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A N I N T E R N A T I O N A L J O U R N A L

Investigation of Some Parameters Affecting Methyl OrangeRemoval by *Fusarium acuminatum*

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ABSTRACT

Dye stuff released to the ecosystem from textile industries cause a serious contamination and become a major environmental problem over the last few decades. As biological decolorization of textile wastewater is an important issue, Fusarium . acuminatum was used to removal of a frequently used textile dye, methyl orange. Live pellet of Fusarium acuminatum was used and decolorization studies performed in various temperatures and pH conditions with different dye concentrations. The highest decolorization rate was observed at 35 ^{O}C. 60 mg/L was found as the optimum initial dye concentration. In the pH range of 3-4, decolorization rate was approximately 70%. It was seen that Fusarium acuminatum have the great ability of the methyl orange removal. To our knowledge, it took place for the first time in the literature.

Keywords: Biosorption, azo dye, methyl orange, *Fusarium acuminatum*, live pellet.

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INTRODUCTION

Environmental pollution has become an important issue with the increase of industrial activities and urbanization, which create more waste than nature can tolerate. Large amount of chemicals, mainly dyes, are utilized during the industrial process of paint, textile, pharmaceutics, paper, pulp, food and release of untreated effluents to ecosystems creates major hazard for the organisms living there. Moreover 17-20% of water contamination is originated from textile effluents; during textile process, the amount of dye released accidentally is up to 50% depending on the type of dye and such release spreads out in to the environment afterwards [1,2]. As a consequence, there has been a considerable interest for the removal of dyes from aqueous waste streams.

Various physicochemical methods are used in dye removal from wastewater such as, adsorption, membrane filtration, ozonation, precipitation, ion-exchange but they have some disadvantages such as high cost and operational complexities. When compared with physicochemical treatment methods, biological dye removal is inexpensive, ecofriendly and simple to use [3,4].

Recently many research have focused on some microorganisms which are capable of removing dyes from wastewater via biodegradation and biosorption. Microorganisms including bacteria, algae and fungi have been used to remove dyes for many years [1]. Methyl orange, which is also an azo dye has been chosen for this study since it is known as highly toxic and complex. Some physical parameters were optimized for methyl orange removal by *F. acuminatum* in this study.

MATERIALS AND METHODS

Microorganisms

Fungal culture used in this study was *F. acuminatum* obtained from the culture collection of Hacettepe University, Department of Biotechnology. The fungus was maintained on potato dextrose agar (Merck) slants and stored at $4^{O}C$.

Dye

Methyl orange (Merck) was dissolved in distilled water and the wavelength of maximum absorption was found spectrophotometrically (Jenway, 105 UV/VIS spectrophotometer).

The stock dye solution was prepared and filtered for the sterilization and it was stored at $4^{O}C$ in complete darkness. In order to obtain the decolorization medium, methyl orange stock solution was diluted to the desired concentration and added to each flask.

Media and Biomass Preparation

The composition of the culture medium was prepared according to Seyis and Subasioglu [5]. The medium contained 2.5 g/L (NH4)2SO4, 2.5 g/L yeast extract, 5 g/L KH2PO4, 0.5 g/L MgSO4•7H2O, 0.13 g/L CaCl2•2H2O and 10 g/L glucose (Sigma). The pH of the medium was adjusted to 6.0.

As for biomass preparation, *F. acuminatum* was incubated for 7 days at $30^{O}C$ in a shaking incubator at 150 rpm. Following the incubation, fungal pellets (0.5g wet pellet per flask) were washed with distilled water for three times, centrifuged and transferred to the medium containing methyl orange. Dye concentrations of the medium varied between 20-100 mg/L.

Decolorization

Flasks were incubated at 30^{O}C, shaking incubator, 150 rpm in complete darkness in order to avoid photodegradation. At the end of the incubation period, biomass was filtered and centrifuged at 6000×g for 15 min. Dye concentrations were read as absorbance at 500 nm using spectrophotometer (Jenway, 105 UV/VIS). Results were expressed as amount of decrease in absorbance at same wavelength. In each experiment dye containing medium was used as control. All experiments were repeated three times.

Effects of Different Physiological Conditions on Decolorization

The effect of temperature, initial dye concentration and initial pH was studied. Inoculated flasks were incubated at 25-45^{O}C, with 5^{O}C increments for 96 h. To investigate the effect of dye concentration, 20, 40, 60, 80, 100 mg/L concentrations of methyl orange was added from the stock solution to the medium. For the pH studies, initial pH of each medium was adjusted between 3.0-8.0. After each incubation, percent decolorization was calculated.

RESULTS AND DISCUSSION

There are various microorganisms which were used for the removal of methyl orange studies, such as; *Candida zeylanoides* [6], *Lactobacillus casei*, [7], *Trametes hirsuta* [8], *Trametes polyzona* [9], *Trametes trogii* [10], *Phanerochaete chrysosporium* [11, 12], *Ganoderma* sp. [13], *Aspergillus ochraceus* [14] and *Humicola fuscoatra* [15].
Optimization of parameters for the removal of methyl orange using *F. acuminatum* should be investigated since none of the previous studies found in literature concentrated on this issue. In this respect, *F. acuminatum* was studied as an alternative for methyl orange decolorization and some physiological conditions affecting the dye removal were investigated.
First of all, in order to find a correlation between decolorization and temperature, incubation was performed between 25-45^{O}C. The highest decolorization rate was observed at 35^{O}C, which decreases slightly above this temperature (Fig. 1). It is considered that temperatures in the range 35-45^{O}C are suitable for the enzymes of fungus, which are used for decolorization.

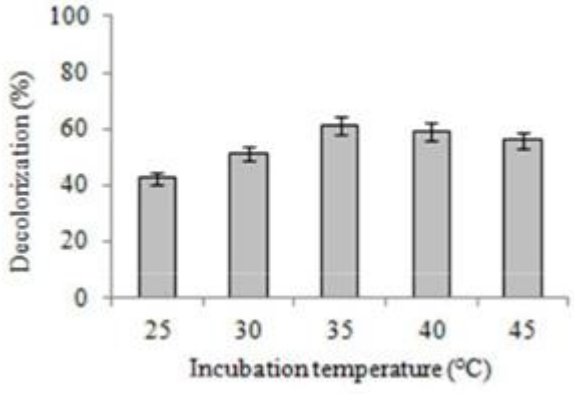

Figure 1- Effect of incubation temperature on decolorization of methyl orange.

Fungus showed activity in a wide temperature range, which is an advantage in terms of industrial use. According to Akar et al. [16], dye removal decreases while the temperature increases from 20^{O}C to 50^{O}C. Anjaneya et al. [17] concluded that

increased temperatures result in better biosorption, but the optimum temperature was found to be 35°C. Hadibarata et al. [18] reported the decolorization of RB5 increases when the temperature increases from 15°C to 40°C. Moreover, different temperatures were reported for the decolorization of methyl orange as 28°C [9], 35°C [7], 37°C [12].

Dye concentration of the effluent is another important factor. Initial concentration of the dye was studied between 20-100 mg/L. Although the maximum amount of decolorization was observed at 60 mg/L, decolorization rate is above 60% in the range 20-60 mg/L and slightly above 50% even at 80 mg/L (Fig. 2). Above the certain concentrations of dye, binding sites of biomass saturates and decolorization rate decreases. Similarly, according to Hadibarata et al. [18], with increasing initial dye concentrations decolorization rate decreases. It is most probably due to the fact that, since the toxicity increases, growth and enzyme inhibition occurs. It was reported that Zhuo et al. [13] performed the decolorization with the concentration of 50 mg/L of methyl orange. Similarly, Couto et al. [8] found the dye concentration of methyl orange as 60 mg/L.

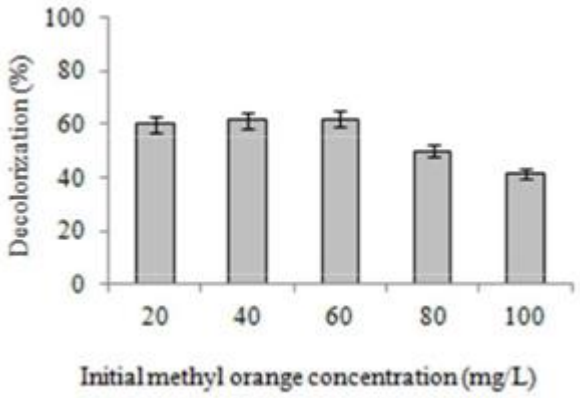

Figure 2- Effect of initial methyl orange concentrations on decolorization

pH is one of the major parameters of decolorization studies. During the reaction, pH affects the enzyme activity and ionization degree of the biosorbent. Hydrogen ions act as a bridge between the dye and the surface of the biosorbent [19, 20]. As seen in Figure 3, the decolorization rate increases up to almost 70% in the pH range 3-4 and decreases with the increasing pH value. Although under the increasing Ph conditions methyl orange decolorization decreases, it still remained almost 30%. This seems to indicate that acidic pH values would be favorable for the removal of methyl orange in terms of dye – biosorbent interactions and enzymes which are capable of decolorization and showed higher activity than the alkaline pH. It was also reported previously that the maximum removal of methyl orange was determined in the pH range 2.5 - 3.0 [11]. Similarly, Couto et al. [21] reported that decolorization of methyl orange was found around 70% at pH 4.0, whereas at pH 5.0 it decreased to 51%.

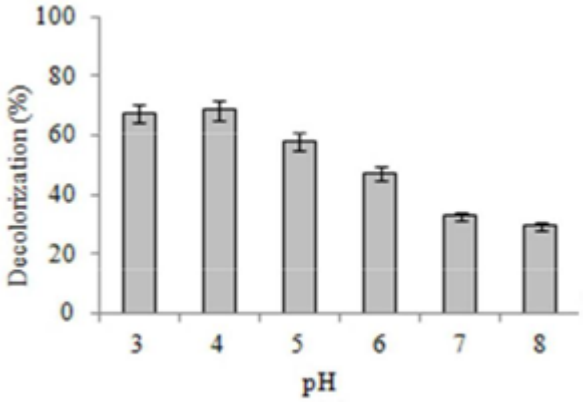

Figure 3- Effect of initial pH on decolorization of methyl orange.

The characteristics of the wastewater samples of textile industry were reported by Sengul et al., [22].

According to these data, pH values of the wastewaters were between 2.15 and 3.5. The results point that the high decolorization rate achieved at acidic pH values in our study would be rewarding in terms of industrial applicability.

In this study, it was performed the decolorization of methyl orange via the pellets of *F. acuminatum* more than 50% of the dye removal was seen even in the first 48 h of the incubation (Fig.4). *F. acuminatum* produces various enzymes that may perform decolorization successfully. Fungus continued to grow in the methyl orange medium and synthesized enzymes necessary for the decolorization activity, while the presence of pellets were performing the biosorption process. Abdel Ghany and Abboud [23] reported that fungal decolorization begins at 2nd day and increases sharply until the 10th day. According to Selvam et al. [24], *Thelephora* sp. was able to remove only 33.3% of Orange G after 216 h. Similarly, Zeroual et al. [25] found that almost all Orange G decolorization was observed between the 4th and the 7th day, which means that it takes longer time for the fungal biomass to begin growing and showing activity of dye removal. Working with live biomass is more convenient than growing cells at this point since there is no need for awaiting the growth phase.

There is only limited data for removal of dyes by *Fusarium* species in the literature. Korniłłowicz- Kowalska and Rybczynska [26] screened 20 strains of *F. oxysporium* and found that only 4 strains have the decolorizing ability of alizarine blue black B with the rate of 40-70%. Przystaś et al. [27] studied removal of the mixture of two dyes, tryphenilmethane brilliant green and azo evans blue with a concentration of 80 mg/L by *F. oxysporium*. Abedin [28] reported the biosorption of crystal violet and malachite green by *F. solani* with 3-10 mg/L dye concentrations. Abd El-Zaher [29] observed 45-66% decolorization with *F. oxysporium* for 24 h with different dyes with a dye concentration of 0.01%. Ansari et al. [30] reported the decolorization activity as 12-55% for 24 h with a 30-80 ppm dye concentrations.

Decolorization rate of the textile dye effluent by non living biomass with *F. acuminatum* was reported as 23% [3].

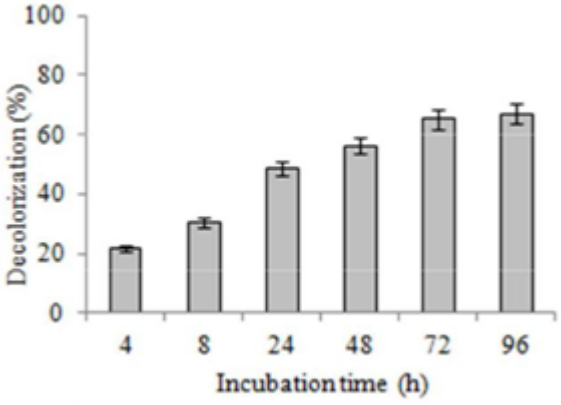

Figure 4- Effect of incubation time on decolorization of methyl orange.

CONCLUSION

Biological treatment of dyes is an important area of interest in wastewater treatment, therefore, some physical parameters affecting the decolorization of methyl orange by *F. acuminatum* have been studied.

In this respect, decolorization was investigated in connection with the temperature, initial dye concentration, incubation time and initial pH. It can be concluded that fungus can achieve maximum decolorization efficiency at pH 4 and 35^{O}C. The fungus has the ability of decolorizing 60 mg/L methyl orange in a short time period with the high percentage. In addition, the fungus is effective in a wide range of temperature, pH and initial dye concentration as discussed above, which makes it a promising alternative for use in industrial applications.

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