Determination of biosorption conditions of Methyl Orange by
Humicola fuscoatra

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This study presents effects of biomass concentration, dye concentration, agitation time, initial pH and temperature on dye (Methyl Orange) removal by dead fungal biomass of Humicola fuscoatra. Biosorption capacity was found dependent mainly on dye and adsorbent dosage. Highest adsorption efficiency was achieved with fungal biomass at 0.5 g/l. Dye concentration (100 mg/l) was found optimum for maximum dye removal. At acidic pH (3-5) and 30°C, Methyl Orange biosorption increased significantly in first 6 h.

Keywords: Biosorption, Dye, Humicola fuscoatra, Methyl Orange

Introduction
Wastewaters of industries using synthetic dyes generally contain hardly biodegradable organic compounds with complex structures. Dyes can be toxic to aquatic life by effecting photosynthetic activity due to reduced light penetration1,2. On treatment of such wastewaters, researches concentrated mainly on physico-chemical methods (chemical coagulation/flocculation, ozonation, oxidation, ion exchange, irradiation, precipitation and adsorption). However, biological methods are more suitable because of convenience and efficiency2-4. Many fungal sources have been used in dye biosorption and decolorization3,5-7. This study presents biosorption of Methyl Orange by Humicola fuscoatra.

Materials and Methods
Microorganism and Adsorbent Preparation
Fungus, H. fuscoatra, was obtained from laboratory culture collection of Hacettepe University, Department of Biotechnology. Fungus was routinely maintained on potato dextrose agar slants and kept at 4°C. Transfers were made at 1 month intervals. Fungus was grown for 7 days in Sabouraud's dextrose broth medium at 30°C in a shaking incubator at 150 rev. min⁻¹. After incubation, biomass was filtered through filter paper. It was washed with distilled water for three times, centrifuged and autoclaved at 121°C for 15 min to obtain dead biomass.

Dye Solution Preparation and Adsorption Studies
Methyl Orange (Merck) was dissolved in distilled water to a desired concentration and filtered for sterilization. Wavelength (λmax, 500 nm) was found spectrophotometrically (Jenway, 105 U.V.-Vis spectrophotometer). Stock solution was stored at 4°C in dark until used.

For decolorization experiments, solution (50 ml) was prepared with dye (conc., 100 mg/l) in Erlenmayer flask (250 ml) at pH 5. Autoclaved fungal biomass (0.5 g) was used for adsorbent and inoculated into dye solution (50 ml). Flasks were agitated in a shaking incubator (150 rev.min⁻¹) for 24 h at 30°C in complete darkness in case of photodegradation. Simultaneously, control experiments were done without any biomass. After 24 h, biomass was filtered and supernatant was centrifuged at 6000 x g for 15 min. Dye concentrations in filtrates were measured spectrophotometrically at 500 nm. Results were expressed as degree of decrease in absorbance against initial absorbance at same wavelength.

Determination of Optimum Biosorption Conditions
For dye concentration studies, different Methyl Orange concentrations (25-200 mg/l) were used. In order to understand effect of biomass concentration, fungal
pellets (0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 g/l) were added to flasks containing test solution (50 ml). Different temperatures (25-50°C) were used to find out optimum temperature. Initial pH of each solution was adjusted between 3.0-8.0 with HCl (0.1 M) and NaOH (0.1 M). To evaluate optimum agitation period, dye solutions were prepared and after inoculation, flasks were monitored for 48 h. Incubation was carried out on a rotary shaker (150 rev.min\(^{-1}\)) at 30°C. Samples of biosorption medium were determined at certain time intervals (2, 4, 6, 8, 24, 48 h). All experiments were repeated thrice.

**Results and Discussion**

Fungi have been screened for decolorization ability and *H. fuscoatra* was found suitable for Methyl Orange\(^6\). Therefore, dead biomass of *H. fuscoatra* was used for dye adsorption capacity and some physiological parameters of Methyl Orange removal process were optimized.

Dye removal has been found to increase by increasing biomass concentration (Fig. 1). Maximum adsorption of Methyl Orange was obtained with biomass concentration of 0.5 g/l. There was a slight decrease in dye adsorption above 0.5 g/l, could be due to aggregation of biomass particles in the solution and reduction of surface area\(^9,10\). Highest adsorption of Methyl Orange was obtained with initial dye concentration (100 mg/l), whereas a slight decrease in dye concentration was observed above concentrations of 150 mg/l (Fig. 2). Binding sites of biomass are reported\(^9,11\) to saturate with higher dye concentrations. In this study, dye concentration results of Methyl Orange adsorption on fungal biomass of *H. fuscoatra* is similar to those observed by Farah *et al*\(^12\) on Astrazone Blue adsorption on dry biomass of Baker’s yeast. Under effect of agitation time (2-48 h) on dye removal (Fig. 3), major removal seemed to occur in first 6 h. After 8 h of agitation, adsorption speed slowed down and after 24 h, there was no significant decrease in dye removal. Methyl Orange removal increased by increasing agitation period of dye and adsorbent.

Chemistry of dye molecules and fungal biomass in an aqueous solution is influenced by initial pH of dye solution\(^13\). Optimal initial pH for Methyl Orange removal was found between 3.0-5.0 (Fig. 4). Adsorption rate decreased rapidly above pH 5.0. When pH was adjusted to 3.0, dye solution prepared with Methyl Orange apparently began to change colour from orange to pink.
Higher pH led to lower adsorption because of characteristics of biomass and Methyl Orange, which can also be seen in many other dyes and most of the researchers claimed that an acidic pH condition of dye solution was more effective for dye removal\textsuperscript{10,14,15}. However, Sadhasivam et al.\textsuperscript{16} reported that removal of rhodamine 6G dye by \textit{Trichoderma harzianum} increased at pH 8.0, indicating that optimum pH depends on the nature of dye and biosorbent. Dye removal at various temperature (20-50°C), using biomass of \textit{H. fuscoatra}, was studied (Fig. 5). Maximum dye adsorption occurred at 30°C and corresponding biosorption capacity was 62.5%. At higher temperatures, dye removal rate decreased. Similar observations reported that adsorption capacity of \textit{Enteromorpha prolifera} for Acid Red 337 increased up to 30°C and decreased at higher temperatures\textsuperscript{8}.

**Conclusions**

Removal of Methyl Orange was obtained using \textit{H. fuscoatra} biomass. For maximum dye removal, various parameters were found as follows: biomass concentration, 0.5 g/l; dye concentration, 100 g/l; agitation time, 6 h; initial pH, 3-5; and temperature, 30°C.

**References**