Atay (2006).

#### Effect of initial dye concentration on biosorption

In order to prevent the dimmer or aggregation of the dye, the initial dye concentration were kept below  $\leq \sim 5 \times 10^{-5}$  M (16.00 mg/l) (Acemioglu, 2005; Inel and Tumsek, 2000). Therefore, the effect of initial dye concentration on biosorption was studied at five different concentrations of 4.00, 4.80, 8.00, 12.00 and 16.00 mg/l at 30°C and natural pH (6.33), respectively. Figure 2 illustrates the effect of initial dye concentration as a function of contact time at 30°C and natural pH. As shown in Figure 1, a rapid biosorption occurs at 5 min for all concentrations and thereafter the gradual increase in biosorption maintains to 120 min which is equilibrium time. With increasing initial dye concentration from 4.00 to 16.00 mg/l, the maximum amount of dye biosorbed onto biomass increases 1.483 to 6.382 mg/g. The biosorption percent of MB increases 74.18 to 79.87 with increasing initial dye concentration from 4.00 to 16.00 mg/l. This increase in proportion of dye biosorbed may be probably due to equilibrium shift during biosorption process. A similar result has been reported for adsorption of Congo red from aqueous solution onto calcium-rich fly ash (Acemioglu,

2004).

#### Effect of temperature on biosorption

The effect of temperature on biosorption was studied at three different temperatures of 30, 40 and 50°C. As shown in Figure 3, the results indicate that the amount of MB biosorbed onto biomass decreases with increasing temperature from 30 to 50°C. For example, for the initial dye concentration of 8.00 mg/l at a contact time of 120 min, when initial solution temperature increases from 30 to 50°C, the maximum amount of MB biosorbed per unit biomass decreases from 3.30 to 2.84 mg/g. The biosorption percent of MB decreases 82.54 to 71.03 with increasing temperature from 30 to 50°C. The fact that the biosorption decreases with an increase in temperature indicates that lower temperature is in favor of biosorption.

#### Effect of pH on biosorption

The effect of the initial pH of dye solution on biosorption was studied at total eight pHs among 3 - 10 for the initial dye concentration of 8.00 mg/l at 30°C and a contact time of 120 min. As shown in Figure 4, the biosorption percent increases from 25.89 to 85.04 while initial solution pH increases 3 to 10. Also, the maximum amount of MB adsorbed per biomass increase 1.035 to 3.40 mg/g while initial solution pH increase from 3 to 10. And therefore, alkaline pH has a high effect on biosorption. As the initial pH of solution increases, the number of negatively

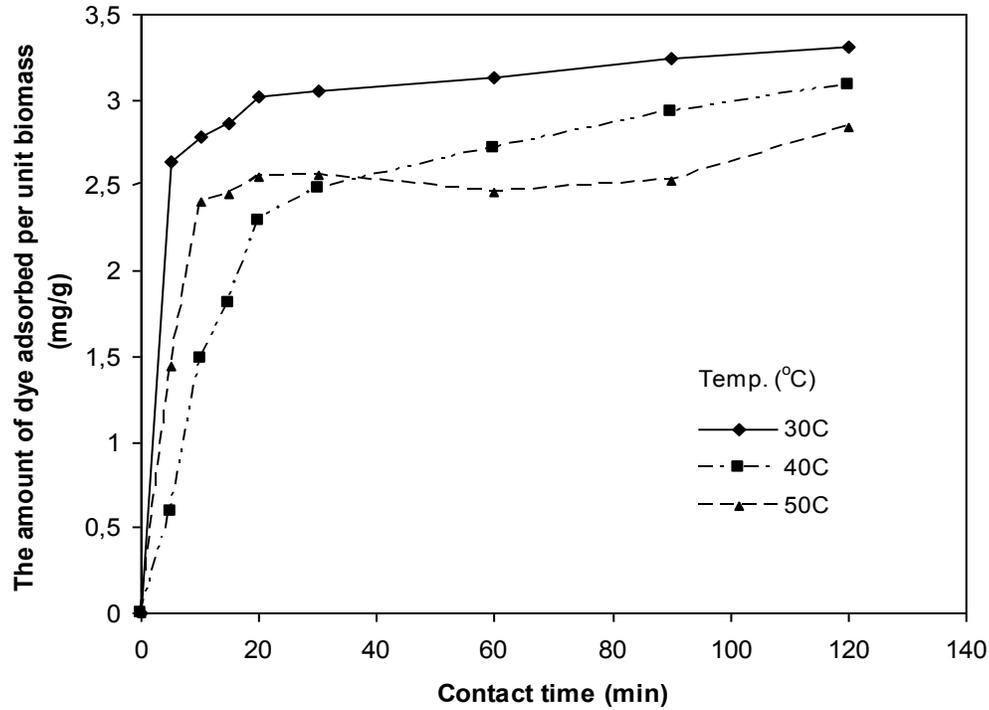


Figure 3. Effect of temperature on biosorption of methylene blue onto *A. wentii*.

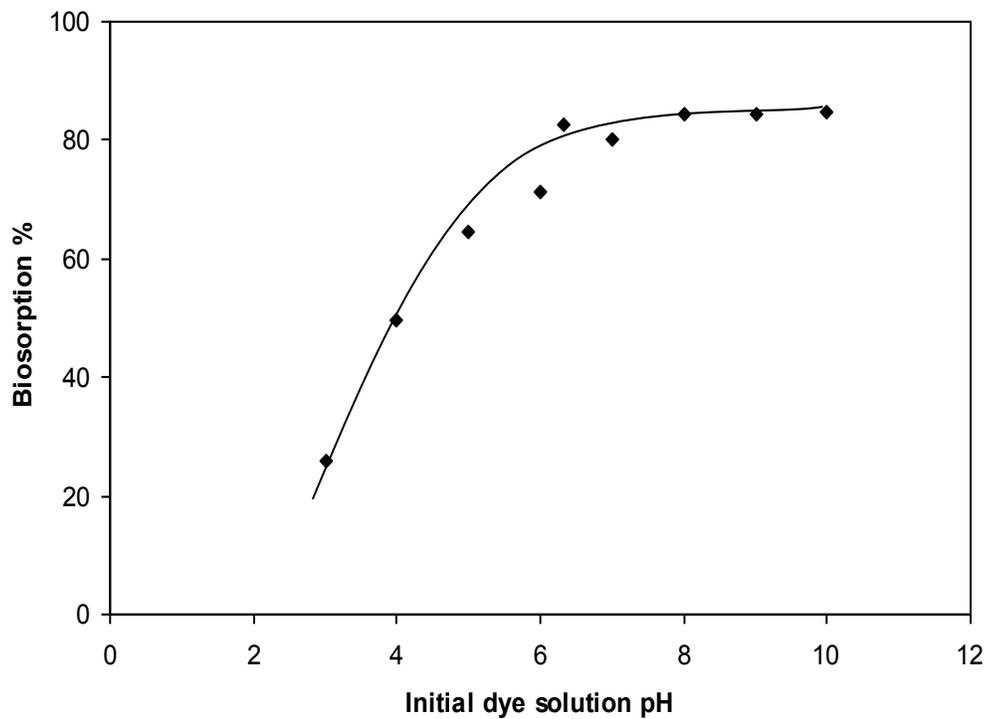


Figure 4. Effect of initial pH on biosorption of methylene blue onto *A. wentii*.

charged biosorbent sites increases. This phenomenon is attributed to more interaction of cationic dye with more negatively functional groups (amine and carboxyl groups)

in the structure of biomass in alkaline medium. Moreover, the fact that biosorption of MB is lower at acidic pH is due to the presence of excess  $H^+$  ions competing with dye

**Table 2.** The comparison of experimental biosorption capacities of *aspergillus wentii* to some sorbents for methylene blue biosorption.

Capacity (in mg/g)	Experimental conditions	Biosorbent/Adsorbent	References
3.30	C <sub>0</sub> = 8.0 mg/l, T = 40°C,	<i>A. wentii</i>	This study
	pH = 6.33, V = 50 ml, W = 0.10 g		
3.40	C <sub>0</sub> = 8.0 mg/l, T = 40°C,	<i>A. wentii</i>	This study
	pH = 10, V = 50 ml, W = 0.10 g		
1.17	C <sub>0</sub> = 50 mg/l, T = 20°C,	<i>A. niger</i>	Fu and Viraraghavan (2000)
	pH = 6.0, V = 100 ml, W = 0.10 g		
0.73	C <sub>0</sub> = 8.0 mg/l, T = 50°C,	Perlite	Acemioğlu (2005)
	pH = 11, V = 75 ml, W = 1.0 g		
249.92	C <sub>0</sub> = 100 mg/l, T = 20°C,	Fomes fomantarius	Maurya et al. (2006)
	pH = 11, V = 50 ml, W = 0.015 g		
263.33	C <sub>0</sub> = 100 mg/l, T = 20°C,	Phellinus igniarius	Maurya et al. (2006)
	pH = 11, V = 50 ml, W = 0.015 g		
15.90	C <sub>0</sub> = 100 mg/l, T = 30°C,	Banana peel	Annadurai et al.(2002)
	pH = 6, V = 100 ml, W = 0.10 g		
13.90	C <sub>0</sub> = 100 mg/l, T = 30°C,	Orange peel	Annadurai et al.(2002)
	pH = 7.2, V = 100 ml, W = 0.10 g		

C<sub>0</sub> = Initial dye concentration (mg/l), T = Temperature, V = solution volume, W = adsorbent/biosorbent amount

cations biosorbed on the surface of biomass. A similar result has also been recorded for the removal of malachite green by *prosopis cineraria* (Gark et al., 2004).

### The comparison of experimental biosorption capacities of *A. wentii* to some sorbents

In this study, for the initial dye concentration of 8.00 mg/l at solution pHs of 6.33 (natural pH) and 10 for a contact time of 120 min, the most biosorption is occurred at 30°C. The maximum amount of MB biosorbed per unit biomass is 3.30 and 3.40 mg/g for pH 6.33 and 10 at 30°C, respectively. And the maximum percent value of MB biosorbed is 82.54 and 85.04 for pH 6.33 and 10, respectively. These values obtained for the biosorption of MB onto *A. wentii* are compared to the biosorption of MB by another sorbents under different conditions and they are presented in Table 2. The maximum amounts of dye adsorbed by various adsorbents in equilibrium time vary as a function of experimental conditions. Especially the amount of adsorbent dose has a very important effect on the estimation of the maximum amounts of dye adsorbed per unit adsorbent. As shown in Table 2, for example, when the mass of *Fomes fomantarius* and *Phellinus igniarius* used for the biosorption of methylene blue is 0.015 g (ratio adsorbent dose/solution = 0.3 g/l), the maximum amounts adsorbed in equilibrium have been found as 249.92 and 263.33 mg/g, respectively. Biosorption percents are 75 and 79 for *F. fomantarius* and *P. igniarius*, respectively (Maurya et al., 2006). When the mass of on banana peel and orange peel used for the biosorption of methylene blue is 0.10 g (ratio adsorbent

dose/solution = 1.0 g/l), the maximum amount of methylene blue biosorbed on banana peel and orange peel has been found as 15.90 and 13.90 mg/g and their percentages are 15.90 and 13.90%, respectively (Annadurai et al., 2002). Herein, when the mass of *A. wentii* used for the biosorption of methylene blue is 0.10 g (ratio adsorbent dose/solution = 2.0 g/l), the maximum percentage of MB biosorbed onto *A. wentii* is 82.54 and 85.04 at pH 6.33 and 10, respectively. These indicate that *A. wentii* will be a favorable biosorbent for the biosorption of MB.

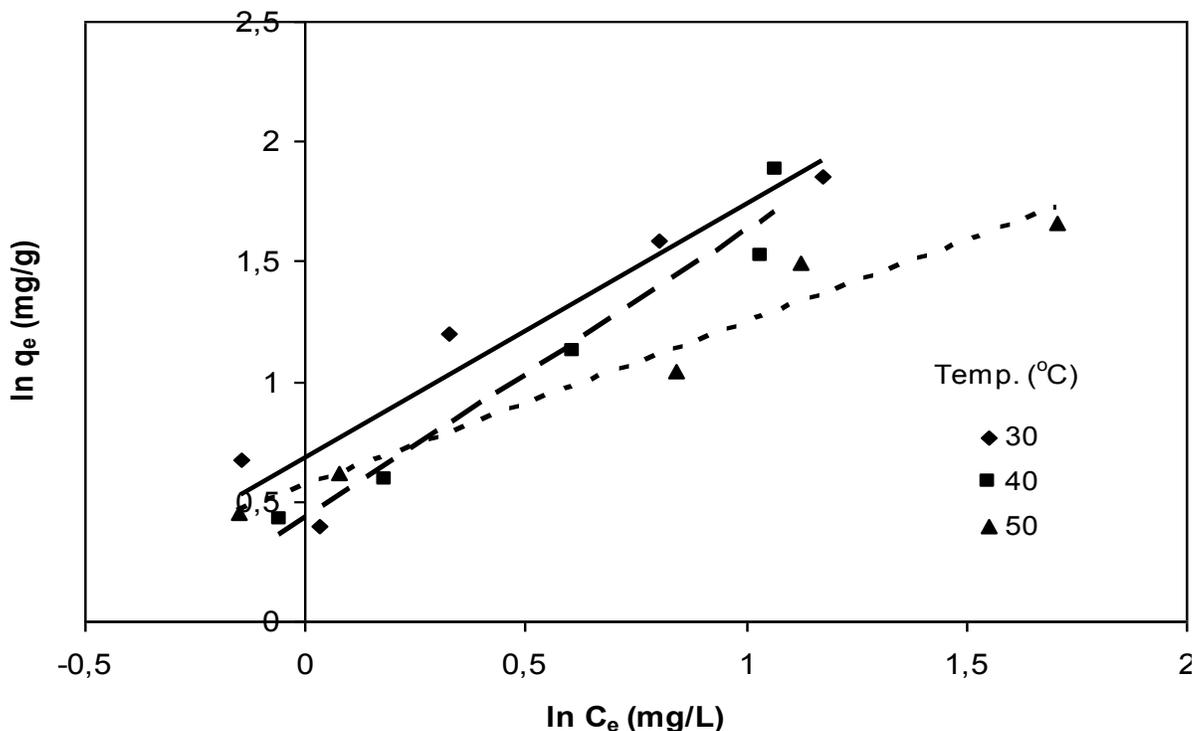
### Biosorption isotherm

The biosorption equilibrium data were fitted for Freundlich and Langmuir isotherms given following, respectively. The isotherm results indicate that the biosorption of MB onto biomass consistent with the Freundlich model. Freundlich biosorption isotherm, which assumes that biosorption take places on heterogeneous surface. The following equation 1 and 2 express Freundlich and Langmuir isotherms, respectively.

$$\ln q_e = \ln k + 1/n \ln C_e \quad (2)$$

$$C_e/q_e = 1/Q_0 \cdot b + C_e/Q_0 \quad (3)$$

where  $q_e$  is the amount of dye adsorbed at equilibrium time (mg/g),  $C_e$  is the equilibrium concentration of the dye in solution (mg/l).  $k$  and  $n$  are isotherm constants which indicate capacity and intensity of the biosorption, respectively.  $Q_0$  and  $b$  are Langmuir constants which indicate adsorption capacity and energy, respectively.



**Figure 5.** Freundlich isotherm of biosorption of methylene blue onto *A. wentii* at different temperatures (— : 30°C, - - - : 40°C, ..... : 50°C).

**Table 3.** Freundlich constants of biosorption of methylene blue on biomass at different temperatures.

Temperature (°C)	n (l/g)	k (mg/g)	r <sup>2</sup>
30	0.94	1.96	0.892
40	0.83	1.28	0.961
50	1.47	1.76	0.963

Figure 5 shows the plots of  $\ln q_e$  against  $\ln C_e$  at different temperatures. The plots are in harmony with Freundlich isotherm with correlation coefficients of 0.89 – 0.96. The values of  $k$  and  $n$  were calculated from the slopes and intercepts of the plots of  $\ln q_e$  vs  $\ln C_e$ . The constants obtained for Freundlich isotherms are shown in Table 3. On the other hand, harmony with Langmuir isotherm is not observed. The values of  $Q_0$  from Langmuir have been determined as -22.22, -15.57 and 9.85 mg/g for 30, 40 and 50°C, respectively. The values of  $b$  from Langmuir have also been found to be -0.026, -0.09 and 0.216 for 30, 40 and 50°C, respectively. The negative values of adsorption capacity and energy indicate contrast with Langmuir isotherm model. Although the values of  $Q_0$  and  $b$  obtained at 50°C are found positively, the correlation coefficient of Langmuir plot has been found to possess a low value of 0.70. The fact that the biosorption obeys only Freundlich isotherm suggests that the surface of the biomass has some heterogeneity and biosorption local.

This situation is attributed to the fact that various active sites on *A. wentii* have different affinities to MB molecules.

### Desorption of methylene blue

Desorption studies can help the researcher to regenerate the spent biosorbent and dye. In order to investigate the possibility of desorption from the *A. wentii*, batch desorption experiments were performed at total eight pHs among 3 - 10 as is in adsorption. Dye-loaded biomass, after biosorption for initial dye solution of 8.00 mg/l onto biomass, was stirred with 50 ml of various alkaline waters for 60 min, separately. The results obtained are shown in Figure 6. As shown in Figure 6, desorption percent decreases with increasing pH. Namely, acidic medium is in favor of desorption. The most desorption occurs at pH 3. The maximum

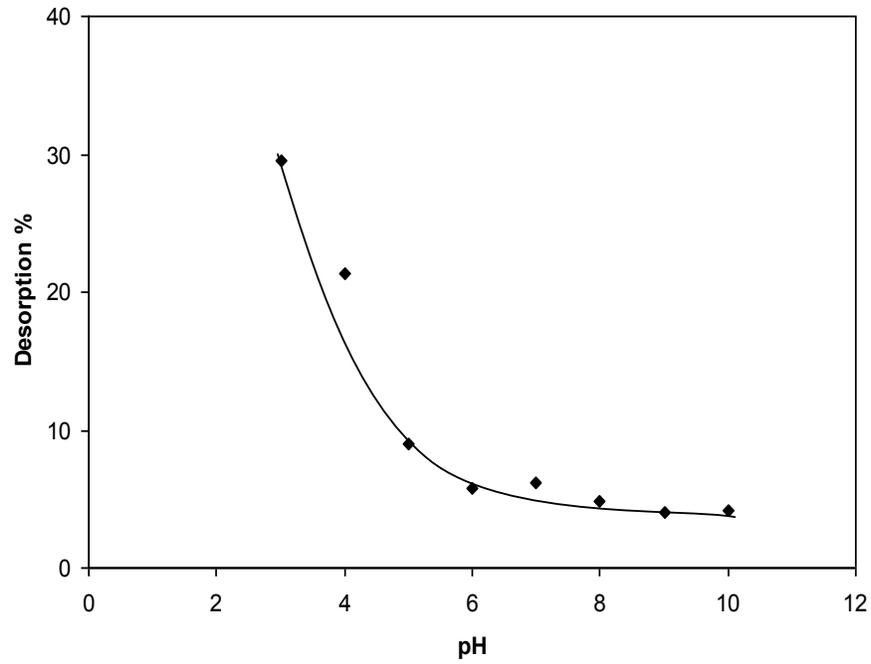


Figure 6. Effect of pH on desorption of methylene blue.

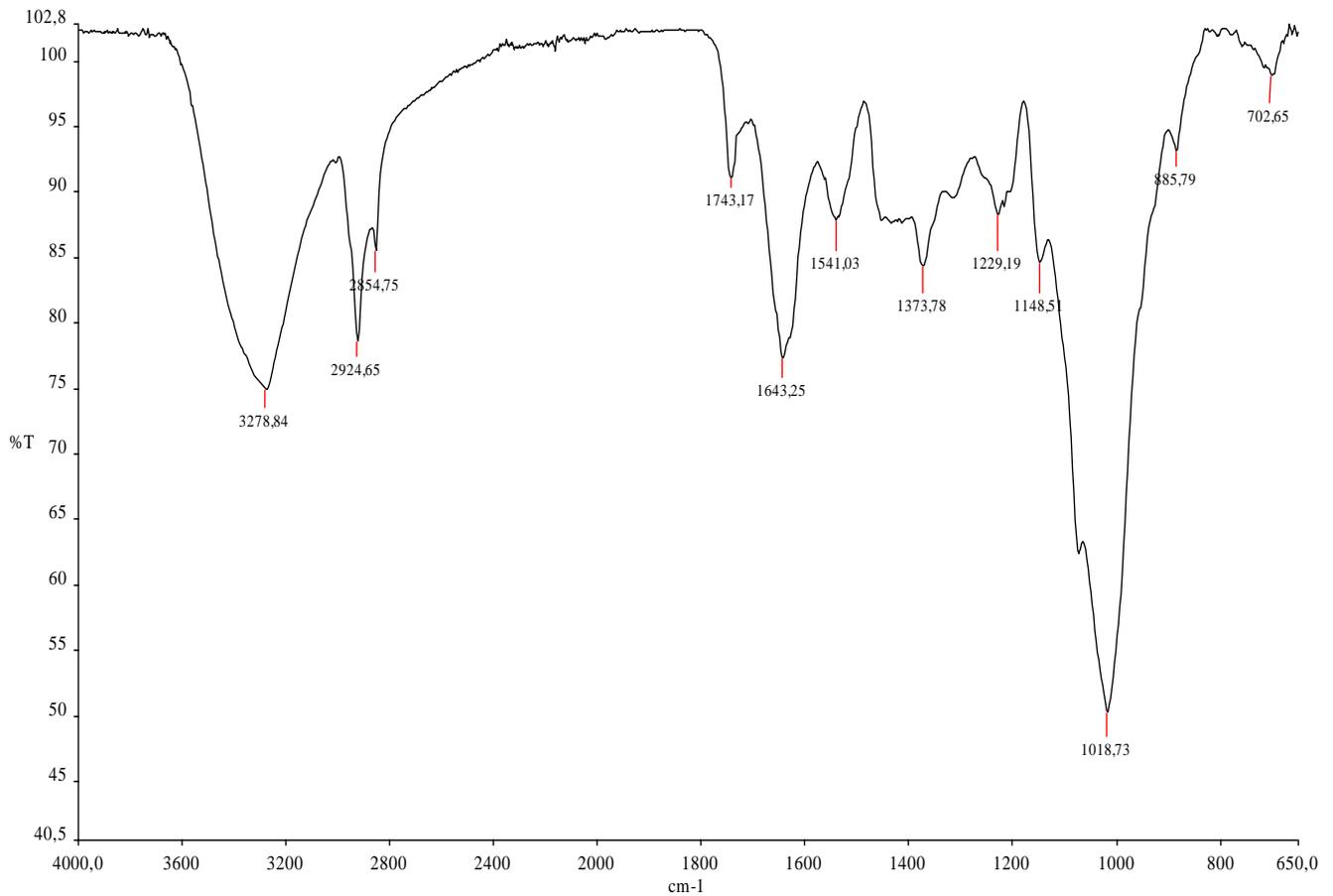


Figure 7. FT-IR spectra of *A. wentii*.

percentage of desorption is 29.51 at pH 3. This is just opposite to the pH effect on biosorption in this work. phenomenon indicates that ion exchange of H<sup>+</sup> ions take place with the cationic methylene blue molecules biosorbed on *A. wentii* because the H<sup>+</sup> ions are excessively present in mediums of low pHs, desorbing the same dye molecules.

### FT-IR study of biosorption

Figure 7 shows the FTIR spectra of *A. wentii*. The broad band at 3274.84 cm<sup>-1</sup> indicates to the NH<sub>2</sub> asymmetric stretch of amines and bonded OH groups. Strong and weak peaks at 2924.65 and 885.79 cm<sup>-1</sup> can be attributed to C-H groups. The peak at 1743.17 cm<sup>-1</sup> refers to carbonyl stretch of carboxylates (Kapoor and Viraraghavan, 1997). The peak at 1643.25 cm<sup>-1</sup> is a result of CO stretching mode conjugated to a NH deformation mode and is indicative of amide 1 band. The band at 1541.03 cm<sup>-1</sup> can be assigned to the presence of amide 2 and results from NH deformation mode conjugated to C=N deformation mode. The peak at 1373.78 cm<sup>-1</sup> indicates to the presence of an amide 3 or sulfamide band. Amide 1 - 3 was observed by Guibal et al. for fungi *Aspergillus*, *Penicillium* and *Mucor* (Guibal et al., 1995). The peak at 1229.19 cm<sup>-1</sup> refers to C=O stretching band of carboxylic groups (Bayramoglu and Arica, 2007). The peaks at 1148.5 and 1018.73 cm<sup>-1</sup> can be attributed to CO or CN stretching vibrations of protein fractions (Vijayaraghavan and Yun, 2008). The < 1000 cm<sup>-1</sup> was the fingerprint zone which were phosphate and sulphure groups (Gulnaz et al., 2006). FT-IR results indicate that the *A. wentii* has several functional groups which are able to react with positively charged methylene blue molecules in aqueous solution.

### Conclusion

*A. wentii* was utilized as a biosorbent for the biosorption of methylene blue from aqueous solution. It was seen that the biosorption of methylene blue was decreased with increasing temperature while it was increasing with increasing solution concentration and pH. The most biosorption of methylene blue on *A. wentii* was occurred at 30°C and pH 10 and the maximum biosorption percent was 85.04 at these temperature and pH. Desorption of methylene blue increased with decreasing pH and the maximum desorption percent was 29.51 at pH 3. Moreover, biosorption process followed the Freundlich isotherm model. As a result, this study shows that *A. wentii* can be used as a potential biosorbent for dye removal from industrial wastewaters.

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