Biodecolorization of textile dye effluent by biosorption on fungal biomass materials

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Abstract
Colored industrial effluents have become a vital source of water pollution, and because water is the most important natural source; its treatment is a responsibility. Usually colored wastewater is treated by physical and chemical processes. But these technologies are ineffective in removing dyes, expensive and not adaptable to a wide range of colored water. Biosorption was identified as the preferred technique for bleaching colored wastewater by giving the best results.

This treatment was based on the use of dead fungal biomass as new material for treating industrial colored effluents by biosorption. We studied the ability of biosorption of methylene blue (MB) by \textit{Aspergillus fumigatus} and optimize the conditions for better absorption.

Biosorption reaches 68\% at 120 min. Similarly, the biosorbed amount increases up to 65\% with pH from 4 to 6, and it's similar and around 90\% for pH from 7 to 13. At ambient temperature 20-22\(^\circ\)C, the percentage of biosorption of methylene blue was optimal. The kinetic of biosorption is directly related to the surface of biosorbent when the particle size is also an important factor affecting the ability of biosorption. Also the biosorption of methylene blue increases with the dose of biosorbent due to an augmentation of the adsorption surface.

In this study, for an initial concentration of 12 mg/L of MB (biosorbent/solution ratio=2g/L) buffered to alkaline pH, and a contact time of 120 min, biosorption takes place at an ambient temperature and reaches 93.5\% under these conditions.

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1. Introduction

The synthetic dyes are frequently used in several recent technologies and industrial sectors (Acemioglu and al., 2010; Bhole and al., 2004). Due to the persistent nature and recalcitrant of dyes, the large quantities of discharge cause the contamination of water and the environment (Crini, 2006). Its

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treatment is therefore a responsibility to eliminate any risk on human health. The effects of dyes on human health can range from gastrointestinal irritation to the cancer (Santhi and al., 2009). Usually colored wastewater is treated by physico-chemical processes (Ho and McKay, 1998). But these treatments have limitations like the formation of toxic by-products and intensive energy requirements (Padmesh and al., 2005; Aksu, 2005), and are ineffective in removing dyes, expensive and not adaptable to a wide range of colored water (Banat and al., 1996; Fu and al., 2001). The decolorization can be realized biologically by three different processes: biosorption, biodegradation and bioaccumulation. The biosorption is considered the most advantageous than others for the treatment of colored waters (Kaushik and Malik, 2009) and is identified as the preferred technique for decolorization by giving the best results (Jain and al., 2003; Ho and McKay, 2003).

In this research we choose to study the biosorption of methylene blue (MB) by a dead fungal biomass: Aspergillus fumigatus. The physicochemical characteristics of the MB are: trade name: Basic blue 9, belongs to the class of thiazine, basic ionization, aqueous solubility 3.55%, maximum absorption at 665nm and molecular mass 319.9 g/mol, chemical structure: C_{16}H_{18}N_{3}SCl (Acemioglu and al., 2010).

The biosorption of colored wastewater by biomass (dead or alive) was the subject of several recent studies reviewed (Aksu, 2005; Pearce and al., 2003; McMullan and al., 2001; Stolz, 2001). The degradation of dyes by several different bacteria such as Aeromonas hydrophila and Pseudomonas putida has reached a high percentage of decolorization in several studies (Ren and al., 2006; Chen and al., 2003; Chen and al., 2007). The role of fungi in the treatment of colored waters has been greatly studied (Azmi and al., 1998; Brar and al., 2006). By using inexpensive media culture, several fungal species dead or alive were used for water decolorization by biosorption (Fu et Viraraghavan, 2002; Mittal et Gupta, 1996; Kaushik et Malik, 2009). Recently, studies move away from the use of bacterial biomass because its isolation is difficult and requires an abundant amount of nutrients while the amount of bacterial biomass obtained is low. In comparison with the bacterial biomass, the separation of dye effluent is easier for fungal biomass (Kaushik and Malik, 2009). Compared with living biomass, dead biomass eliminates the problems of toxicity and nutritional requirements. In this case, dead organisms are not affected by toxic waste (Brady and al., 1994). Their use is effective when the conditions are not always favorable for growth and maintenance of the microbial population (Crini and al., 2006). Their operation is easy and simple. It is therefore an effective biosorbent. Mou and al. (1991) compared the living and autoclaved cells and found that those autoclaved are more effective in fading waters. Fu and Viraraghavan (2001) showed that the capacity of dead biomass Aspergillus niger to remove a dye (either basic or acidic) is better than living. Tatarko and Bumpus (1998) used the two cultures of the fungus P. Chrysosporium living and dead (autoclaved) to treat water. They found that the autoclaved cells have a higher percentage of biosorption (90%) than living cells (70%). The isotherms of the biosorption data of MB by Aspergillus fumigatus was studied also.

1. Materials and Methods

1.1. Preparation of dead biomass

A liquid growth medium was prepared, composed of 20g glucose, 7g brain heart (BIOMERIEUX, France) in one liter of distilled water. After autoclaving and cooling, liquid gentamicin (PANPHARMA, France) is added to the medium (240 mg/L of the medium). The medium is then inoculated with the fungal species, and then placed in a rotary platform shaker (Edmund Buhler, Germany) for 10 days at room temperature 22±2°C and rotation speed of 150 rpm. The fifth day of culture, 150 ml of a liquid culture medium similar to that prepared initially is introduced into the medium to ensure nutritional requirements. After 10 days of incubation, the biomass obtained is autoclaved at 120°C /15 min, then filtered under vacuum using Whatman filter paper grade1, well washed with distilled water and finally dried in an oven at 80°C (ONTHERM, New Zealand) for 6 hours to prepare the dead biomass. This treatment leads not only to inactivate the fungal organisms but also to increase the adsorption efficiency (Bhole and al., 2004). Biomass is obtained in the form of a black small plate (obtaining of 5 g of dead biomass per liter of liquid media culture) crushed using a mortar and finally sieved to obtain two different particle sizes: 0.25 and 0.5 mm that will be used as biosorbent in this biosorption studies.
1.2. Preparation of dye solution

Methylene blue, a cationic dye, obtained by the Rainbow Company, Lebanon, unpurified. Several dilutions and calibration lines are prepared with distilled water.

1.3. Sample preparation and biosorption studies

Biosorption studies are carried out in 50 ml Erlemeyer flasks by mixing 20mg of biomass with 10 ml of methylene blue solution 12 mg/L, neutral pH at room temperature and 0.25 mm particle size. The samples were placed in the shaker in a constant speed of 150 rpm at room temperature. After a determined contact time, 1 ml of the supernatant was taken and analyzed using a UV-Vis spectrophotometer (Thermo Fischer Scientific, Evolution 60, USA), to determine the concentration of dye remaining in the sample at the maximum wavelength of the MB, obtained at 665 nm. The amounts of MB biosorbed onto biomass were calculated using the following equation:

\[ Q_e = \frac{(C_0 - C_e)V}{W} \]

Where \( C_0 \) is initial concentration (in mg/L), \( C_e \) 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) (Santhi and Manonmani, 2009). Several parameters were studied: the contact time, pH, temperature, type of agitation, particle size, and the effect of dose and concentration of the dye.

2. Results and discussion

2.1. Effect of contact time

The effects of contact time on the adsorption capacity of *Aspergillus fumigatus* were studied in all experimental conditions, concentration, temperature and pH. The results (fig.1) show that a rapid biosorption of MB by *Aspergillus fumigatus* occurs at 30 min and reaches 58%. At 120 min it becomes 68%. Beyond 120 min, biosorption reaches a maximum of 71% at 210 min and remains constant. Therefore, we assume that 120 min is determined as the optimum contact time.

![Figure 1: Contact time influence on the biosorption of MB by *A. fumigatus*]

2.2. Effect of pH

The effect of the initial pH of the dye solution, on the biosorption was studied at pH values ranging from 3 to 13 for 120 min.

![Figure 2: Effect of pH on the biosorption of MB by *A. fumigatus*]
The result (fig.2) shows that the biosorption was 0 at pH 3, and the percentage of the biosorption at 120 min increase from 32 to 92% when the initial pH increase from 4 to 13. For pH from 7 to 13, biosorption percentages are almost similar and around 90%. So it's not necessary to significantly increase the pH, a pH of 7 is suitable. To do so, in this study the pH 7 was chosen for use in subsequent experiments.

In order to see if the initial pH of dye solution remains constant throughout the process of biosorption, a final pH measurement is performed for each solution. The initial pH below 7 remains constant until the end of biosorption, while the pH decreases at the end of biosorption for initial pH above 7, which involves the release of positively charged protons during the process. This can be explained by the fact that at high pH, fungal cells transfer their protons and lead to decrease the pH of the medium. The increase of the removal percentage of MB with increasing pH was studied by Kanamadi and al. (2006) and Acemioglu and al. (2010).

2.3. Effect of temperature
The effect of temperature on biosorption was studied at five different temperatures 20, 30, 40, 50 and 60°C. The results indicate that ambient temperature is optimum for biosorption of MB by *A. fumigatus*.

2.4. Effect of agitation type
Two samples were prepared under the same conditions. One of them is placed in the rotary shaker, the other one on a magnetic stirrer (WiseStir, Korea) at the same speed 150 rpm and at room temperature. The results (fig.3) indicate that the percentage of biosorption of MB obtained in the sample placed in the shaker continues to increase and reached 62% at 120 min. the magnetic agitation increases the percentage of biosorption from 47 to 45% until 120 min. This can be explained by an increased turbidity of the colored solution due to cell destruction of the fungal biomass by rotation of the bar magnet.

2.5. Effect of particle size
The initial conditions were applied to two different particle sizes: 0.25 and 0.5 mm, the percentage of dyes adsorbed increases as the particle size of the sorbent decreases.

The results (fig.4) show that to increase biosorption depends on the broad outer surface of small particles of biosorbent. A study published by santhi and al. (2009) shows that the percentage of biosorption on MB increases with decreasing particle size.
2.6. Effect of the dose

This study determines the optimal amount of biosorbent for better biosorption. The percentage of biosorption of the dye increases with an increasing dose of the biosorbent due to an augmentation in the surface adsorption. For all doses studied, we note (fig.5) that an augmentation of the biomass quantity from 1 to 6g/L leads to a significative augmentation in the percentage of the biosorption. A dose greater than 6g/L is not necessary because above this dose all results have almost the same percentage. Kanamadi and al. (2006) determined the influence of the dose on the biosorption of MB and found a higher biosorption when the dose is increased.

2.7. Effect of initial dye concentration

Different concentrations of MB are prepared to determine their influence on biosorption. The amount of biosorbed MB (qe) increases from 0.8 to 9 mg/g with increasing dye concentration from 2 to 24 mg/L. It is therefore clear that the removal of dyes depends on the concentration of the colored solution. A study by Acemioglu and al. (2010), shows that when increasing the concentration of MB from 4 to 16 mg/L, the maximum amount of dye biosorbed by \textit{A.} \textit{wentii} increases from 1.48 to 6.38 mg/g, compared to results obtained in our study (fig.6), the augmentation of the concentration leads to an increase in the amount of MB biosorbed by \textit{A. fumigates} from 1.56 to 5.92 mg/g. a study was conducted by Fu and Viraraghavan (2000) on the biosorption of MB by \textit{Aspergillus Niger}, and the biosorption capacity obtained was 1.17 mg/g. under the same conditions, \textit{A. fumigatus} showed a biosorption capacity of 4.816 mg/g in our study.
3. Biosorption isotherm

The biosorption equilibrium data are evaluated for Freundlich and Langmuir isotherms. The following equation expresses Langmuir isotherm: $1/q_e = \left(1/Q_{max} \cdot b\right) \cdot (1/C_e) + 1/Q_{max}$. Results indicate that biosorption of MB by *A. fumigatus* depends on the Langmuir isotherm. The figure 7 shows $1/q_e = f(1/C_e)$ at different concentrations with a regression coefficient of 0.9906.

![Langmuir isotherm](image)

$1/Q_{max} \cdot b$ is the slope and its value is 0.4445. From the value obtained $1/Q_{max}$ (0.1), we know that the adsorption capacity of MB by *A. fumigatus* $Q_{max}$ is 10 mg/g while the ratio of the rate of adsorption $b$ is 0.2247 L/mg. The essential characteristics of a Langmuir isotherm is expressed by a balance parameter $R_L$ defined by $R_L = 1/(1 + bC_0)$. In this case, $R_L = 0.27$ and the isotherm is considered favorable.

![Freundlich isotherm](image)

The Freundlich isotherm is expressed by: $\ln q_e = \frac{1}{n} \ln C_e$ + $\ln K_f$, where $K_f$ is the adsorption capacity (mg/g) and $n$ is a constant. The figure 8 shows $\ln q_e = f(1/\ln C_e)$ at different concentrations: 2, 4, 8, 12, 16 and 24 mg/L.
The following equation expresses the Freundlich isotherm: \( \ln q = (\ln C_e) \cdot (1/n) + \ln K_f \). The curve \( \ln Qe = f(\ln Ce) \) is obtained in the figure 8. \( 1/n \) is the slope of the line, its value is 0.8253<1. This is favorable biosorption. A regression coefficient of 0.9749 is obtained with the Freundlich isotherm. The values of \( K_f \) and \( n \) are 0.5 mg/g and 1.2117 respectively (\( n \) is between 1 and 10, the adsorption is favorable).

The adsorption obeys not only to the Langmuir model but also the model of Freundlich. This suggest that the surface of the biomass is heterogeneous and biosorb locally. This is attributed to the fact that several active sites of \( A. fumigatus \) have a different affinity to the molecules of the MB.

4. Conclusion

In this research, we studied the biosorption capacity of fungal species \( Aspergillus fumigatus \) overlooked a basic textile dye methylene blue as there is no literature that use this strain of \( Aspergillus \) with this dye. In this study, for an initial concentration of 12 mg/L (compared biosorbent/solution=2g/L), buffered to alkaline pH and a contact time of 120 min, biosorption takes place at an optimum 30°C. The percentage of maximum biosorption of MB is 93.43%. Adsorptions isotherms were evaluated and showed that the adsorption system studied is favorable and follows the Langmuir and Freundlich isotherms.

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