

Claviceps purpurea fungus: a promising biosorbent for wastewater treatment

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Abstract

In this study, Claviceps purpurea was successfully used, for the first time, to remove methylene blue dye from aqueous solutions and treat wastewater. This fungus was as efficient as commercially activated carbon in terms of the dye per cent removal, and it required low processing without a significant cost as it is a readily available biomaterial. The adsorption equilibrium was described by Langmuir isotherm and obeyed a pseudo-second order kinetic model. External mass transfer and intra-particle diffusion mass transfer are the main controllers of the adsorption process. The adsorption process is exothermic and spontaneous as well as proceeds with a positive entropy. SEM micrographs allowed for visualization of the particle morphology before and after adsorption. According to EDS spectra, the adsorbent consists of 64 per cent carbon and 32 per cent oxygen. Furthermore, the CP fungus was evaluated for its ability to treat wastewater samples. Based on the results, CP fungus enabled the adsorption of 94 -98 per cent of the contaminants. CP fungus could then be considered a highly efficient water treatment agent.

Keywords: adsorption; methylene blue; *Claviceps purpurea*; isotherms; kinetics; wastewater treatment

1. Introduction

Dyes are used in several industries, such as the leather, paper, textile and plastics industries. A substantial level of colored wastewater is then generated. Small levels (less than 1 ppm) of some dyes are highly perceptible and offensive for toxicological and esthetical reasons [1]. Indeed, dyes represent a major environmental and health concern because they are hazardous to nearly all living organisms. Moreover, these substances are known for resisting pH effects, microbial attack and solar light. Therefore, it is beneficial to eliminate them from wastewater prior to discharging them into nature [2].

In recent years, several alternatives have been proposed to exploit waste products from agriculture and industry as economical and efficient adsorbents [3, 4, 5, 6, 7, 8, 9, 10, 11].

Recently, a promising alternative to substitute or supplement existing wastewater treatment processes using fungal decolorization has been a topic of intense research [12, 13]. The white-rot fungi were efficiently used to degrade synthetic dyes owing to their ability to produce enzymes that can modify the lignin structure [14]. Several plant-associated fungi

were assayed, and their decolorization efficiencies have been demonstrated [15].

The most frequently used dye in the cotton, wood and silk processing industries is methylene blue (MB). This dye has several side effects, including mental confusion, vomiting, nausea, profuse sweating and methemoglobinemia (with oral ingestion). The cardiovascular side effects of MB include hypertension and precordial pain. MB could be responsible for permanent eye injury in humans and animals. MB can cause respiratory tract irritation when inhaled. It can also produce delayed pulmonary edema^a and cause methemoglobinemia, cyanosis, convulsions, tachycardia, dyspnea and death.

Therefore, the treatment for and removal of this dye from industrial effluents is receiving significant attention in many industries.

Due to the ease of its use, high flexibility and comparably low cost of application to the discoloration process, adsorption is widely used in water decontamination applications [16, 17].

The aim of this study was to perform adsorption of a pollutant, MB dye, at a lower cost and with the minimum number of processing steps. *Claviceps purpurea* (CP) fungus, hosted by *Elytrigia repens*, was used as a readily available biosorbent for MB

adsorption. Equilibrium and kinetic data studies were performed to understand the mechanism of MB adsorption onto CP. Different parameters were considered, including the MB concentration, CP dosage and pH. SEM analyses were used to visualize the morphology of CP particles before and after MB adsorption, whereas EDS analyses were performed to determine the chemical composition of the biosorbent.

2. Materials and methods

2.1 Adsorbent specification

The *Elytrigia repens* plant was collected at Guelma University in May-June 2011; the plant was authenticated by the Ecole Nationale d'Agronomie, (Guelma, Algeria), and a voucher specimen is deposited in the Laboratory of Applied Chemistry, Guelma University.

Elytrigia repens is one of the hosts of *Claviceps purpurea*, also known as ergot, which is an endophyte that lives most of its life inside graminaceous plant cells. This fungus generates a mycelial mat that substitutes for the ovarian tissues of the plant and develops into black sclerotia, which can be found growing from the inflorescence of the plants [18, 19]. More than 50 ergot alkaloids have been identified in the sclerotia of ergot, [19]; they could play an important role in protecting sclerotia or the plant host from predation [19].

The black water insoluble sclerotia powder, which has the same physical aspect as carbon, was manually collected and tested for its ability to remove MB from aqueous solution as well as to treat wastewater samples by adsorption technology.

2.2 Methylene blue adsorption

Analytical grade MB dye ($C_{16}H_{18}ClN_3S$, MW: 319.87 g.mol⁻¹) was from Sigma (Germany) and commercially activated carbon was from Merck. MB solution was prepared at a concentration of 10⁻⁴ mol.L⁻¹ and diluted as needed. To perform the adsorption experiments, 0.1 g of CP were added to 25 mL of MB solution at a concentration of 0.15-1.00 g.L⁻¹.

of 60 min was assumed to be sufficient to perform all adsorption tests.

Absorbance measurements were achieved using a UV-VIS spectrophotometer (Shimadzu, Japan). The MB solution detection was performed at 660 nm, which is the maximum wavelength for MB.

2.3 Effects of pH

The influence of the pH on the adsorption of MB onto CP was experimentally studied by adjusting the MB solution to the desired pH values (3-8), using 0.1 N HCl and 0.1 N NaOH, for 60 min at room temperature.

2.4 Kinetic studies

A study of the adsorption kinetics was performed by mixing 25 mL of methylene blue solutions at the following three concentrations: 150, 350 and 550 mg.L⁻¹ with 0.1 g of CP. Mixtures were homogenized by constant magnetic stirring. Samples were collected at different time intervals. After adsorbate-adsorbent separation, the MB concentration was deduced from measuring the UV-VIS absorbance.

2.5. Morphological examination

Morphological examination was achieved using scanning electron microscopy (JEOL JSM-5600LV SEM).

3. Results and discussion:

3.1 Effects of the initial MB concentration and contact time.

The initial MB concentration varied from 150 to 1000mg.L⁻¹. As shown in Figure 1, MB was effectively removed for the concentration range 0.1-1.0g.L⁻¹. In Figure 2, the MB adsorption was instantaneous and within approximately 10 min, 99 per cent of MB was removed, indicating the high affinity of the dye towards CP biosorbent. To evaluate the required contact time for each adsorption to reach equilibrium, 25ml of MB solution (at a concentration of 150mg.L⁻¹) was mixed with 0.1g of CP for different time intervals, ranging from 5 to 90 min. The equilibrium was reached at 60 min of stirring; as a consequence, a contact time

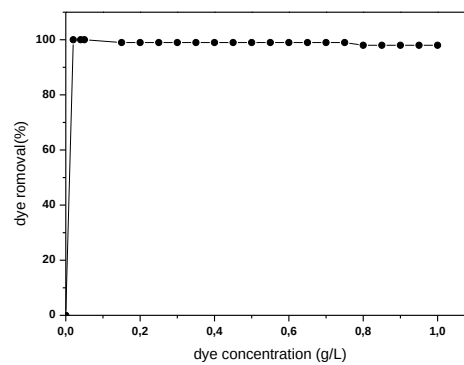


Figure 1. Effect of MB concentration on MB removal.

3.2 Effect of pH

Because the adsorption was found to be instantaneous, changes in the pH did not considerably affect the dye uptake and adsorption efficiency; hence, all MB adsorption tests were performed at normal solution pH (6.3) [20].

3.3 Effect of adsorbent dosage

The CP dosage was also an important controlling parameter in the adsorption reaction. We initially

tested higher CP dosages, according to different studies undertaken by several authors, but there was significantly fast removal of MB. CP loadings were then decreased to the range 0.01-0.2 g (with an increment of 0.02 g). Figure 3 shows that MB adsorption onto CP rapidly increases with increasing CP loading until a certain value is reached. This is due to the accessibility of the adsorption sites on the surface area; afterwards, the per cent MB elimination remained constant irrespective of the amount of CP that was added to the solution.

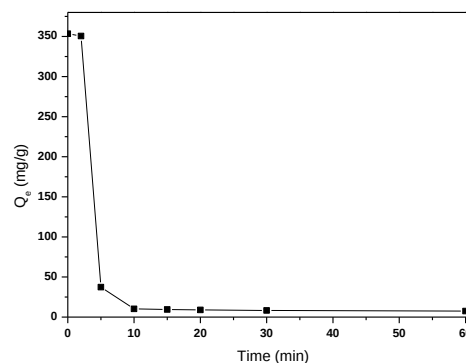


Figure 2. Effect of contact time on MB removal

Figure 3 also shows that 95 per cent and 99 per cent MB removal were obtained for 0.025 and 0.1g of adsorbent, respectively; this indicates that MB could be eliminated with small levels of CP.

The per cent MB removal was calculated using equation (1):

$$\%R = \frac{C_0 - C_e}{C_0} .100 \quad (1)$$

The MB uptake (mg.g⁻¹) for each concentration of CP at equilibrium was determined as follows:

$$Q_e = \frac{(C_0 - C_e)}{W} \quad (2)$$

Where C_e is the equilibrium concentration of MB (mg.L^{-1}) in solution and W is the dose of adsorbent (g.L^{-1}).

The uptake of the adsorbate at time t , Q_t (mg.g^{-1}), was calculated by the following equation:

$$Q_t = \frac{(C_0 - C_t)W}{W} \quad (3)$$

Where C_0 is the initial MB concentration, C_t is the concentration of MB (mg.L^{-1}) in solution at any time, t , and W is the dose of adsorbent (g.L^{-1}). The decrease in the adsorption uptake with increasing adsorbent dosage is mainly due to the unsaturation of adsorption sites through the adsorption reaction [21].

3.4 Adsorption equilibrium study

The equilibrium between the liquid and the solid phases can be described by the adsorption isotherms determined at a constant temperature. Langmuir and Freundlich isotherms are the most commonly used models for obtaining a suitable description of the adsorption equilibrium.

3.4.1 Langmuir and Freundlich isotherm models

In this study, the linear form of the Langmuir isotherm was used:

$$Q_e = \frac{K_b \cdot C_e}{1 + bC_e} \quad (4)$$

$$\frac{C_e}{Q_e} = \frac{1}{Kb} + \frac{C_e}{K} \quad (5)$$

$$\frac{1}{Q_e} = \frac{1}{1 + bC_e} = \frac{1}{KbC_e} + \frac{1}{K} \quad (6)$$

Where Q_e (mg.g^{-1}) is the adsorption density, C_e (mg.L^{-1}) is the equilibrium concentration of the adsorbate in solution, b (mg.g^{-1}) is the adsorption capacity, and K (mg.L^{-1}) is the Langmuir constant. The two latter constants are determined from the plot of C_e/Q_e versus C_e .

The Freundlich, [22] isotherm is commonly presented as:

$$Q_e = k_f C_e^{\frac{1}{n}} \quad (7)$$

$$\log Q_e = \log k_f + 1/n \log C_e \quad (8)$$

where k and $1/n$ are the Freundlich constant related to the adsorption capacity and adsorption intensity of the adsorbent, respectively.

Table 1 lists the Langmuir and Freundlich constants. The highest correlation coefficient ($R^2 = 0.99$) was obtained for the Langmuir isotherm model. The maximum adsorption capacity for CP was 241.6 mg.g^{-1} , which indicates that CP biosorbent is characterized by larger mesoporosity than other adsorbents, as is displayed in section (3.7).

Table 1: Langmuir and Freundlich constants for methylene blue adsorption onto CP

Isotherm model	$K(\text{mg.g}^{-1})$	$b(\text{L.mg}^{-1})$	n and $k_f(\text{mg.g}^{-1})$	Correlation Coefficient
Langmuir	241.6 ± 7.23	0.24 ± 0.0085	-	0.978
Freundlich	-	-	$n = 1.36 \pm 0.032$ $k_f = 353.4 \pm 0.26$	0.846

3.5 Adsorption kinetics study

The study of adsorption kinetics is essential because it provides important information and insights into the reaction mechanisms. Kinetic models describe the process of mass transfer of a solute from a liquid to a solid phase involving the following steps: (i) transport of the solute from the external liquid phase to the liquid film bound to the adsorbent, (ii) transport of the solute from the liquid film to the surface of the adsorbent and (iii) diffusion of the solute through the sites of the adsorbent under the influence of the concentration gradient.

It is apparent from Figure 2, which illustrates the effect of the contact time on MB adsorption onto CP, that the adsorption process follows two steps: a

rapid first step (from 0-10 min), where MB adsorption was fast and significantly contributed to equilibrium uptake, and a slower second step (from 10-30 min) that had a relatively small contribution to the total MB adsorption. It is worth noting here that the equilibrium is reached in a relatively low period compared to many other studies [23].

To explore the adsorption mechanism and characteristics, three simple kinetic models were used, including the pseudo-first-order, pseudo-second-order and Elovich equation, whereas, intraparticle diffusion and external diffusion models were used to explore the diffusion process.

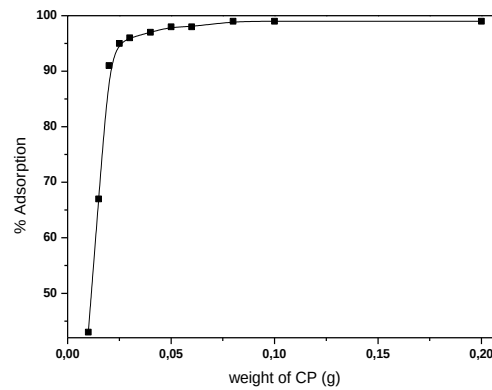


Figure 3. Effect of CP dosage on % MB removal (initial concentration of MB: 150 mg/L, CP dosage: 0.00 - 0.2 g/L).

3.5.1 Pseudo-first-order kinetics

The pseudo-first-order kinetics are expressed by the equation of Lagergren [24, 25]:

$$\frac{dQ_t}{dt} = k_1(Q_e - Q_t) \quad (9)$$

Where Q_e and Q_t are the amount of solute adsorbed (mg.g^{-1}) at equilibrium and at any time t (min), respectively, and k_1 is the rate constant of pseudo-first-order adsorption (min^{-1}).

The constants of pseudo-first-order reaction were determined by extrapolation of $\ln(Q_e - Q_t)$ vs t plot. Values of Q_e , k_1 and regression coefficients (R^2) at the three concentrations used in this study are given in Table 2. The R^2 values, varying from 0.93 to 0.96, are relatively low compared to those obtained when

applying other kinetic models. On the other hand, the calculated Q_e values for the three concentrations studied here are lower than the experimental ones. These observations allowed us to conclude that the MB adsorption cannot be represented by a Lagergren pseudo-first-order reaction; hence, the adsorption process cannot be expressed by controlled diffusion [21].

Table 2: Kinetic parameters of MB adsorption onto CP at three working concentrations

Pseudo first-order kinetics				Pseudo second-order kinetics			Elovich kinetics		
C_0 mg L^{-1}	K_1 min^{-1}	Q_e mg.g^{-1}	R^2	K_2 $\text{L.mg}^{-1} \cdot \text{min}^{-1}$	Q_e mg.g^{-1}	R^2	A	B	R^2
150	0.7100 ± 0.0025	120.52 ± 1.05	0.93	0.00730 ± 0.00002	36.96 ± 1.12	0.999	34.800 ± 0.265	0.820 ± 0.067	0.92
350	1.7400 ± 0.0024	75.82 ± 0.98	0.96	0.00240 ± 0.00005	85.87 ± 0.87	0.999	79.330 ± 0.238	2.410 ± 0.064	0.99
550	0.8200 ± 0.0021	22.35 ± 0.85	0.95	0.00311 ± 0.00008	135.87 ± 0.68	0.999	134.550 ± 0.238	0.810 ± 0.054	0.95

Where, A and B are Elovich constants, presented in Table 2.

3.5.2 Elovich kinetics

Elovich equation expressed as [21]:

$$Q_t = A + B \ln t \quad (10)$$

The trend of the straight lines obtained, for the three concentrations studied, as well as the R^2 values, which were lower than for the other kinetic models, led us to conclude that this model is not the best candidate for representing the adsorption kinetics (Table 2).

$$\frac{t}{Q_t} = \frac{1}{k_2} t \quad (12)$$

3.5.3 Pseudo-second-order kinetics

The pseudo-second-order kinetics is expressed by the following equation [24, 26]:

$$\frac{dQ_t}{dt} = k_2(Q_e - Q_t)^2 \quad (11)$$

After integration, eq. (11) gives:

Where Q_e and Q_t are the same as defined above and k_2 is the rate constant of pseudo-second-order adsorption (min^{-1}). Q_e , k_2 and R^2 values are displayed in Table 2.

Table 3: Intraparticle diffusion parameters for different concentrations of MB

C_0 mg.L^{-1}	k_{id} $(\text{m.L}^{-1}.\text{min}^{-0.5})$	C	R^2
150	0.4258 ± 0.0018	35.31 ± 0.15	0.96
350	1.3119 ± 0.0024	80.56 ± 0.05	0.97
550	0.4355 ± 0.0013	135.01 ± 0.12	0.97

Q_e increases with increasing initial MB concentration, while k_2 decreases. Moreover, the R^2 values (>0.99) are greater than those obtained for pseudo-first-order kinetic and Elovich models. In addition, the calculated Q_e values (37.00, 86.00 and 136.00 mg g^{-1}) were closer to those that were experimentally determined (37.37, 87.33 and 137.22 mg.g^{-1}). These results confirm that the adsorption process of MB onto CP follows pseudo-second order kinetics.

3.6 The diffusion process

3.6.1 External diffusion process

The transfer of a solute from a liquid phase to a solid phase is given by the expression:

$$\frac{-dC}{dt} = k \left(\frac{a}{v} \right) \cdot (C_e - C_t) \quad (13)$$

Where C_e is the concentration of the solute in the solution at equilibrium, k is the external diffusion constant, a is the solid/liquid interface area and v is the volume of the solution. After integration, eq. 13 becomes:

$$\ln \left[\frac{C_0 - C_e}{C_t - C_e} \right] = k \left(\frac{a}{v} \right) \cdot t = kt \quad (14)$$

From the plot of $\ln[C_0 - C_e / C_t - C_e]$ versus time t , the experimental data do not fit the equation of this model ($R^2 = 0.92$); external diffusion of MB through CP particles does not represent a decisive step in the adsorption process. The pseudo-second-order surface reaction is faster and should prevail over external diffusion around the adsorbent particles.

3.6.2 Intraparticle diffusion model

The rate constant for intraparticle diffusion, k_{id} , is determined using the following equation, [25]:

$$Q_t = k_{id} \cdot t^{1/2} + C \quad (15)$$

k_{id} is the rate constant for intraparticle diffusion and C is a constant.

The plots of adsorbate uptake Q_t versus $t^{1/2}$ for different concentrations of MB dye (150, 350 and 550 mg.L^{-1}), were linear with regression coefficients (R^2) higher than 0.95 (Table 3), indicating that the intraparticle diffusion is rate-limited for the MB adsorption process onto CP. The k_{id} and C values are displayed in Table 3.

3.7 Thermodynamic parameters

In Figure 4, we can see that the dye retention rate on the adsorbent decreases with increasing reactor temperature, suggesting that the surface reaction is exothermic.

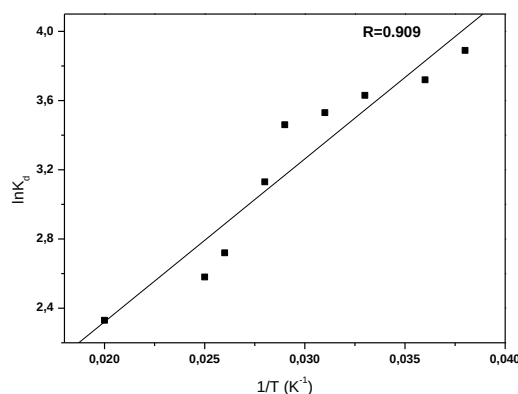


Figure 4. Van't Hoff plot ($\ln(KF)$ vs $1/T$) for MB adsorption onto CP. Each data point is the mean $\pm$ SD of three replicates.

The thermodynamic parameters (ΔH° and ΔS°) presented in table 4 were determined from the slope and intercept of the Vant' Hoff plot (equation 16):

$$\ln K_d = \left(\frac{\Delta S^\circ}{R} \right) - \left(\frac{\Delta H^\circ}{R} \right) \frac{1}{T} \quad (16)$$

Where K_d is the apparent equilibrium constant:

$$K_d = \frac{Q_e}{C_e} \quad (17)$$

The variation in Gibbs free energy (ΔG°) is given by equation (18)

$$\Delta G^\circ = -RT \ln K_d \quad (18)$$

Where R is the universal gas constant ($8.314 \text{ J.mol}^{-1}.\text{K}^{-1}$) and T is the absolute temperature (K). The negative values of ΔH° indicate that the reaction is exothermic and negative values of Gibbs free energy signify that the adsorption process is spontaneous. The positive value of ΔS shows that the order of distribution of MB molecules on the adsorbent increases with respect to that in the solution and that MB dye has a high affinity for the adsorbent. Moreover, the high enthalpy value indicates that the adsorption of MB onto CP is chemical in nature, [27].

Table 4: Thermodynamic parameters of MB adsorption onto CP

$\ln K_d$	$T (\text{K}) \pm 0.005$	$\Delta S (\text{KJ.mol}^{-1})$	$\Delta H (\text{KJ.mol}^{-1})$	$\Delta G (\text{KJ.mol}^{-1})$	R^2
2.52	299	308.22	-157.78	-6.18	-0.92
2.72	303			-6.76	
3.72	313			-9.55	
3.89	323			-10.30	

3.8 Comparison of the efficiency of CP fungus with Merck-activated carbon and other low-cost adsorbents.

As seen in Figure 5, MB per cent removal by CP and commercially activated carbon (MAC) were

almost identical for the same experimental conditions (normal pH and room temperature, $C_{MB} = 150 \text{ g.l}^{-1}$). Indeed, the MB percent removal is 98 for 0.05g and the maximum adsorption (≈ 100 per cent) is obtained for 0.2g loading of both adsorbents loading.

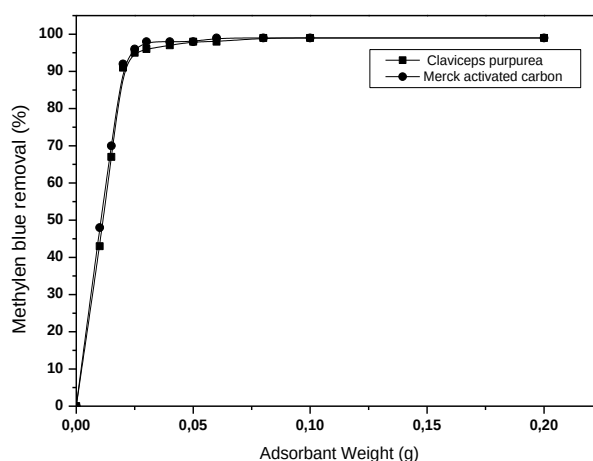


Figure 5. Comparison of MB removal using CP and Merck activated carbon.

On the other hand, the adsorption capacity value for CP fungus was higher than many low-cost adsorbents used for MB removal (Table 5).

Table 5: Comparison of adsorption capacity of CP fungus to other low-cost adsorbents, [28].

Adsorbent	k(mg.g ⁻¹)
<i>Claviceps purpurea</i> fungus (from this study)	241.5
Palm kernel fiber	217.95
Modified rice straw	208.33
Broad bean peels	192.72
Gulmohar (<i>Delonix regia</i>) plant leaf powder	186.22
Castor seed shell	158.73
Cedar sawdust	142.36
Pumpkin seed hull	141.92
Saw dust	133.87
Chemically treated guava leaves	133.33
Meranti sawdust	120.48
Pineapple stem	119.05
Dehydrated peanut hull	108.6
Coconut husk	99
Coffee husks	90.1
Phosphoric acid treated <i>Parthenium hysterophorus</i>	88.49
Teak wood bark	84
Garlic peel	82.64

3.9 SEM and EDS analyses

Scanning electron micrographs of CP before and after adsorption are shown in Figure6.

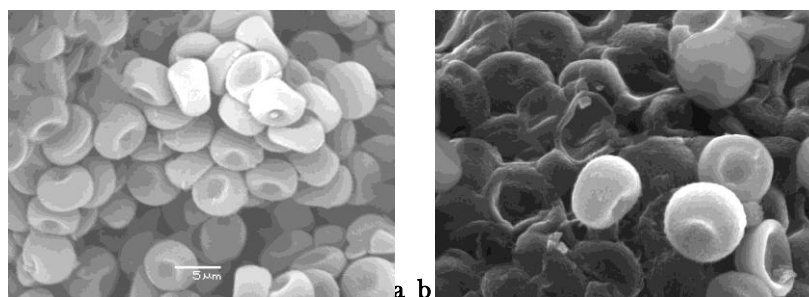


Figure 6. SEM micrographs of CP, a) before adsorption of MB, b) after adsorption of MB.

The morphology of this fungus promotes dye adsorption owing to its regular surface; consequently, the adsorption of adsorbate in different parts of this material is possible. However, surface of MB loaded adsorbent (Figure 6b) shows that the particles of CP are more dispersed, offering more sites for the dye molecules to be adsorbed. It can be concluded that this material offers an appropriate morphology to maintain dye ions.

The EDS analyses showed that the adsorbent mainly contains carbon and oxygen but lower levels of chlorine, potassium and other elements (Figure 7).

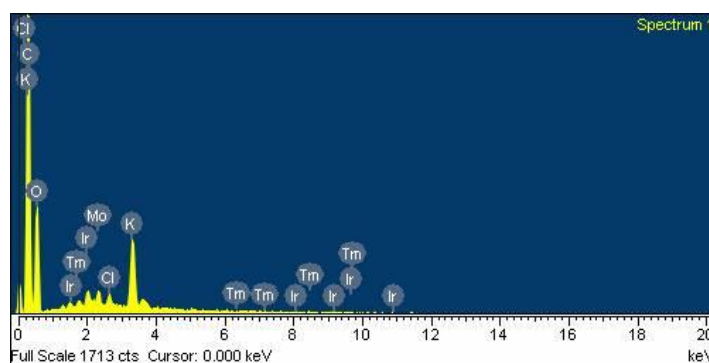


Figure 7. EDS spectra of *Claviceps purpurea*

The high adsorptive efficiency of the fungus appears to be due to its chemical composition. Additionally, according to several authors [29, 30], ergot fungi contain many secondary metabolites, including indole alkaloids, bearing several functional groups (OH, NH, COOH, CONH...) with unshared electron doublets allowing for fixation of the cationic dye to the adsorbent via different mechanisms, donor acceptor complexation, H-bonding or π - π interactions, [31]. The higher enthalpy variation value supports these suggestions.

3.10 Use of *Claviceps purpurea* for on-site wastewater treatment

Two wastewater samples were treated by adsorption onto CP and were collected from the following locations:

1) Oued Rhumel, a river close to an industrial zone in the region of Rhumel in Constantine town (east of Algeria).

The results show that the adsorbent entrapped an important level of contaminants from this wastewater. Indeed, 81 per cent adsorption was obtained for 0.4g of added adsorbent and 94 per cent pollutant removal was obtained for 2g of added adsorbent. The solution volume was 25ml and detection was performed at 350 nm.

2) Household and domestic wastewater taken from a building in an urban area in the region of Constantine.

For this wastewater sample, the addition of 0.1 and 2g of CP led to 80 per cent and 98 per cent removal of pollutants, respectively. Volume of the wastewater sample was 20 mL and detection was performed at 380 nm, which is mean value of the mixture's wavelength. In this case, the CP efficiency was higher, which could, on one hand, be due to the complexity of the first wastewater sample. On the other hand, it could be due to the affinity of the wastewater molecules for the adsorbent.

4. Conclusion

The aim of this study was the use of a new biosorbent, CP fungus, for removing a cationic dye, methylene blue. The use of CP as a biosorbent reduced both the cost of dye adsorption and operation time. Indeed, the main features of CP fungus are as follows: (i) high percentage adsorption (99 per cent), (ii) relatively low loadings (≤ 0.2) and (iii) mostly reduced contact time (10 min).

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