Decolorization and Mycoremediation of Methyl Orange using *Beauveria* bassiana

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Abstract. The purpose of this study is to evaluate the application of *Beauveria* bassiana in the decolorization and degrade of Methyl Orange (MO) as a model sulfonated azo dye from aqueous solution. *B. bassiana* was acclimated to higher concentration of MO dye (25 – 250 mg/L) in MSM after repeated sub-culturing. *B. bassiana* could completely decolorize of MO in a large variety up to 100 mg/L of MO concentration, at 28 °C and pH 8. This study showed that methyl orange is fully decolorized by *Beauveria* bassiana at optimized operational conditions within 7 days of incubation period.

1. Introduction

In terms of volume as well as composition of effluents the wastewater from textile industry is listed as the most polluting industries of all. Textile products are colored by dyes, and about 10 – 15% of dyes used in effluent throughout the process of dyeing are lost due to the low level of dye-fiber fixation [1]. Wastewater discharges into the ecological system not only affect the natural systems, but also humans and animals. The remediation and maintenance of water quality of dye-contaminated waters are thus one of the major issues in the field of environmental technologies [2, 3].

The important category of pollutants is Azo-dyes which are synthetic ones primarily utilized in the cloth, food additives, textile, oil, and cosmetics field [4]. The dyes without proper treatment can last extensively in the environment and are harmful to all living organisms, as degradation can lead to carcinogenic substance [5].

In the present study, methyl orange MO was candidate as the model dye. Methyl Orange (MO) or Orange III (547-58-0) is a toxic, carcinogenic, and mutagenic sulfonated azo-dye [6]. MO is bright and highly soluble in water, and low biodegrading ability; making it hard to remove by a traditional method of treatment [5]. Methyl orange inhalation causes allergies, hypersensitivity and dermatitis. It has wide used in textiles, printing, paper manufacturing, pharmaceutical, food industries, and research laboratories [7, 8]. For the azo-dyes degradation, there were various physicochemical techniques have been used, including adsorption, precipitation, coagulation, electrocoagulation [9, 10], electrocoagulation/electroflotation (EC/EF) [11] and electrochemical [12]. Some of these techniques are expensive for decolorization of and often generate amine residues after treatment. The regular consumption of this water causes diseases such as cancer [8, 13]. As a partly or completely bio-converted pollutants into stable non-toxically products, biodegradation has also been assumed an efficient, less intensive, and environmentally sound process.

Mycoremediation is the biodegradation technique which uses fungi in the degradation of toxic organic and inorganic compounds [14]. Also, by-products that may be produced during the biodegradation
The process may be the substrate origin of species of the same strain or of other strains [14]. Fungi are popular for their colonizing capacity and adapt to extreme environmental conditions in a wide variety of heterogeneous environments. In addition, organic matter can be broken down and abiotic surfaces easily colonized [15]. Fungi can form an extensive mycelial network and synthesize several specific enzymes typically has a greater resistance than bacteria to high levels of contaminant. In addition, most of the fungal strains grow to a great extent producing high biomass quantities in order to treat large amounts of colored water. Diverse fungal strains have been demonstrated more successful in decoloring textile dyes [16]. Many of researchers used isolated and mixed fungal strains to decolorize azo dyes. Kirby and others observed Phlebia tremellosa decolorized eight synthetic-dyes at a concentration of (200 mg/L) by greater than 96% through 14 days under static conditions [17]. While Sam and Yeşilada optimized the cultural and operational conditions for Orange II dye decolorization by Coriolus versicolor and Funalia trogil [18]. Aksu and Tezer studied the bio-sorption of Remazol Black B from water solutions on Rhizopus arrhizus in a batch system with respect to different incubation conditions [19]. Ali and others adapted isolated fungi, Aspergillus niger SA1 from the sludge of textile wastewater for decolorization of four different dyes (Orange II, Sb and Db K2RL); to high concentrations under shaking conditions [16].

Beauveria bassiana has been utilized historically for insect pest prevention like caterpillars such as pathogens of mosquitoes [20]. B. bassiana is one of the microbial hydroxylation fungi most popularly used and is available all times and could be handled with simple manners since it is a natural pathogen of many insects and other invertebrates. It has been successfully utilized for the hydroxylation of a range of natural products, aromatic compounds, and hydrocarbon substrates with no toxic by-product [21]. The first step in the oxidative degradation of a variety of compounds in the air is hydroxylation. In detoxifying compounds, it is extremely important.

The present study aims to assess the ability of Beauveria bassiana to decolorize and degrade MO which was chosen as the candidate dye in aqueous solution at the optimum operational conditions.

2. Material and Methods

2.1. Chemicals and Fungal Strain

The chemicals utilized in this study have been analytically approved. Methyl Orange (MO), C14H14N3NaO3S, \( \lambda_{max} = 466 \text{ nm} \), and a molecular weight = 327.34 g/mol, has been obtained from the laboratory of Environmental Engineering Department, College of Engineering, at Tikrit University (figure 1).

![Figure 1. Methyl Orange (MO).](image)

The isolated fungal strain of B. bassiana has been obtained from College of Agriculture, Tikrit University as shown in figure 2. The chemicals to use in this analysis were of the maximum available analytical quality.
2.2. Mineral Salt Medium (MSM)
Mineral Salt Medium (MSM) was created by adding per liter of water; Acetic Acid (99.9%) 0.150 mL, KH$_2$PO$_4$ 67.0 mg, (NH$_2$)$_2$CO 100.0 mg, NaHCO$_3$ 840.0 mg, FeCl$_3$·6H$_2$O 7.0 mg, MgSO$_4$·7H$_2$O 38.0 mg, and glucose 6 g. pH of the medium was balanced to 8 by utilizing 0.1M HCl and NaOH. Agar (15 g/L) was utilized as solidifier in the medium if necessary [16].

2.3. Preparation of Fungal Inoculum
Fungal biomass 0.0012 % w/v of distilled water was mixed for 5 minutes in the blender and then utilized in experiments as an inoculum [16].

2.4. Adaptation of B. bassiana to higher MO Concentrations
The fungi B. bassiana implemented simultaneously for increased MO concentrations (25 -250 mg/L) by cultivating it with MSM several times. New fungal inoculated dyes containing plate have been incubating at 28 °C for 7 days. A microscope may identify the apparent decolorization due to fungal strain behavior and analyze coloring zones in the media [16].

2.5. Cultural Conditions
The dye watery medium was made by dissolving a weight of 0.01 g MO in 50 mL of distilled water and volume completion to 100 mL mark [22]. The decolorization experiments of sulphonated azo-dye MO were carried out in 250 mL Erlenmeyer flask having 100 mL of dye synthetic solutions and 25 mL of MSM. The decolorization was carried out by inoculation the flasks with growing exponentially fungus (28 °C; 7 d) at fixed times under static conditions. All the experiments were performed in duplicates. The effects of pH, temperature, inoculum size and MO dye concentration on mycoremediation were studied in static cultures [6, 16, 17, and 19].

2.6. Analytical methods
Dye decolorization was measured photometrically at the maximum dye visible wavelength (466 nm) using a UV–Vis spectrophotometry. Decreases in methyl orange were observed in the dye (methyl orange) calibrated curve versus absorption. The activity for decolorization as a percentage (R) was analyzed using absorbent (D) and dye concentration, as follows [23]:

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D(\%) = \frac{(OD)_{initial} - (OD)_{final}}{(OD)_{initial}} \times 100
\] (1)
Where OD is the optical density, $C_0$ and $C_t$ are initial and at any time ($t$) concentration of MO Dye in (mg/L), respectively.

3. Results and Discussion
Optimization of operating conditions in mycoremediation phase must always be considered as a prerequisite since the dye degradation properties by fungal strains tend to change with various nutritional and cultural factors [24, 25]. Degradation is a process followed by the dye decolorization [6].

3.1. Effect of Initial pH
The effect of pH on MO decolorization was assessed for 72 hours in MSM at varying pH (4, 6, 7, 8, 9, and 10). The pH buffered by adding 0.1 N HCl / NaOH; was pre-autoclaved at different levels [22]. The optimum range of pH was 7.0–9.0, with a maximum rate of decolorization (95 %) being observed at pH 8.0 as shown in figure 3. The fungus was suggested to be able to decolorize the azo color in the alkaline medium. This is an important and economical conclusion and the reduction of pH of effluent increase the cost and time-consuming during degradation process [22]. This is in accordance with previous studies [16, 22 and 25]. It was clearly understood that decolorization was lower in acidic pH than alkaline pH.

![Figure 3. Effect of pH on decolorization of MO by B. bassiana.](image)

3.2. Effect of Temperature
Generally, the temperature is important for the microbial culture's physiological efficiency and influences the rate of color decolorization [25]. The optimal MO decolorization temperature was found at 28 °C as shown in figure 4. Decolorization decreased due to the reduction of bioactivity and catabolic enzymes of the fungal strain as temperature increases [6, 8]. This is in line with prior studies [16, 19 and 25]. Since temperatures outside the ambient range require an additional source of energy, making the process expensive, it is necessary to identify if the microbes selected will spread out in natural warm environment. Further, increase in the temperature resulted in the decrease in the percent MO decolorization. This could be because the degradation enzyme may be thermally inhibited at higher temperatures [6].
3.3. Effect of Inoculum Size

Another factor to be considered in developing MO decolorization strategy is the dose of inoculum. The effect of the inoculum size on the rate of decolorization process tends to vary with the microbial strains used [25]. The experiments illustrated that MO decolorization process improved with the increase inoculum size of *B. bassiana* about 5 to 20 % (w/v) and the optimum MO decolorization was observed at inoculum size 0.0012 % (w/v) as shown in figure 5. However, further increase in inoculum size beyond 25 % (w/v) did not cause any change in the decolorization process. This could be attributed to the increased biomass which causes overlapping and competition the active sites of the substrate particles.

![Figure 5. Effect of inoculum size of *B. bassiana* on MO decolorization.](image)

3.4. Effect of MO Concentration

Another important factor affecting MO decolorization and mycoremediation is the concentration of the dye itself [25]. The fungal strain *B. bassiana* was utilized to decolorize increased MO concentrations which range from 25 to 250 mg/L at pH 8 and 7 days incubation. Concentrations above 200 mg/L were shown to be inversely proportional to the dye concentration (see figure 6). This is because of increased dye toxicity that consequences in cellular activity inhibition and fungal growth [6, 24]. It was clear from the observation that, percent decolorization of MO increased with an increase in time, irrespective of initial MO concentration. Further, percent decolorization of dye decreased with an increase in dye concentration. i.e. lower the concentration higher the decolorization (%) and vice-versa.
4. Conclusions
According to the previous results, it is clear that many of fungal strains are capable to decolorize important category of pollutants; azo-dyes. In this study, which used at first time the fungal strain *Beauveria bassiana* to decolorize an important sulfonated azo-dye, Methyl Orange MO. Maximum ability of *Beauveria bassiana* to decolorize MO dye was accomplished by optimizing various cultural and operational factors including pH, temperature, inoculum size and dye concentration. The decolorization percentage of 95% was observed at the optimum conditions (pH 8, 28 °C, 0.0012 % (w/v) inoculum size of *Beauveria bassiana* strain, and 200 mg/L of MO concentration for 7 days incubation time. Complete decolorization of MO by *B. bassiana* was observed at dye concentration of 100 mg/L.

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Figure 6. Effect of MO concentration on the decolorization process.
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