

Full Length Research Paper

Comparison of live and dead biomass of fungi on decolorization of methyl orange

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Decolorization of methyl orange with live and autoclaved biomass fungi were studied. Sixteen different fungi including *Fusarium*, *Trichoderma*, *Humicola*, *Aspergillus*, *Penicillium*, *Paecilomyces*, *Alternaria* and *Beauveria* were evaluated. The highest decolorization activity was observed with *Fusarium acuminatum* and *Humicola fuscoatra*. Decolorization efficiencies of live and autoclaved biomasses were compared. In addition, spores of eight fungi showing high decolorization efficiency were inoculated for decolorization ability. The most successful fungi were *Penicillium* sp. and *Fusarium* sp. For adsorption studies, methyl orange decolorization with live and autoclaved biomasses were found most suitable with malt extract compared to yeast extract peptone medium.

Key words: Decolorization, methyl orange, *Fusarium* sp., *Humicola* sp., fungi.

INTRODUCTION

One of the most important environmental problems that the textile industry is facing today is to eliminate color from the dyes. More than 10.000 different dyes are used in industries such as textile, leather, pharmaceutical, food, pulp and paper, paint and electroplating (Maurya et al., 2006). Especially, textile industry generates large quantities of wastewater because of the azo dyes, which are very toxic and difficult to be disposed by physical, chemical and microbiological methods (Sadhasivam et al., 2005). The total world textile dye production is estimated at about 800 kt annually (Zollinger, 1991). Therefore, the discharge of highly colored synthetic dye effluents from these industries can result in serious environmental pollution problems. Even though some physical and chemical methods such as adsorption, membrane filtration, photocatalytic degradation, ion exchange, precipitation, flocculation, flotation and ozonation are quite effective in decolorization of dyes, all have some disadvantages such as high cost per unit volume of waste water treated, unfriendly for nature or unreliability

in operation (Aksu, 2005). On the other hand, biological processes are frequently applied to decolorization of textile and dyestuff wastewater due to cost effectiveness and simplicity.

Many microorganisms belonging to different taxonomic groups of bacteria (Wu et al., 2005), fungi (Zheng et al., 1999; Terasawa et al., 2000; Neelamegam et al., 2004; Couto et al., 2006) and algae (Dilek et al., 1999) have been reported for their ability to decolorize different dyes. In this study, we evaluated the ability of 16 different fungi for efficient decolorization of methyl orange, which is classified as both acid and azo dye, with complex structure.

MATERIALS AND METHODS

Microorganisms

Fungal strains used in this study were: *Fusarium acuminatum*, *Fusarium culmorum*, *Fusarium equiseti*, *Fusarium* sp., *Trichoderma harzianum* D2, *T. harzianum* D3, *Trichoderma viride* A2, *T. viride*, *Humicola fuscoatra*, *Humicola insolens*, *Aspergillus niger*, *Penicillium* sp., *Paecilomyces lilacinus*, *Alternaria alternata*, *Gibberella fujikuroi*, *Beauveria bassiana*, obtained from the culture collection of Hacettepe University, Department of Biotechnology. They were maintained on PDA and slants were kept at 4°C. Transfers were made at one month intervals.

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Media

The composition of the basal medium used in this study consisted of 10 g/L glucose, 2.5 g/L $(\text{NH}_4)_2\text{SO}_4$, 2.5 g/L yeast extract, 5 g/L KH_2PO_4 , 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.13 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. The pH was adjusted to 6.0 and sterilized. The second medium used in biomass studies was peptone-yeast extract medium and it contains 4 g/L peptone and 4 g/L yeast extract.

Dye solution preparation

Methyl orange was used as a dye, dissolved in distilled water to a desired concentration and filtered for the sterilization. The stock methyl orange solution was stored in the dark. The dye was added into the basal medium with the concentration of 0.1 g/L.

Biomass production

For the biomass studies, the fungi were inoculated into 200 ml of peptone-yeast extract medium in 500 ml flasks and incubated at 30°C, 150 rev. min⁻¹ for 5 days. After the cultivation, 0.5 g of fungal pellets were inoculated into 50 ml of fresh basal medium with methyl orange in 250 ml flasks. Similar to the live biomass method, in order to determine dead biomass decolorization, 0.5 g of fungal pellet was derived and inoculated after autoclaved at 121°C for 15 min.

For the spore decolorization studies, the spore concentrations of respective strains were adjusted to 10⁵ spores/ml and inoculated into 50 ml of fresh basal medium with methyl orange in 250 ml flasks.

Three sets of flasks were incubated at 30°C in the incubator shaker at 150 rev.min⁻¹ for submerged cultivation in complete darkness in case of the photodegradation. After the cultivation, the flask contents were filtered through Whatman filter paper. To determine biomass in terms of dry weight, fungal pellets were washed and filter papers were dried until constant weight. The dry weight of the biomass was determined as gram per 50 ml. The filtrates were then used for the determination of decolorization and pH.

Decolorization assay

Culture broth was centrifuged at 6000 g for 15 min. Dye concentrations in aqueous solutions were measured spectrophotometrically at 500 nm (Jenway, 105 u.v., vis spectrophotometer). The results were calculated as the difference in decolorization and the decolorization yield was expressed as the degree of decrease in the absorbance at 500 nm against the initial absorbance at the same wavelength.

RESULTS AND DISCUSSION

The aim of our study was to investigate alternative fungi in decolorization process of methyl orange. In addition, these fungi were compared with living biomass and autoclaved biomass and their respective efficiencies were calculated. As a result, an alternative fungal source that realizes color elimination biologically is offered. In this respect, pellet production of various fungi in 5 days at 30°C, 150 rev.min⁻¹ (*Beauveria*, *Gibberella*, *Alternaria*, *Paecilomyces*, *Penicillium*, *Aspergillus*, *Humicola*, *Trichoderma*, *Fusarium* sp.) were carried out. Following,

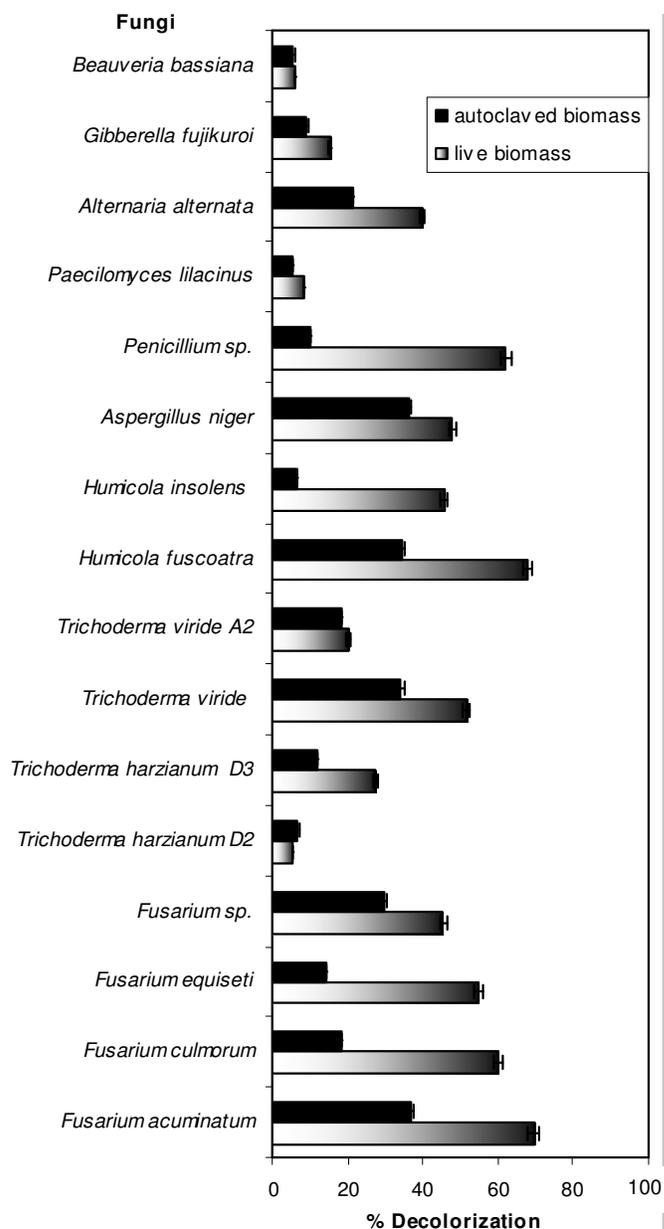


Figure 1. Decolorization activities of autoclaved biomass and living biomass.

living biomass of these fungi was inoculated into methyl orange media and methyl orange decolorization efficiencies after 5 days at 30°C, 150 rev.min⁻¹ incubation were compared.

As shown in Figure 1, decolorizations by living pellets of *F. acuminatum* as well as *H. fuscoatra* were higher when compared with other fungi. In *H. insolens*, decolorization efficiency was slightly lower. There is no study in literature that has been carried out with *H. fuscoatra* and *F. acuminatum*. In addition, 48% decolorization was observed with *A. niger*, which is a fungus known as showing high efficiency in dyes other than methyl orange decolorization.

Table 1. pH and dry weights of living biomass study.

Fungi	pH	Dry weight (g/50 ml)
<i>Fusarium acuminatum</i>	6.7	0.81
<i>F. culmorum</i>	7.9	1.02
<i>F. equiseti</i>	7.5	0.69
<i>Fusarium</i> sp.	7.3	0.68
<i>Trichoderma harzianum</i> D2	5.9	1.05
<i>T. harzianum</i> D3	6.9	0.61
<i>T. viride</i>	6.6	1.15
<i>T. viride</i> A2	6.8	0.60
<i>Humicola fuscoatra</i>	7.7	0.59
<i>H. insolens</i>	7.4	0.58
<i>Aspergillus niger</i>	6.6	0.65
<i>Penicillium</i> sp.	7.2	0.91
<i>P. lilacinus</i>	6.2	0.65
<i>Alternaria alternata</i>	6.9	0.74
<i>Gibberella fujikuroi</i>	7.4	0.59
<i>Beauveria bassiana</i>	7.1	0.66

rization in literature. Similarly, decolorization efficiencies of *Penicillium* sp. were high in our study (Figure 1) (Zheng et al., 1999; Zhang et al., 2003).

No studies were recorded in literature using *T. viride*, although *T. harzianum* was used for decolorization of trypan blue (Sadhasivam et al., 2005). When the results of our study with *Trichoderma* sp. are investigated, decolorizations in the range 6 - 52% show that some species are suitable for methyl orange decolorization. *A. alternata* and *G. fujikuroi* have 35 and 15% decolorization efficiencies, respectively (Figure 1). This can be considered as a promising result as no studies on decolorization with *Alternaria* sp. have been reported in literature. On the contrary, *B. bassiana* and *P. lilacinus* were not studied before and their results were not satisfactory in our study. But it should be noted that there are a number of studies in literature using different *Paecilomyces* sp. (Terasawa et al., 2000; Thakur, 2004).

From pH point of view, both *Fusarium* sp. showing high decolorization efficiency and *B. bassiana* showing almost no decolorization have pH values around 7 after incubation (Table 1). Therefore, it was concluded that pH value after incubation has no relation with decolorization efficiency.

Although growth of *T. viride* and *T. harzianum* D2 was higher than others, decolorization rates of these two fungi were rather different (5% in *T. harzianum* and 50% in *T. viride*). This implies that even growth rate of *T. harzianum* D2 was high, it cannot show high decolorization neither with the enzymes it produces nor with biomass. Figure 1 presents the results of *T. harzianum* D2 with autoclaved pellets, and as it can be seen from these results it does not show high decolorization, therefore it is not a successful methyl orange adsorbent as biomass.

Same decolorization efficiencies were recorded for *F. acuminatum* and *H. fuscoatra*, although growth of *H. fuscoatra* was rather low when compared with *F. acuminatum* (Figure 1 and Table 1). Therefore, *H. fuscoatra* can be considered to be more advantageous as it is expected to have much higher decolorization efficiency when same amount of growth rate with *F. acuminatum* is achieved. At the same time it can be concluded that decolorization efficiency does not have a direct relation with growth rate.

High decolorization with low growth rate is most probably due to enzymes produced. Therefore, as growth increases, enzyme production also increases. These increased amount of enzymes will help much more efficient methyl orange decolorization.

Biodegradation of dyes is extremely difficult; therefore much research is being carried out on this subject. Selection of the most appropriate method for decolorization in industrial scale is based on the economics, feasibility and efficiency of the processes used. Although adsorption is one of these methods, adsorbents used in these processes are rather expensive. Using biomass in adsorption, which is another decolorization method, is quite advantageous. Much research has been carried out for biomass, especially for metal bonding. Although efficiency of adsorption is highly dependent on decolorization efficiency of adsorbent, availability and cost of the adsorbent are the limiting factors for its effective usage in industrial applications. In industrial processes, large amount of biomass is generated as a result of fermentation, which increases its availability to be used for decolorization of fungal biomass.

In this respect, biomasses of different fungi were used in the first part of this study and in the second part, ad-

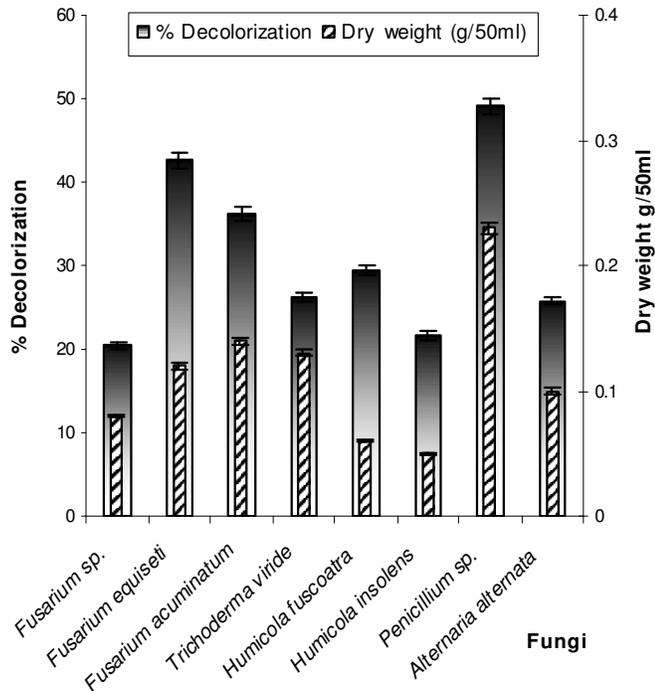


Figure 2. Decolorization activities and dry weights of fungi*, *inoculated as 10^5 spores/ml.

sorption characteristics of these fungi with dead biomass were studied. Pellet production of different fungi in 5 days at 30°C , $150 \text{ rev. min}^{-1}$ was carried out. Following, decolorization efficiencies of biomass of these fungi in methyl orange after autoclave process were compared.

Decolorization efficiencies of *H. insolens* and *Penicillium sp.* with live pellets were higher than autoclaved pellets. This result indicates that fungi are not successful biosorbents with pellets but perform decolorization with the help of enzymes that they produce. In fact, decolorization with microorganisms is carried out either with adsorption of the pigment on to mycelia or its enzymatic degradation (Nakajima-Kambe et al., 1999).

Similar to living biomass, decolorization efficiencies of autoclaved pellets of *H. fuscoatra* and *F. acuminatum* were higher than other fungi. Higher decolorizations of living biomass when compared with autoclaved biomass show that these perform decolorization both with adsorption and with the help of enzymes that they produce.

In dye adsorption studies, chitin, chitosan and lignocellulose are used as adsorbents. As the cell walls of the fungi used in our study contain chitin, cellulose and lignocellulose, high adsorption rate is an expected result. In some studies found in literature, the cell wall of *Myrothecium verrucaria* was used in decolorization of azo dyes (Brahmi-Horn et al., 1992). But on the contrary, presence of fungi with low adsorption rate is dependent on several factors. These factors are different culture conditions used for preparation of biomass, differences

encountered by these fungi from media and different composition of cell walls. In addition, pH, temperature and ionic concentration of media affect decolorization results of different fungi as these are important factors in adsorption studies.

Methyl orange decolorization with dead biomass will be more advantageous. In living biomass, additional nutrients in the medium increase costs. In addition, products of living biomass may cause problems in adsorption.

Decolorization rates for autoclaved biomass are not greater than those of living biomass in our study. In fact, higher results can be obtained with these alternative fungi with optimization. In literature, majority of previous works concentrated on physicochemical methods rather than biological decolorization as methyl orange is a recalcitrant material. Although there are a lot of studies about the decolorization of different types of dyes with different fungi, there is no study of methyl orange decolorization with fungi which we studied and showed in Figure 1. Methyl orange decolorization has been carried out with *Phanerochaete chrysosporium*, *Trametes versicolor*, *T. hirsuta*, *Bacillus sp.* and *Pleurotus ostreatus* initially (Ollikka et al., 1993; Couto et al., 2004; Neelamegam et al., 2004; Pourbabae et al., 2005; Couto et al., 2006).

In our study, spores of fungi showing high decolorization efficiency were also inoculated into the basal medium and decolorization efficiencies were compared. In this respect, dye biodegradation during the growth was investigated. Decolorization was high in *Penicillium sp.* and *Fusarium sp.* similar to the biomass study. In addition, it was observed that decolorization as a result of spore inoculation was not as high as with living biomass (Figures 1 and 2). Therefore, it was concluded that using biomass is more advantageous in decolorization studies carried out with these fungi. Higher efficiencies were observed with living biomass as it enables both enzymatic decolorization and adsorption. It should be noted that, products of living biomass may prevent adsorption; therefore, media conditions in biomass production and decolorization are extremely important.

Biosorption capacity of biomass depends on several factors. One of these factors is the culture conditions of biomass preparation. In this respect, in the last stage of our study, decolorization efficiencies of different biomass grown in yeast extract peptone medium and malt extract broth medium were compared. Biomass was grown in *F. acuminatum* and *H. fuscoatra* as decolorization efficiencies of these fungi were high. As it can be seen from Figure 3, decolorization efficiencies of two fungi in yeast extract peptone were lower. Therefore, it was concluded that malt extract broth was more suitable for growing biomass of these fungi. Bonding capacity of dye to biomass depends on factors such as charge of the dye, pH, salt content, ionic force and size. In this respect, high efficiencies in autoclaved pellets of malt extract broth medium are probably due to the fact that surface of these pellets are suitable for adsorption.

As a result, it was concluded that *H. fuscoatra* and

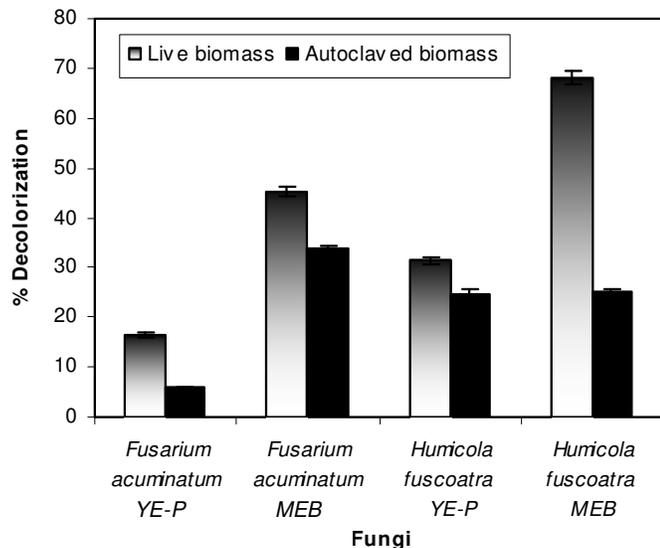


Figure 3. Decolorization activities of autoclaved biomass and living biomass in yeast extract-peptone medium (YE-P) and Malt extract broth medium (MEB).

Fusarium sp. are suitable for methyl orange decolorization and using autoclaved biomass is advantageous. *Humicola* sp., though less studied, could be an alternative for decolorization of different dyes. In addition, decolorization efficiencies obtained with *A. alternata* (35% with living biomass and 21% with autoclaved biomass) could offer promising results, as it has not been studied earlier. When decolorization efficiencies of yeast extract peptone and malt extract broth were compared, it was seen that malt extract broth was more suitable.

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