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Biosorption capacity of methylene blue by *Alternaria alternata*

Badour Salim Ali Alfarsi and Sedky H. A. Hassan*
Department of Biology, College of Science, Sultan Qaboos University, Muscat, 123, Oman.
**Corresponding authors' email address: s.hassan@squ.edu.om (S.H.A.H.)*

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Abstract. Industrial effluents containing colored dyes have become a significant contributor to water pollution, and since water is the most critical natural resource, it is a duty to treat it. In this study, *Alternaria alternata* was used as a biosorbent for the adsorption of methylene blue (MB) from an aqueous solution. The effects of contact time, initial dye concentration, pH, and temperature on biosorption were examined. The obtained results showed that the contact time required for maximum dye biosorption was 90 minutes. Furthermore, the amount of dye biosorbed increased as the pH of the solution increased, with 8 being the optimum pH. Biosorption reached its highest amount at 40 °C. Similarly, an initial concentration of 100 ppm of MB was preferred the most in biosorption. The results concluded that *Alternaria alternata* could be an attractive option for removing methylene blue from dye wastewater because of its high biosorption capacity and low cost.

Keywords: *Textile, Alternaria alternata, methylene blue, biosorption, adsorption, biosorbent*

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1. Introduction

The manufacture of dye effluent has developed rapidly due to the increasing number of industries (dying, fabric, cosmetics, paper production, printing, food, and coloring) that use various dyes to paint their products (Bilal et al., 2010). The high demand for synthetic dyes is a result of the deficiency in natural resources. The attraction of using synthetic dyes is their highly soluble properties in water, which can be absorbed easily. Furthermore, synthetic dyes offer various colors and are very rapid at coloring (Jamee & Siddique, 2019). There are approximately 100,000 commercial dyes produced annually, totaling over 700,000 metric tons. However, nearly 10 to 15% of the dyes used in manufacturing procedures are discharged into the processing waters (Bouras et al., 2021). Generally, synthetic dyes are considered an essential source of wastewater since they are produced in large quantities. In addition, synthetic dyes pose great threats not only to aquatic life but also to human health and cause serious illnesses, including cancer and gastrointestinal irritation; hence, they release noxious chemicals, including chlorides, amines, aromatics, and metals (Kabbout & Taha, 2014). Synthetic dyes play a significant role in inhibiting the penetration of the sunlight that enters the water and thus lead to a serious problem in photosynthetic activity and disturb the food chain (Bilal et al., 2010). By considering the nature of the dye, many types of dyes are conceived to be teratogenic, mutagenic, and oncogenic when breathed or applied to the skin (Abdallah & Taha, 2012). Dyes have various categories and complex structures that make them hard to treat and resistant to temperature, oxidation, and photolysis. It has been suggested that there are over 5,000 types of dyes, including reactive, basic, acidic, azo, diazo, anthraquinone, cationic, and anionic dyes (Rani et al., 2014).

Methylene blue (MB) is considered a thiazine cationic dye, and it belongs to the azo dye group. The other name for methylene blue is tetramethylthionine chloride. Methylene blue dye is represented as a heterocyclic aromatic chemical compound. MB is most likely used as a model for cationic dyes in numerous types of research. Frequently, it has various applications, including coloring hair, cotton, paper, and wool. Besides its widespread utilization in the dye textile and house industries, it has an important role in the medical field, particularly in diagnostics and surgery. Furthermore, it has been found to act as a photo-oxidation sensitizer (Abbas et al., 2018). MB is not considered an enormously hazardous dye. However, it can result in side effects since it contains aromatic amines, which are regarded as carcinogenic (Abbas et al., 2018). Also, Nharingo et al. (2013) highlighted that MB can increase the heart rate and cause cyanosis, regurgitation, shock, jaundice, and tissue necrosis. It also results in the formation of Heinz's body, quadriplegia, and diarrhea.

The menace of synthetic dyes had been eliminated by improving several conventional techniques. The strategies used to treat the dyes include chemical, physical, and biological procedures. Among all dye removal techniques, biological treatments are mostly preferred because they are regarded as "green." The biological treatment is environmentally friendly and produces a small amount of sludge compared to the chemical treatment. Moreover, this approach breaks down the bonds of synthetic dyes and converts them to a less harmful inorganic compound (Bhatia et al., 2017).

Biosorption has been known as an effective alternative to bleach-colored wastewater by using biomass as an adsorbent. Various biomasses could be used in biosorption methods, including algae, bacteria, fungi, seaweeds, and sludge from biological wastewater treatment plants, as well as fermentation manufacturers' by-products (Aksu et al., 2010). Several studies have revealed that natural biological, agricultural, and industrial waste materials are considered the best choice for bio-adsorbents in terms of economic alternatives (Abbas et al., 2018). In recent times, significant attention has been paid to biosorption because of the high performance in removing the dyes, the requirement of simple equipment, and the high level of selectivity and flexibility. In addition to that, it has been suggested that the low cost and simple operation of the biosorption approach give more interest to using this method and make it more affordable than other types (Abdallah & Taha 2012). Other treatments than adsorption have several disadvantages, such as utilizing a huge amount of chemicals, insufficient removal of dyes, and producing a large amount of undesirable toxic sludge. Bhatia et al. (2017) said that the success of biosorption depends on a number of factors, such as contact time, pH, temperature, dye concentration, adsorbent dose, dye structure, type of microorganism, and ionic quality.

A wide variety of fungi can eliminate various types of dyes. The cell wall of fungi consists of acidic polysaccharides like chitin. In addition to chitin, chitosan is another component of the cell wall, which is composed of various functional groups like phosphate, hydroxyl, and amine. Both chitin and chitosan play a significant role in the biosorption of dyes, heavy metals, and phenolic compounds by forming a strong force or bond between the cell wall of the adsorbent and the synthetic dyes. Several genera of fungi have been utilized, whether in living or inactivated form, in biosorption (Rani et al., 2014). A study conducted in 2013 highlighted the ability of a fungus called *Alternaria alternata* to decolorize and biodegrade a type of anionic synthetic dye known as Congo red (Chakraborty et al., 2013). As the *Alternaria alternata* was capable of removing a type of synthetic dye, this indicates it may have the same effects on another synthetic dye, such as methylene blue, that will be estimated through this study. This research aims to estimate the biosorption capacity of the cationic dye methylene blue produced by *Alternaria alternata* and to study the effects of pH, contact time, MB concentration, and temperature on biosorption capacity.

2. Materials and Methods

2.1. Sampling and isolating microorganisms

The biomass used in this study was derived from *Alternaria alternate*, which was isolated from the advertisement board of the College of Science at Sultan Qaboos University. After sterilizing the media in an autoclave at 120 °C for 2 hours, the sample was grown in Petri dishes containing SDA (sour dextrose agar). After 7 days of incubation at room temperature (25±2 °C), the sample was purified to isolate *Alternaria alternata* from other microorganisms.

2.2. Preparation of an inoculum of the fungal spore

First, liquid SDB (Sabouraud Dextrose Broth) media was prepared to grow the fungi by mixing 60 grams of SDB in 1 liter of distilled water. Then, media was distributed into 10 Erlenmeyer flasks (250 mL) containing 100 mL, and then each Erlenmeyer flask was autoclaved at 120 °C for 2 h. The final step was to keep them in incubation for 7 days with shaking at 28 °C and a speed of 150.

2.3. Preparation of fungal biomass

The biomass was harvested and then centrifuged for 10 minutes at 4,000 rpm; after that, the pellet was taken and oven-dried at 60 °C for 24 h. Next, the biomass was ground into fine pieces, and then it was used as a biosorbent for batch biosorption studies.

2.4. Dye solution preparation

The dye used in this study was methylene blue ($C_{16}H_{18}ClN_3S.2HO$; molecular weight: 319.86), which is a cationic dye. The chemical structure of MB is shown in Figure 1. The stock solution of 1,000 ppm of MB was prepared by mixing 50 mL of ultrapure water with 0.05 g of MB powder, which was from the Sigma brand, and other concentrations were obtained by successive dilutions.

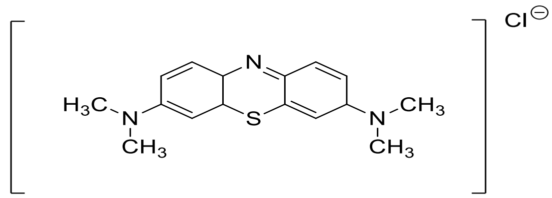

Figure 1. The chemical structure of methylene blue (ChemDraw)

2.5. Dye concentration analysis

Standard curves were made by using a UV/Visible Spectrophotometer (P SELECTA model v-1100D Spectrophotometer) to measure the absorbance of the dye solutions at their maximum wavelength of 663 nm, as shown in Figure 2.

2.6. Batch biosorption experiments

The batch biosorption studies were carried out at various parameters such as pH (2–8), contact time (5–120 min), initial dye concentration (5–150 ppm), and temperature (28 °C and 40 °C) at pH 8; for the pH experiment, 8 test tubes were prepared, and 0.03 g of fungal biomass was added into each tube with 900 µL of 1,000 ppm methylene blue solution, and then distilled water was added to adjust the final volume to 30 mL. Next, the pH value was adjusted to (2, 3, 4, 5, 6, 7, and 8) for each test tube. Then the tubes were placed in a rotating shaker at 28 °C at a speed of 150 rpm for 30 min. After that, the mixtures were centrifuged for 10 minutes at 4,000 rpm (using Bio-Rad Centrifuge Company) to separate the *Alternaria alternata* from the MB. The absorbance of the supernatant solution was measured by UV-spectrophotometer and repeated three times; only the average was obtained. Based on this absorbance data, the final dye concentration was calculated. For other parameter experiments, the same previous steps were performed, and only the tested parameter was changed while other factors were kept constant and the optimum value of the previous parameter experiment was used.

Based on the graph, the slope of the line was determined using the equation:

$$slop = \frac{y_2 - y_1}{x_2 - x_1} \quad (1)$$

Then, methylene blue's remaining amount was measured using the following equation:

$$\text{Unadsorbed amount of the dye} \left(\frac{\text{mg}}{\text{g}}\right) = \frac{\text{Absorbanceat 663 nm}}{\text{slope}} \quad (2)$$

The adsorbed amount of the dye (MB) was measured using the following equation:

$$\text{The adsorbed amount of the dye} \left(\frac{\text{mg}}{\text{g}}\right) = \text{Initial dye concentration} - \text{Unadsorbed amount of the dye} \quad (3)$$

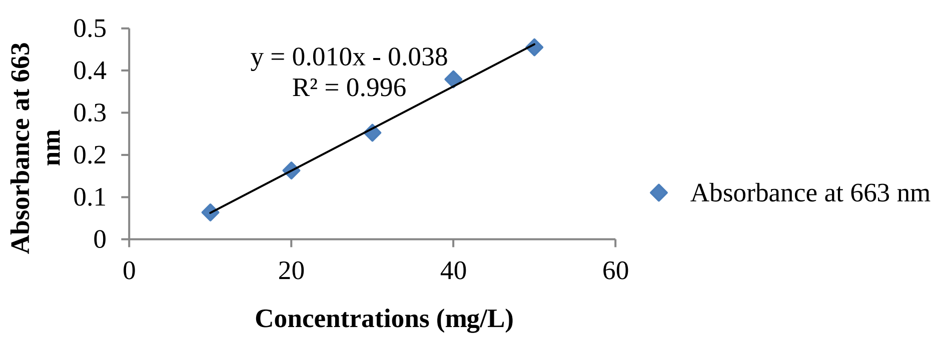

Figure 2. Standard absorbance curve at 663 nm of methylene blue using different concentrations

3. Results

3.1. The effect of solution pH on biosorption capacity

The effect of pH was studied at different ranges (2–8). It is clear from Figure 3 that as the pH value increased, the biosorption capacity increased. The highest adsorbed amount was at pH 8, around 19±0.41 mg/g. At pH 2, however, no biosorption occurred. It can be shown that the biosorption of MB is more effective in an alkaline solution rather than an acidic one.

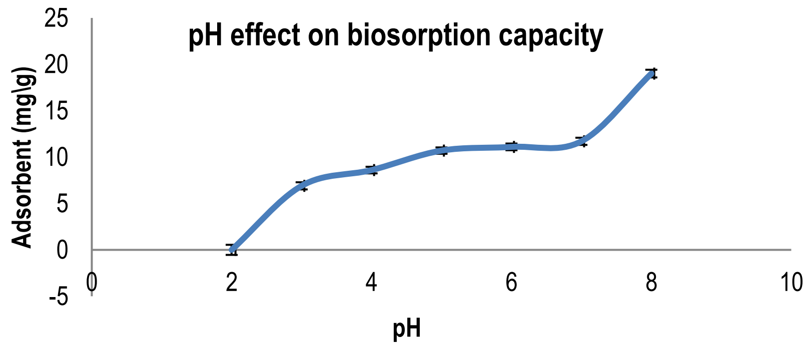

Figure 3. The effect of solution pH on the ability of *Alternaria alternata* to biosorb MB ions at 28 °C, 1000 mg/L MB, and 0.03 g of biomass

3.2. Effect of contact time on biosorption capacity

The effect of contact time was investigated at 5, 10, 20, 30, 60, 90, and 120 min. It is obvious from Figure 4 that the adsorbed amount increased with increasing contact time and then remained constant. The biosorption reached equilibrium after 90 minutes. The adsorbent amount of MB at equilibrium time was found to be 21±0.25 mg/g.

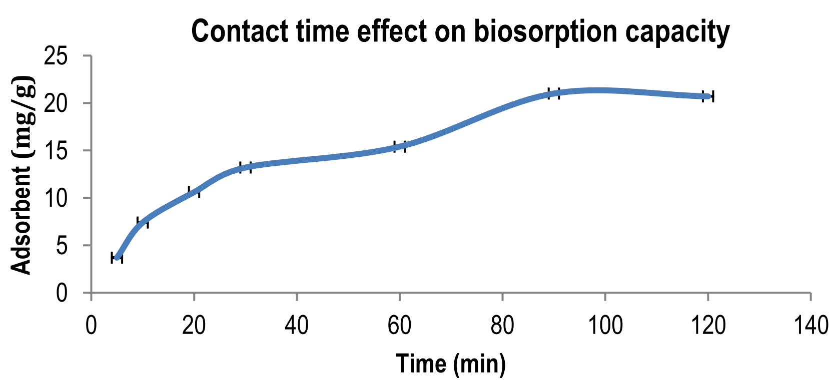

Figure 4. Effect of contact time on the biosorption capacity of MB ions using *Alternaria alternata* at 28 °C, Co = 1000 mg/L of MB, and 0.03 g of biomass

3.3. Effect of initial ion concentration on biosorption capacity

The initial concentration of methylene blue played a significant effect on biosorption capacity. This effect was studied at different concentrations, including 5, 10, 20, 30, 50, 70, 100, and 150. Based on Figure 5, the biosorption capacity showed an increase with the initial concentration until it reached the saturation point, where there was no more increase in the adsorbent amount. The highest adsorbent amount was found at 100 mg/L, which was 83±0.31 mg/g.

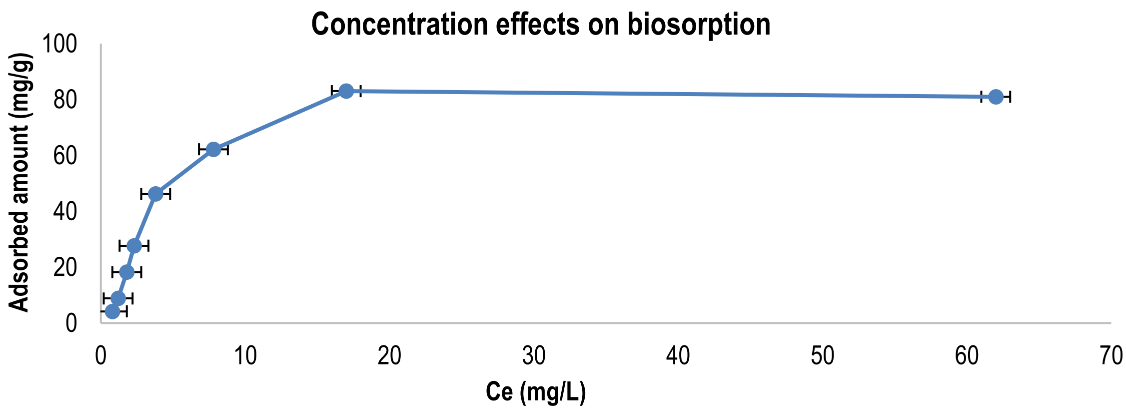

Figure 5. Effect of initial concentration on uptake capacity of MB ions using *Alternaria alternata* at 28 °C, 0.03 g of biomass, and different initial concentrations (5, 10, 20, 30, 50, 70, 100, and 150) mg/L

3.4. Effect of temperature on biosorption capacity

The influence of temperature on the adsorbent amount of MB was tested at two temperatures (28 °C and 40 °C). According to Figure 6, there was a difference in adsorbent amount between 28 °C and 40 °C. At 28 °C, biosorption increased to 83.0±0.31 mg/g, while at 40 °C, it reached 98.9±0.23 mg/g.

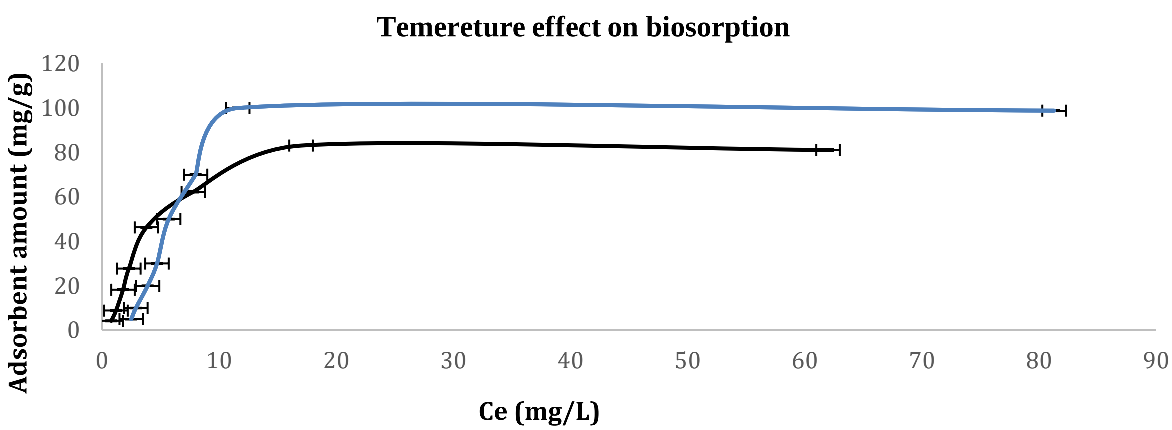

Figure 6. A comparison of the effects of 28 °C (black line) and 40 °C (blue line) temperature on the biosorption capacity of MB ions in Alternaria alternata under the following conditions: 0.03 g of biomass at different initial concentrations (5, 10, 20, 30, 50, 70, 100, and 150) mg/L

4. Discussion

4.1. The effect of solution pH on biosorption capacity

It is thought that the pH of the water solution influences biosorption capacities. The effect of pH on the removal of methylene blue dye from wastewater by *Alternaria alternata* was examined over the range of 2.0 to 8.0 at 28 °C, Co = 1000 mg/L, and 0.03 g of biomass, as shown in Figure 2. It is clear from this figure that the biosorption capacity increases from 0±0.54 to 19±0.41 mg/g as the pH increases from 2 to 8, and then will remain constant with further increases in the pH of the solution. The pH effect on the methylene blue adsorption is more favorable in an alkaline solution, and that can be clarified by the fact that at lower pH values, the biosorbent surface is positively charged, and as the pH value rises, the sorption of methylene blue ions increases and then the biosorbent surface gradually becomes negatively charged. With lower pH values, there will likely be a higher concentration of H^+ ions in acidic solutions, and this will result in high competition with the MB ions for available sorption sites (Abbas et al., 2018). The highest amount of biosorption was observed at pH 8, with a percentage of 63.3%, while at pH 2, the biosorption percentage was 0%. As reported by a study to investigate the biosorption of MB from solution white rot fungi, the optimum range was from 5.5 to 7.5, which varies from this study and can be explained by the different surfaces of the biosorbent.

4.2. Effect of contact time on biosorption capacity

The effect of contact time was studied at initial concentrations of 50 mg/L at 28 °C and pH = 8.0 for (5, 10, 20, 30, 60, 90, and 120) min. It is obvious from Figure 3 that the removal of MB increased with contact time and the adsorption achieved equilibrium after about 90 min, which was 21±0.25 mg/g, and then after this time the adsorbent amount will keep constant, and that can be explained by the fact that during the initial phase, a large number of vacant surface sites were available for biosorption, whereas when the equilibrium stage is reached, the remaining vacant surface sites are difficult to occupy because of repulsive forces between solute molecules in the solid and bulk phases. The highest removal percentage was observed at 70.0% after 90 min; accordingly, 90 min was selected for the next parameter experiment. According to a study done by Abdallah and Taha (2012) to investigate the biosorption capacity of methylene blue from aqueous solution by nonviable *Aspergillus fumigatus,* the optimum contact was after 30 min, which is different from the obtained result in this study, which was after 90 min, and that may be because of the difference in the biosorbent fungi used to eliminate the MB.

4.3. Effect of initial ion concentration on biosorption capacity

The biosorption capacity is highly affected by the initial ion concentration. This factor was implemented at initial concentrations of 5, 10, 20, 30, 50, 70, 100, and 150 mg/L at 25 °C and 8 pH for 120 min of contact time, as shown in Figure 5. The adsorbent amount increases from 4.2±0.21 mg/g to 83±0.31 mg/g, with an increase in the initial methylene blue concentration from 5 to 100 mg/L. The percent MB removal at 100 mg/L was found to be 83%. After 100 mg/L, the biosorption capacity remained unchanged. The reason for this is that with an increase in the initial concentration of MB, the concentration of MB at the biosorbent interface also increases, which increases biosorption. Consequently, this occurred due to a higher probability of collision between the MB ion and the biosorbent particles. Biosorption reaches its saturation point when the active sites at the surface of the biosorbent are filled with MB ions. According to a 2018 study that looked into the biosorption of methylene blue from aqueous solutions by waste mycelium of fungal biomass type white rot fungi (Abbas et al., 2018). The result of this study showed that the biosorption reached equilibrium at 100 mg/ around 23.1 mg/g, which agrees with the result obtained in the current study.

4.4. Effect of temperature on biosorption capacity

The effects of temperature on the *Alternaria alternata* biosorption capacity were investigated at 28 °C and 40 °C. As illustrated in Figure 6, the MB elimination increased with increasing temperatures from 28 °C to 40 °C, from around 83.0±0.31 mg/g to 98.9±0.23 mg/g, respectively, at equilibrium. The possible explanation is that as the temperature increased, the adsorbate molecules were diffused more quickly over the external boundary layer and through the internal pores of the adsorbent particle.

5. Conclusions

In this study, the biosorption ability of the fungus *Alternaria alternata* was tested to see if it could remove the color from methylene blue basic textile dye. This was done because no previous research had mentioned using this strain of *Alternaria* with this dye. The amount of MB adsorbed by this fungus depends on the initial dye concentration, contact time, temperature, and pH of the solution. The results show that the best conditions for the uptake of methylene blue are at 5–120 min, pH 8, 100 ppm MB concentration, and 40 °C temperature. The findings of this study show that *Alternaria alternata* is a successful biosorbent in the removal of methylene blue from aqueous solutions in terms of cost, efficiency in removing dyes, and abundance in natural and environmentally friendly material.

Conflicts of interest. The authors declare that there are no conflicts of interest regarding the publication of this paper.

ORCID

Badour Salim Ali Alfarsi: https://orcid.org/0000-0001-5378-8928
Sedky H.A. Hassan: https://orcid.org/0000-0003-2481-5231

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