Biodecolorization of methylene blue using *Aspergillus* consortium

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**Abstract.** To establish sustainable circular ecosystems, the immense knowledge of different fungal strains as pure and mixed *isolates* and application them in biodecolorization of dye-laden wastewater is required. In this study, the biodecolorization of methylene blue (MB) dye was investigated by using mixed microfungal strains of *Aspergillus* consortium consisting of *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*. The experiments were conducted in batch-mode. Analytical measurements were performed by UV-VIS spectroscopy. Optimization of operating conditions was carried out in order to achieve the optimal biodecolorization of methylene blue dye (MB). Furthermore, the results illustrated that 36 hrs., 30°C, 9, 2g/L and 150mg/L for incubation time, temperature, initial pH, fungal inoculum size and MB dye concentration, respectively, were chosen as the optimum conditions with the maximum biodecolorization of MB dye was 92%.

**Keywords:** *Aspergillus* consortium, biodecolorization, methylene blue

1. Introduction

The contamination of freshwater supplies with a vast amount of xenobiotic compounds leaching from different industrial sectors is becoming a major issue. Synthetic dyes are considered xenobiotic compounds which pose a potential threat to the environment as they are disposed of to water bodies [1]. Textile wastewater is of particular concern due to the discharge of large fractions of synthetic dyes into the aquatic environment [2]. Effluents from the textile industry contained dye concentrations ranging from 10 to 200 mg / L that are considered to be mutagenic, carcinogenic and lethal to marine organisms when introduced to the aquatic environment [3]. Synthetic dyes are issues of a challenge to remove when discharged directly to the environment because they are purposely created to be resistant to water, light, and oxidants [4].

In spite of the fact that several industries have used various traditional and advanced physical and chemical methods and techniques such as adsorption [5], flocculation [6], electro-coagulation [7], coagulation / flocculation [8], chemical precipitation [9], membrane-filtration [10], and advanced oxidation [11], for the treatment of textile-industry effluents; these methods and techniques are energy-intensive and require complex and expensive strategies, so undesirable by-products (e.g. sludge) are eventually produced [12]. Biological dye-wastewater treatment technologies are extensively considered as having technological and economic benefits over physical and chemical treatment approaches. In addition, biodegradation and biosorption are two major mechanisms involved in the biodecolorization [13]. Various species and strains as isolated and mixed ones of organisms have shown fairly great results in the bio-removal of synthetic dyes as these organisms play a key function in the decolorization and degradation of dyes. These organisms include bacteria [2,3,14,15], macrofungus and microfungus strains [16], white-rot fungi [17-19], algae and microalgae [20,21], and yeast [22,23].

Methylene blue (MB) was a nominee for the function of representative dye in this study. Methylene blue MB is an aromatic thiazine heterocyclic cationic dye. MB has a wide use in biological and medicine applications in addition to textile-processing industries. Also, it is often utilized in house dyeing. It is considered a prevalent pollutant due to its destructive effects on the ecosystems and environment [24,25].
Mycoremediation is one of the biological strategies have been employed to remediate colored-wastewater by synthetic dyes which is a technique that uses various fungus species as pure and mixed ones in the degradation of dyes [26]. In general, the fungus Aspergillus spp. has a potential applications in biotechnology and mycoremediation as it is consisting of adaptable species capable of tolerating elevated levels of a broad variety of toxic contaminants. In addition, it has been shown that these fungal strains have the potential to grow and exist in a spectrum of dye-contaminated water and soil, without influencing the rate of mycoremediation and biodecolorization [13].

Many studies investigated these fungal strains to decolorize different synthetic dyes. Singh L. and Singh VP. [27] studied the biodegradation of Congo red and Bromophenol blue dyes using fungus species of Aspergillus flavus in Potato Dextrose Agar (PDA) medium. This fungus has indicated positive results for the biodecolorization for two dyes. Kadam and others [3] investigated the ability of the consortium of Pseudomonas sp. SUK1 and Aspergillus ochraceus NCIM-1146 (consortium-PA) to decolorize textile dye reactive navy blue RNB HE2R. Esmaeili and Kalantari [28] examined the ability of Aspergillus flavus, a fungus was isolated from a soil, for biodecolorization of Reactive Red 198 (RR198) using batch-mode experiments. Hamad and Soliman [29] assessed the Congo red dye decolorization by immobilized Aspergillus niger under different experimental conditions like incubation time, temperature, initial pH, fungal inoculum size and MB dye concentration.

The main objective of the present study is investigating the potential ability of Aspergillus consortium; Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus, for biodecolorization of methylene blue dye (MB) from synthetic aqueous solutions taking into account the main parameters affecting biodecolorization such as initial pH, inoculum concentration, and MB dye-concentration.

2. Material and Methods

2.1. Materials and Fungus Species

The materials used in the current experiments have been verified by the analytical methods. Methylene Blue dye (MB) IUPAC name 3,7-bis (Dimethylamino) - phenothiazin-5-iium chloride, has a chemical formula: C_{16}H_{18}N_{3}SCl; molecular weight 319.86 g/mol; and maximum wavelength λ_{max} = 660 nm [25,30,31]. MB dye included in this work was commercially pure elsewhere without any purification [30]. The chemical structure of MB dye is shown in figure 1 [32].

![Figure 1: Chemical structure of methylene blue dye](image)

The micro-fungi Aspergillus consortium including Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus is defined as a species of conidial fungi which has been gained from Agriculture College, Tikrit University. The chemicals and reagents were of high experimental quality available.

2.2. Mineral and Biological Salts Medium

Mineral and Biological Salts Medium was constituted via introducing each liter of water; KH₂PO₄ 1 g, NaNO₃ 2 g, NaHCO₃ 1g, MgSO₄·7H₂O 0.5 g and glucose 3 g. pH of the medium was adjusted to 5
± 0.5 by applying 0.1M NaOH and HCl. In addition, Dextrose Agar (SDA) medium consist of 10 g agar, 10 g dextrose, and 5 g peptone per a liter of distilled water was utilized as in the experimental medium [13][16][28].

2.3. Preparation of Fungal Inoculum
To enhance the growth of fungal biomass of Aspergillus consortium, Dextrose Agar (PDA) medium has been primed by dissolving the medium ingredients in 2 % (w/v) of mineral salt medium. An Erlenmeyer flask involving a 50 mL autoclaved solution of cultural medium with dye at the desired concentration was injected with the fungal suspension on a plate of PDA. The medium was autoclaved for 15 minutes and about 20 mL of medium was poured into each previously sterilized Petri plates. The apical part of 5-day old fungal mycelium was used as inoculant. All inoculated Petri plates were kept in an incubator at 28°C. The fungal cell biomass of Aspergillus consortium was added as dry weight by 0.5 % w/v of distilled water and then was blended for 8 minutes. As an inoculum, the homogenized solution is used in experiments.

2.4. Biodecolorization in Batch –Mode Experiments
The growth of soil-derived fungal cells of Aspergillus consortium was enhanced by maintaining a determined volume of fungus suspended on a SDA medium and conveyed to the Erlenmeyer flask with BMSM. The flask was incubated at 30 °C in a rotary shaker (150 rpm) for 4 hours. Then the expansion of fungus on BMSM as wet cells was added in an Erlenmeyer flask involving an autoclaved solution of methylene blue MB [31].

2.5. Cultural Conditions
The aqueous solution of MB dye was made by dissolving the required amount of MB dye in distilled water. The biodecolorization experiments of heterocyclic cationic MB dye were investigated using Erlenmeyer-flask involving 100 mL of MB model solution and 25 mL of BMSM. Biodecolorization was studied by injection the flasks with spreading cells exponentially Aspergillus consortium at different operational conditions and surroundings. All the experiments were carried out in duplicates. The effects of incubation time, temperature, initial pH, MB concentration and volume of fungal inoculum on biodecolorization were investigated in batch-mode experiments [24][25].

2.6. Analytical methods
The biodecolorization of MB cationic dye was studied by photometrical analysis at the maximum MB dye-visible wavelength (660 nm) utilizing a UV–Vis spectrophotometer. The decreasing in MB dye concentration was observed in the MB calibrated curve versus the absorption. The activity for biodecolorization as a percentage (B) was experienced using absorbent (A) and dye concentration, as follows [14]:

\[
A(\%) = \frac{(OD)_{\text{initial}} - (OD)_{\text{final}}}{(OD)_{\text{initial}}} \times 100
\]

\[
B(\%) = \frac{C_i - C_f}{C_i} \times 100
\]

Where OD is the optical density, \(C_i\) and \(C_f\) are initial and at any time (t) dye concentration of MB in (mg/L), respectively.

3. Results and Discussion
To investigate the biodecolorization of MB dye, optimal operational conditions must often be considered as prerequisites, since the biodegradation properties of fungal living cells appear to vary with various cultural and nutritional surroundings [19][29]. The effect of following factors; incubation...
time, temperature, initial pH, fungal inoculum size and MB dye concentration were investigated and then optimized.

3.1. Effect of Incubation Time
To evaluate the influence of incubation-time to the biodecolorization rate process, investigations were attended with various time-intervals ranged within (6-48) hrs. As presented in figure 2, the optimal incubation time on MB dye biodecolorization (92%) was 36 hrs. The biodecolorization strategy could be divided into two stages: a rapid biodegradation stage within the first 8 hrs. and consequently a slow biodecolorization stage [33]. In comparison with some previous studies [13][22][29], the incubation time in this study for the higher biodecolorization efficiency as (%) was shorter than in them, due to the combined effect of Aspergillus consortium in the MB decolorization process. The use of Aspergillus consortium use provides intensive and rapid biodecolorization due to mutual and collective catabolic activities of the mixed fungal population [1].

![Figure 2. Effect of incubation time on biodecolorization of MB by Aspergillus consortium](image)

3.2. Effect of Temperature
In general, temperature is an essential factor for the living cells’ physiological-efficiency. In addition, it affects the percentage of dye biodecolorization [1]. The dye biodecolorization by the fungus cells was thus examined in the range of 25 to 36 °C as shown in figure 3. The optimal MB biodecolorization; 92% when temperature was estimated at 30 °C. The reduction in MB biodecolorization as temperature increases could be attributed to the mortification of biological activity and metabolic processes enzymes of living cells [34]. This is in line with previous studies [12,29]. However, the increase in the temperature negatively affects the MB biodecolorization. This could be attributed to the loss of the degradation enzymes which are thermally inhibited at higher temperatures [12][29][34].

3.3. Effect of Initial pH
The biodecolorization of MB as percentage at various pH values was shown in figure 4. The impact of pH on MB biodecolorization was tested for 48 hours within BMSM at developing pH (5, 6, 7, 8, 9, 10 and 11). The pH solution samples adjusted by using HCl/NaOH 0.1 N [24]. The experiments were performed at the optimal conditions. The optimum value of pH was 9 as shown in figure 4. For a farther rise in pH beyond 9, there is no dramatic increase the amount of dye bioremoved [22]. This would be in consistent with prior researches [16,35]. That one was simply established that decolorization in acidic nature was lesser than alkaline ones. This could be attributed to acidic or
highly alkalinity may be inhibited MB dye biodecolorization by fungal living cells [24]. These findings were valid in large-scale biodecolorization techniques, since most textile wastewater treatments were alkaline pH values, and biodecolorization in alkaline environments was commonly desired in industrial treatments, as dyeing based on the alkaline conditions [35,36].

![Figure 3: Effect of Temperature on biodecolorization of MB using Aspergillus consortium at incubation time](image)

![Figure 4: Effect of pH on biodecolorization of MB by Aspergillus consortium at optimal conditions: 36 hrs., 30ºC, 2g/L and 150mg/L for incubation time, temperature, fungal inoculum size and MB dye concentration, respectively](image)

3.4. Effect of Fungal Inoculum Size
The study involved the examination of the influence of another important factor; which is fungal inoculum size[12]. The experimental tests depicted MB biodecolorization enhanced from a raise in mycelial growth of Aspergillus consortium. To identify the optimum amount of the inoculation to
also MB biodecolorization be efficient, the experiments were conducted with various inocula size; 0.5, 1, 1.5, 2, 2.5 and 3 g/L of the synthetic Mb dye-laden solution. From the results shown in figure 5, it illustrated that any farther raise in inoculum density of fungus over 2g/L, biodecolorization procedure didn't seem to affect any measurable influence. It may have been due to the competition between the excess living cells Functional locations of nutrients. These results were in the line of previous studies [1, 4, 27]; that illustrated the microbial consortia have greater dominance than single/pure microorganisms in the treatment of dyes-laden wastewater and this property improves the development board of bio-remedial processes by reducing total cost as overall.

Figure 5. Effect of inoculum concentration of Aspergillus consortium on MB biodecolorization at optimal conditions; 36 hrs., 30ºC, 9 and 150mg/L for incubation time, temperature, pH and MB dye concentration, respectively.

3.5. Effect of MB Concentration
In order to evaluate the effect of the concentration of MB dye on biodecolorization performance, the experiments were performed at 30 °C for 36 h with different concentrations of MB dye between 50 and 250 mg/L as illustrated in figure 6. The chosen concentration as the optimal one is 150 mg/L. This seems to be due to the rising toxicity of the dye, which has repercussions in fungal growth and activation of cellular functions [14, 35]. Remarkably, MB biodecolorization as percentage was inversely proportional with the initial-concentration of MB. The latter indicates that the less the concentration leads to increased biodecolorization (%) and so forth.
Figure 6. Effect of MB-concentration on the biodecolorization by Aspergillus consortium at optimal conditions; 36 hrs., 30°C, 9 and 2g/L for incubation time, temperature, pH and fungal inoculum size, respectively.

4. Conclusions
The following is concluded on the basis of the experimental findings of this study; firstly, Aspergillus consortium consisting of Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus was considered as an efficient fungal consortium in biodecolorization of methylene blue dye (MB). The use of Aspergillus consortium has greater dominance than single/pure fungal isolates in the biodecolorization of MB dye-laden wastewater and this property improves the development board of bio-remedial processes by reducing total cost as overall. The experiments were achieved at several values of the most important factors affecting the biodecolorization process to determine the optimal conditions. To conclude, the optimal conditions at which MB biodecolorization was 92% were: 36 hrs., 30°C, 9, 2g/L and 150mg/L for incubation time, temperature, initial pH, fungal inoculum size and MB dye concentration, respectively.

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