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Biodecolorization of a persistent organic dye from model wastewater using *Curvularia spp*

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ABSTRACT

The textile industry produces a huge volume of wastewater, out of which 10–50% of these effluents are discharged into the aquatic environment without being treated. The discharge of these colored compounds in the environment causes considerable nonaesthetic pollution and serious health-risk factors. In this study an effort has been made to biodecolorize simulated dye wastewater containing Reactive Red 195 by isolated fungus *Curvularia spp*. The experiments have been performed by varying the influencing parameters, such as initial concentration, pH, and temperature. Maximum decolorization of 66% was obtained for 0.01 g/l of dye concentration and at pH 5. Maximum decolorization of 75.33% was obtained at a temperature of 40°C (in 48 h). The optimum condition for maximum dye uptake capacity is: initial dye concentration 0.02 g/l, temperature 40°C, pH 5, and an inoculum size of 5 ml (approximately 1.0×10^5 cells/ml).

Keywords: Biodecolorization; *Curvularia*; Dye bath; Fungi; Reactive Red 195

1. Introduction

Wastewaters coming from the textile industries containing dyes are highly colored and are therefore visually identifiable [1]. Textile industries consume considerable amount of water for wet processing of fabrics for various processes. It was estimated that over 100,000 commercially available dyes existed over 7×10^5 metric tons of dyestuffs produced annually [2–4]. A majority of azo dyes are quite resistant to biodegradation under aerobic conditions and easily pass through conventional aerobic wastewater treatment systems [3]. On the other hand, azo dyes are readily decolorized by splitting the azo bond(s) in anaerobic environments. Biodecolorization is the initial stage of the dye-removing process, whereas in the biodegradation process, complete mineralization from aromatic amines takes place, which results in the biodecolorization process.

Due to the most extensive use of these dyes in industries, they become an integral part of the industrial wastewater. In fact, out of the 450,000 tons of organic dyes annually produced worldwide, more than 11% is lost in effluents during manufacture and application processes [5]. The complex aromatic structure of the dyes is designed to be resistant to light, biological activity, fading, and other degradative environmental conditions. Thus, conventional wastewater treatment remains ineffective. Also, anionic and nonionic azo dyes release toxic amines due to reactive cleavage of the azo groups [6].

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Up to date, many methods exist for the treatment of effluents by various physical processes, namely adsorption using activated carbon [7], coagulation [8], membrane filtration [9], nanofiltration [10], and chemical processes such as electrochemical oxidation [11], Fenton's reactions [12], photocatalytic oxidation [13], and sonochemical methods [14]. But these processes can be summarized as expensive, power consuming but not environment-friendly, and usually dependent on the concentration of the waste. Therefore, the search for environment-friendly and cost-competitive alternative, efficient remedies for dye degradation have been initiated. Complete mineralization of dyestuffs can be effected by chemical or biological oxidation [3,4,15–19] .

Many micro-organisms belonging to different taxonomic groups of bacteria, fungi, actinomycetes, and algae have been reported for their ability to decolorize azo dyes [20–21]. The active role of fungi in the treatment of wastewater has been extensively researched especially on white-rot fungus *Phanerochaete chrysosporium* [22–26]. The fungus has proved to be a suitable organism for the treatment of textile effluents and for dye removal. Several other wood-rotting fungi capable of decolorizing a wide range of structurally different dyes were also isolated and found to be more effective than *P. chrysosporium* [23–26]. White-rot fungi (WRF) produce various isoforms of extracellular oxidases including laccase, manganese peroxidesm and lignin peroxides (LiP), which are involved in the degradation of lignin in their natural lignocellulosic substrates. This ligninolytic system of WRF is directly involved in the degradation of various xenobiotic compounds and dyes.

While many studies were devoted to biodecolorization of the textile dyes, far less attention has been paid to textile wastewaters and simulated dye effluents in which the presence of salts and high dye concentration may be inhibitory to biological agents. The aim of our study was to assess the degrading potential of isolated fungal strain *Curvularia* on model wastewater under aerobic conditions. To achieve this objective, in the present study the isolated fungal strain *Curvularia* has been tested on model wastewater containing Reactive Red 195. This fungus is a facultative pathogen of many plant species and of the soil. The experiments have been performed by varying the parameters such as initial concentration, pH, and temperature. Growth kinetics and half-life period have also been estimated.

2. Materials and methods

2.1. Micro-organism

The white-rot fungus *Curvularia* was obtained from the Bharathidasan University, Tamilnadu, India, and the stock cultures were maintained by periodic subculture on potato dextrose agar medium at 4°C [27].

2.2. Inoculum

The fungus *Curvularia* was inoculated on Potato dextrose agar and incubated at 37°C until extensive spore growth occurred. The basal medium [28–29] used to study the fungal biomass and decolorization test consisted of: D-glucose, 5.0 g/l; (CAS No. 50-99-7), KH_2PO_4, 2.0 g/l; (CAS No. 7778-77-0), NH_4Cl, 0.050 g/l; (CAS No. 12125-02-9), $MgSO_4 \cdot 7H_2O$, 0.5 g/l; (CAS No. 10034-99-8), $CaCl_2 \cdot 2H_2O$, 0.1 g/l; (CAS No. 10035-04-8), thiamine HCl, 100 mg; (CAS No. 67-03-8), trace element solution, 10 ml and the final pH of the medium was maintained at pH 4.5. Trace element solution consisting of $MnSO_4$, 0.5 g/l; (CAS No. 7785-87-7), $FeSO_4.7H_2O$, 0.1 g/l; (CAS No. 7782-63-0), $ZnSO_4 \cdot 7H_2O$, 0.1 g/l; (CAS No. 7446-20-0) was prepared separately and 10 ml was added to the medium.

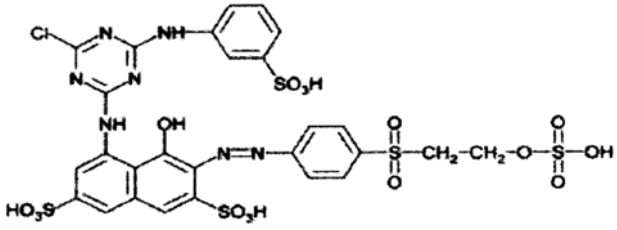
2.3. Preparation of dye-bath effluent and decolorization studies

The properties and structure of Reactive Red 195 (CAS No. 93050-79-4) are shown in Table 1. Simulated dye wastewater was prepared according to the composition that is commonly used in cotton dyeing with Reactive Red 195. Reactive Red 195 hydrolyzed dye-bath wastes were collected from a dyeing industry located in Tiruppur, Tamil Nadu, India, and was dried by keeping these wastes in a hot air oven. To prepare the study sample, the dried dye sample was dissolved in double-distilled water (to make a concentration of 1 g/l) [30]. Serial dilution was done from the stock solution prepared to get the desired concentration of the dye effluent. Synthetically prepared dye-bath effluent was added to Erlenmeyer flasks (250 ml) containing 100 ml of the medium, which was inoculated with approximately 1×10^5 cells. The experiments were carried out in an orbital shaking incubator at 150 rpm for 4 days at 37°C. The dye concentrations were measured from samples that were collected at regular intervals using a spectrophotometer (Jasco V502 spectrophotometer). Control experiments for each test were carried out using an uninoculated medium with all the ingredients and dye addition. Samples were withdrawn everyday and analyzed based on their maximum absorbance value.

2.4. Analytical methods for the decolorization study

The absorbance peak (λ_{max}) of Reactive Red 195 dye was determined by UV scanning and found to be

Table 1
Structure and properties of Reactive Red 195 used in this study

S. No	Dye	Molecular structure	Molecular weight	Absorption maxima (λ_{max})
1	Reactive Red 195 (CAS No. 93050-79-4)		1,136.31	540 nm

at 540 nm. For the decolorization study, 5 ml of the sample was taken and centrifuged at 3,000 g for 10 min. The absorbance of the supernatant was spectrophotometrically determined at 540 nm at different time intervals (on the first day and thereafter every 24 h on subsequent days for a total period of 4 days). Percentage decolorization calculated by the equation is as follows:

$$\%\ \text{Decolorization} = \frac{C_o - C_i}{C_i} \times 100 \quad (1)$$

where C and C_o are the initial and final concentrations of the dye-bath effluent [31–35].

Specific growth rate was calculated from the plot of fungal growth Vs incubation period. A plot of ln (C/C_o) vs. time represents a straight line, the slope of which on linear regression equals the pseudo-first-order rate constant $k\,(hr^{-1})$ [36–37].

$$\ln\left(\frac{C}{C_o}\right) = kt \quad (2)$$

$$t_{1/2} = \frac{0.693}{k} \quad (3)$$

where $t_{1/2}$ is the half-life period, k is the rate constant, and C and C_o are the initial and final concentrations

3. Results and discussion

3.1. Effect of the dye-bath effluent on initial concentrations

Curvularia was used to study the percentage decolorization along with the maximum time requirements for the decolorization process. For initial experiments, keeping the parameters such as initial pH and temperature as constant, the initial concentrations of the individual dyes were varied from 0.01 to 0.1 g/l.

The maximum time taken for decolorization varies with the nature of individual dyes and the longer time taken for decolorization is a result of the production of extracellular peroxidases, which are inhibited with the growth of the fungus [31]. Decolorization of model wastewater containing Reactive Red 195 by Curvularia at 37°C and pH 7 under aerobic conditions at 150 rpm is given in Fig. 1. Dye-bath effluent containing Reactive Red 195 had shown considerable decrease in color by the aerobic liquid cultures of Curvularia. The absorbance decreased for 4 days and no significant change was observed thereafter, except 0.01 g/l.

The results showed that an increase in concentration suppresses the rate of biodecolorization. Spadaro et al. [32] established that *P. chrysosporium* was capable of mineralizing a variety of toxic azo dyes and the mineralization of aromatic rings of azo dyes was dependent on the nature of the ring substituent.

Maximum decolorization obtained for dye-bath effluent containing Reactive Red 195 is 66% for 0.01 g/l and 48% for 0.02 g/l, respectively, on the fourth day. Decolorization of Reactive Red 195 of concentration ranging between 0.04 and 0.1 g/l was less extensive, ranging between 15 and 23%.

The growth of the fungi may be effected by the presence of dyes at toxic higher concentrations. This also has an effect on the dye decolorizing efficiency of the fungi. Also, the class of the dye, which defines its structure, is also influential in deciding the extent to which the dye is decolorized. Curvularia showed a maximum activity for laccase at 24 U/l on the fourth day [19]. Parshetti et al. Kapdan et al. and Aksu et al. [33–35] found that decolorization showed an adverse effect at a higher concentration.

3.2. Effect of pH

Decolorization of model wastewater containing Reactive Red 195 by Curvularia at 20 mgL^{-1} and 37°C under aerobic conditions is represented in Fig. 2. The

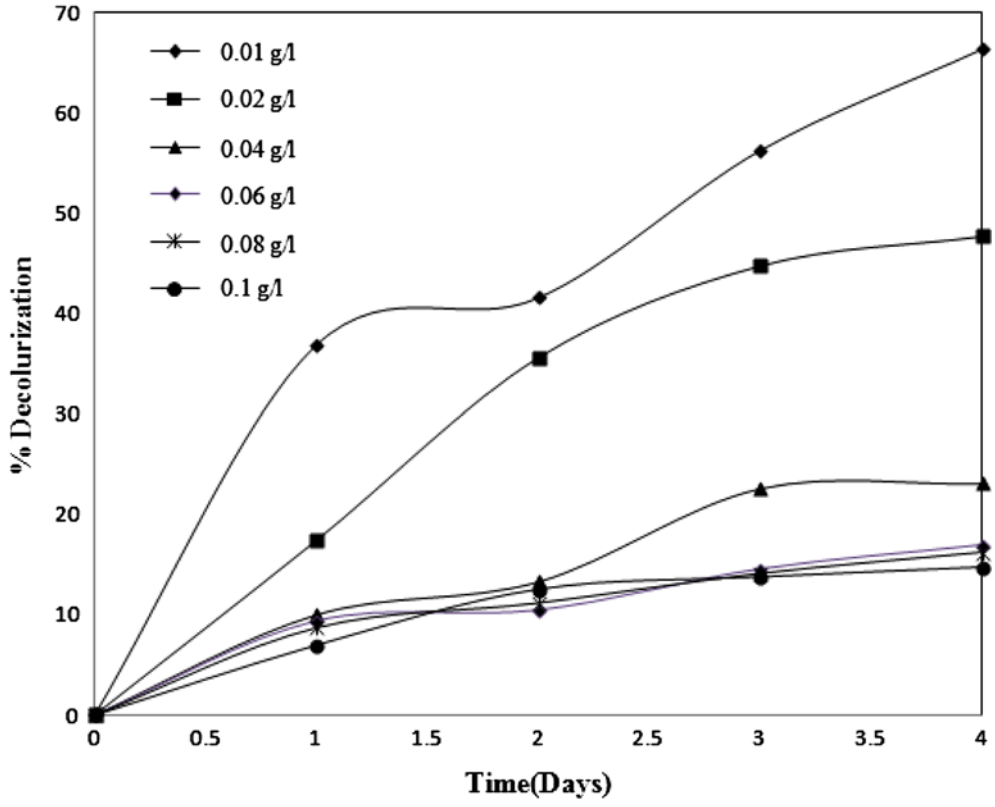


Fig. 1. Decolorization of model wastewater containing Reactive Red 195 by *Curvularia* at 37°C and pH 7 under aerobic conditions at 150 rpm for 4 days.

fungal ligninolytic enzymes show maximal activity at low pH [25]. Therefore, efficient dye decolorization is also observed at low pH. Kapdan et al. [34] reported the optimum growth pH of Coriolus versicolor as 4.5 but lowered at pH 6 and 7. Parshetti et al. [33] obtained complete decolorization of Reactive Blue-25 A. ochraceus NCIM-1146 at pH 5.0 in seven days. However, less decolorization was obtained at pH 7 and 9, respectively. The efficiency decreased from 59 to 8%, as the pH was increased from 5 to 6 [33]. Hence, it was observed that for a majority of the fungi, the optimum pH for dye decolorization lay in the acidic range. Nearly 66% efficiency was obtained at pH 5. Whereas at pH 3, pH 7, and pH 9 the efficiency stood at 31.4, 5.35, and 3%, respectively.

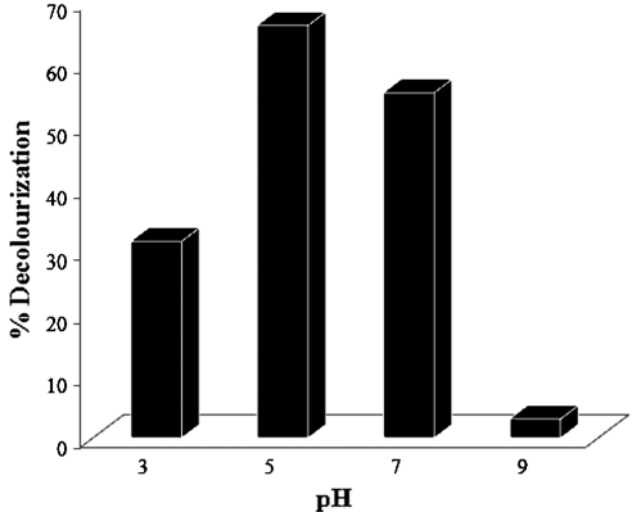


Fig. 2. Decolorization of model wastewater containing Reactive Red 195 by *Curvularia* at 20 mg L^{-1} and 37°C under aerobic conditions at 150 rpm for 4 days.

3.3. Effect of temperature

The effect of temperature on dye decolorization was extensively studied [29]. A majority of these reports indicate that decolorization capacity of the fungal biomass increases with increase in temperature. In order to study the variation in temperature on decolorization, studies were carried out at temperatures ranging from 20 to 50°C. The experimental results for decolorization of model wastewater containing Reactive Red 195 by Curvularia at 20 mgL^{-1}

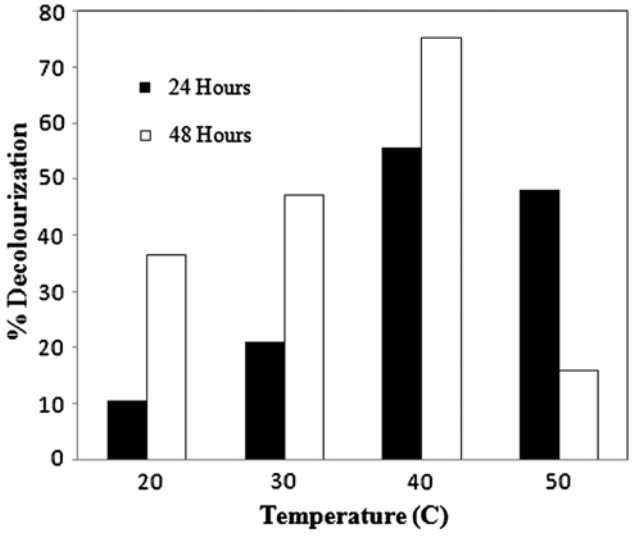


Fig. 3. Decolorization of model wastewater containing Reactive Red 195 by *Curvularia* at 20 mg L^{-1} and pH 5 under aerobic conditions at 150 rpm for 2 days.

Table 2
Specific growth rate and half-life period for the decolorization of model wastewater containing Reactive Red 195 by Curvularia at 37°C pH 7 under aerobic conditions at 150 rpm for 4 days

S.No.	Concentration (mg/l)	μ (hr^{-1})	T_d (Half-life) Day^{-1}
1	10	0.4579	1.51343088
2	20	0.0783	8.850574713
3	40	0.0926	7.483801296
4	60	0.0494	14.02834,008
5	80	0.0392	17.67857143
6	100	0.0366	18.93442623

and pH 5 under aerobic conditions is given in Fig. 3. At higher (>40°C) or lower (<30°C) temperatures, the decolorization activity of the fungus gets reduced, which indicates that either the fungus is not able to grow for decolorization or it gets denatured. Based on the results of the screening study, for the decolorization of dye-bath effluent containing Reactive Red 195 by *Curvularia* was selected for examining the effect of temperature from 20 to 50°C. It can be noted that Reactive Red 195 showed a higher rate of decolorization of 75.33% at 40°C than at 30°C. It has shown very poor decolorization of 15.92% at 50°C in four days. Whereas in the experiments performed at 20 and 30° C, the percentage decolorization was 35.5 and 48%, respectively [20,23,25,29].

3.4. Growth rate for decolorization

The plot between ln OD vs. incubation period gives the specific growth rate of Curvularia on Reactive Red 195, which is seen in Table 2. From the table it is inferred that Reactive Red 195 has shown a maximum growth rate occurred for 0.01 g/l is 0.4579 day^{-1}. But 0.1 g/l has shown a very poor growth rate of 0.0366 day^{-1}. Half-life period has been calculated for Reactive Red 195 to be 1.5134 for 0.01 g/l [36,37].

4. Conclusions

Many researches have been done into the ability of WRF to decolorize and degrade textile dyes. This is the first report on the biodecolorization of dye-bath effluent containing Reactive Red 195 by the isolated fungus *Curvularia*. The mycelium of this fungus was able to decolorize the azo dyes. The purpose of this study was to investigate the possibilities of facilitating microbial decolorization of dye-bath effluent. The results indicate that this is a possible and potential application in the bioremediation of textile effluents contaminated with azo dyes and other toxic compounds (i.e. it can be used along with any other hybrid process like electrochemical treatment method). Maximum percentage decolorization for Reactive Red 195, which is studied in the present work, was found to be more than 76% (In 48 h) at the following optimum condition: initial dye concentration 0.02 g/l, temperature 40°C, pH 5, and an inoculum size of 5 ml (approximately 1.0×10^5 cell/ ml), thus the high effectiveness in decolorization was attained.

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