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A Study on the Decolourization of Methyl Red by *Acremonium sclerotigenum*

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Abstract

Dyes are extensively used in textile, leather and paper industries. But unsafe disposal of these dyes into water bodies and other places causes serious damage to the environment and negative impact on the human, plant and animal life. Textile effluent treatment is one of the greatest challenge. Use of microbes is a frequently applied process for the decolorization of textile dyes. Microbial decolorization offers an eco- friendly and cost competitive alternative to chemical decomposition process. Methyl red is a carcinogenic textile dye which is used for the colouration of various products. The aim of this research was to explore the ability of the fungal isolate *Acremonium sclerotigenum* to decolorize textile dyes. The fungus was isolated from soil of industrially contaminated areas and identified as *Acremonium sclerotigenum* at NFCCI, Pune. The isolate had shown maximum dye decolourization after 72 hrs (89.85%) in a medium containing dextrose (40mM), soya peptone (1.4%), yeast extract (1.6%) and beef extract (0.8%),inoculum size of 9.5×10^5 spores/ml, pH-6.0. with 10mM methyl red. Present study showed the potential of *Acremonium sclerotigenum* as microbial dye decolourization agent.

Keywords: Dye, Decolourization, *Acremonium sclerotigenum*, Methyl red

1. INTRODUCTION

Dyes are an important class of chemicals widely used in many industrial processes, like in leather, textile and printing, food, and cosmetics industries. They become an integral part of industrial wastewater due to their extensive use [1]. Inefficient dyeing processes, poor handling of dye effluent and insufficient treatment of dye wastes of industries lead to contamination of the soil and water bodies [2].A conventional biological wastewater treatment process is not very efficient in treating a dye wastewater due to the low biodegradability of dyes. Physical or chemical-treatment processes, used for treatment of dyes do not offer a solution as they generate a significant amount of the sludge and cause secondary pollution due to the formation of by products [3].

Azo dyes are major synthetic dyes extensively used in various industries. The process of applying dye on fabric is inefficient and approximately 10-15% of the dyes are released in to environment .The first contaminant observed in waste water is color and are visible in water even at concentrations as low as 1 ppm [4]. Dyes escape the conventional wastewater treatment process as they are recalcitrant's due to their synthetic origin and complex stable structure [5, 6]. Synthetic dyes are extensively used in textile dyeing paper printing, colour photography, pharmaceutical, and food, cosmetic and other industries [7].

Approximately 10,000 different dyes and pigments are used industrially, and over 0.7 million tonnes of synthetic dyes are produced annually worldwide. In 1991, the world production of dyes was estimated at 668,000 t [8] of which an estimated 70 percent were azo dyes [9]. A bulk of azo dye production is lost to domestic and industrial wastewater r[8 ,10]. Major classes of synthetic dyes include azo, anthroquinone and triaryl methane dyes, and many of them are toxic or contain carcinogenic compounds with long turnover times [11].The ability of microorganisms to carry out dye decolorization has received much

attention as it is a cost effective method for removing dyes from the wastewater [12,13].

A variety of microorganisms has been reported which are capable of decolorization of dyes. Keeping in view the application of various microorganisms in the field of dye decolourization and degradation the present study was focused on exploitation of *Acremonium sclerotigenum* as potential textile dye decolourization agent.

2. MATERIALS AND METHODS

2.1 Microorganism

The fungal isolate *Acremonium sclerotigenum* NFCCI 3463 was isolated from soil samples of industrially contaminated areas of Himachal Pradesh (India) and identified as *Acremonium sclerotigenum* at NFCCI ,Pune (India).

2.2 Chemicals

Congo red and media components were purchased from HIMEDIA (India). All the other chemicals were of analytical grade and were obtained from Sigma and HIMEDIA, unless otherwise specified.

2.3 Mode of fermentation

Cultivation of fungus was carried out by submerged fermentation in 250ml flasks using the production medium containing ,dextrose (40mM), soya peptone (1.4%), yeast extract (1.6%) and beef extract (0.8%),inoculum size of 1.25×10^6 spores/ml, pH-6.0. autoclaved at 121°C for 20 minutes. The medium was supplemented with filter sterilized penicillin G at 1% concentration as substrate inducer. Then inoculated with inoculum size of 1.25×10^6 spores/ml and incubated for 48 hours at 25°C at 150 rpm in an incubator shaker.

Five different dyes (5mM) were tested for decolourization efficiency of fungus *Acremonium sclerotigenum*. The dyes used were Reactive Blue - 4,Remazol Brilliant Violet 5R, Methyl red ,Indigo Bismarck Y. Out of five different dyes, the dye which was decolorized to a highest extent was further used for decolourization. 5mM of dye concentration, which was used initially for the optimization of various parameters? Extent of decolourization was estimated with respect to increase in transmittance and decrease in O.D at 472 nm.

$$\% \text{ Decolourization} = \frac{\text{Transmittance of test} - \text{transmittance of control}}{\text{Transmittance of test}} \times 100$$

2.4 Decolourization of Methyl red dye

Decolourization experiment was performed with *Acremonium sclerotigenum*

2.4.1 Optimization of decolourization of methyl red with fungus *Acremonium sclerotigenum.*

To get highest decolourization, it is necessary to optimize various nutritional and physicochemical parameters e.g. Carbon sources, nitrogen sources, pH, temperature, inoculum size and dye decolourization profile.

2.4.2 Optimization of carbon sources for dye decolourization

6- Different carbon sources (Starch, Sucrose, Fructose, Galactose, Maltose, and Dextrose.) were used to get best carbon source for evaluation of dye decolourization potential of *Acremonium sclerotigenum.*

2.4.3 Optimization of nitrogen sources

6 different nitrogen sources (Peptone, Soya peptone, Tryptone, Urea, Gelatin, Acetamide, Ammonium sulphate) were used to get the highest decolourization, nitrogen source for the assessment of dye decolorizing potential of *Acremonium sclerotigenum.*

2.4.4 Optimization of pH for the dye decolourization

pH of the medium varied from 3.0 to 9.0.For the evaluation of dye decolorizing potential of *Acremonium sclerotigenum.*

2.4.5 Optimization of inoculum size.

The inoculum size was varied from 8.5×10^5 spores/ml-1.65×10^6 spores/ml size for the evaluation of dye decolorizing potential of *Acremonium sclerotigenum.*

2.4.6 Optimization of decolourization profile of methyl red

Dye decolorization profile was studied for 5 days; samples were taken at the interval of 24 hrs and assayed as described earlier.

2.4.7 Optimization of temperature for the dye decolourization

Temperature of medium was varied from 25^0C to 45^0C for evaluation of dye decolourization of methyl red by *Acremonium sclerotigenum* s mentioned above.

2.4.8 Spectrophotometric analysis

After the optimization of various parameters, decolourization of dye by fungus *Acremonium sclerotigenum* and penicillin acylase was further confirmed by UV-spectroscopy. The spectrum of the dye decolourization by fungus was plotted in shimadzu UV-Spectrophotometer.

3. RESULTS AND DISCUSSION

3.1 Fungal growth conditions

An optimal environment is necessary for fungal growth is and it enhances the ability of the fungi to decolourize dyes. Therefore various nutritional parameters for optimum fungal growth have been optimized.

3.2 Optimization of medium

Fungi were grown in the medium with methyl red to develop a biosorbent. The medium mainly composed of carbon, nitrogen source and other nutrients. Out of five different dyes used for decolourization,(Table-1) methyl red showed highest decolourization with *Acremonium sclerotigenum* (Fig.1).

Table 3.1: Dye decolourization (%) potential of *Acremonium sclerotigenum*

Dye	**Decolourization(%)using *Acremonium sclerotigenum***
Reactive Blue 4	0
Remazol Brilliant Violet 5R	11.75
Methyl red	29.5
Indigo	8.25
Bismarck Y	4.2

(A) **(B)**

Fig.1 Dye decolourization of methyl red by *Acremonium sclerotigenum* (A) Control (B) Test

3.3 Optimization of carbon source

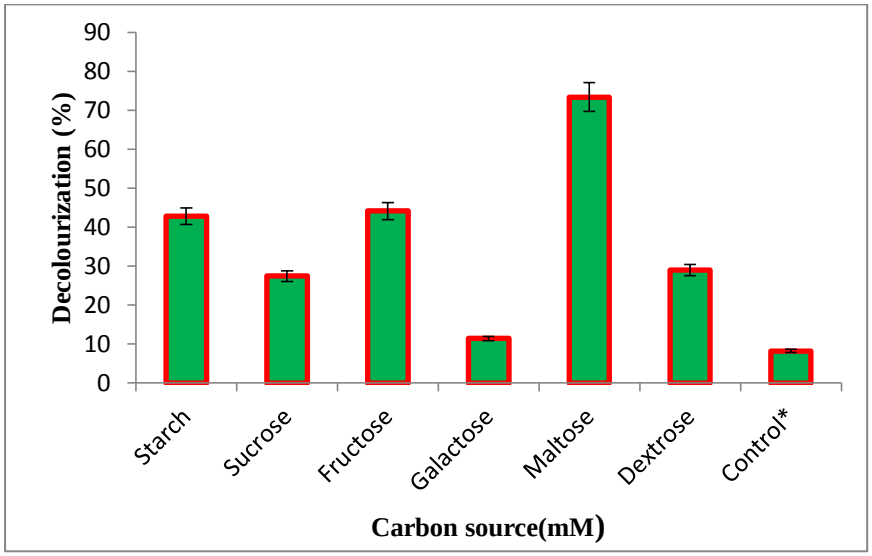

Fig. 3.3 Effect of different carbon sources on dye decolorization

To determine the effect of different carbon sources on dye decolorization by *Acremonium sclerotigenum* six carbon sources, namely starch, sucrose, fructose, galactose maltose, and dextrose, were used and the highest decolourization was recorded when maltose was used as carbon source and decolourization was 73.45 % (Fig.3.3) Similarly [14] reported that maximum decolourization of azo dye- Red 3BN by *P .chrysogenum*, *A. niger*, and *Cladosporium sp.* was observed when maltose was used at a concentration of (1%). Whereas [15] in his study found that dextrose was most effective for RBBR dye decolorization by *Mucor hiemalis* MV04 (KR078215). (38.17% free and 64.5% immobilized biomass) among all carbon sources used. Decolorization ranged between 80 to 100% in 8 days. The primary mechanism of decolorization may involve dye adsorption/degradation by mycelium of fungi as well as the reduction of dye intensity in solution because of changes caused by them [16]. Furthermore, the rate of dyes removal can be linked with the available co-substrates and with the exponential growth phase [17]. However at higher concentrations the same carbon sources on metabolism produced organic acids, which in turn decreased the pH of media. The fungi needs readily usable carbon source for their growth and production of secondary metabolites and extracellular enzymes [15].

3.4 Optimization of nitrogen source

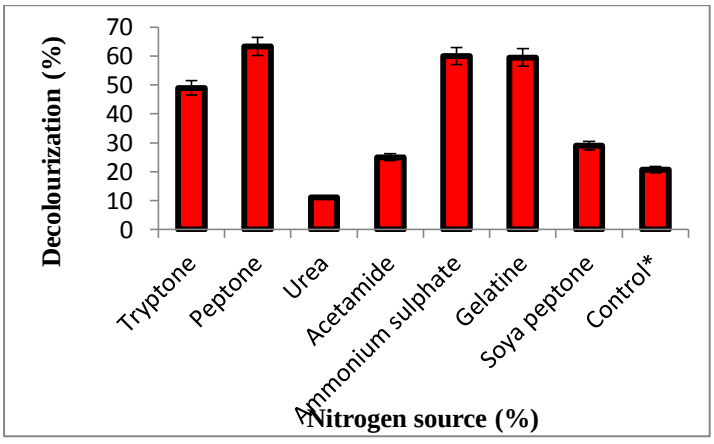

Fig. 3.4 Effect of nitrogen source on dye decolourization

Nitrogen source supplementation in medium plays a critical role in dye decolorization activity. The amount of nitrogen present in the media effects dye decolorization by altering the enzyme production by fungi. Nitrogen in the form of ammonium ions acts as a nutrient for the growth of fungal mycelium. The rate of decolourization of dye molecules depends on the rate of breaking of azo (-N,N-) bonds in the dye molecule [18]. Seven different nitrogen sources were used and highest decolourization was recorded when peptone was used as nitrogen source and decolourization was 63.3% (Fig.3.4). This indicates that the rate of decolourization increased up to [16] reported that peptone supported the decolourization of Red 3BN by *A. niger*, and *Cladosporium sp* (90%). For several fungal species the ligninolytic enzyme activity is suppressed rather than stimulated by high nitrogen concentrations (20-100mM) [15].In contrast [20] found the range of decolorization of Brown GR with ammonium chloride, ammonium sulphate, ammonium nitrate, yeast extract and peptone ,72%, 64%, 68%, 68 % and 76% respectively with *Aspergillus* sp .

3.5 Optimization of temperature

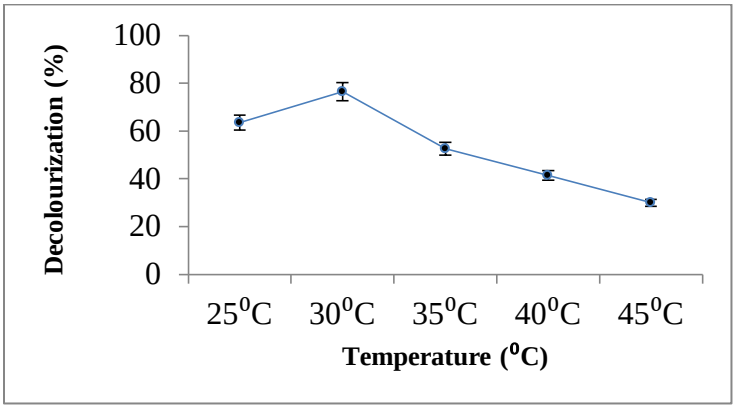

Fig.3.5 Effect of incubation temperature on dye decolourization

The incubation temperature affected the growth and decolourization potential of the *Acremonium sclerotigenum*. To study the effect of varying temperature on dye decolorization efficiency of the fungus, the medium pH was adjusted to initial optimum pH -6 and incubated at 25, 30, 35 and 45 °C 50 for 2 days. It was found that with an increase in temperature from 25 to 30°C decolorization rate increased (maximum observed was at 30°C) and then a further increase in temperature to 45°C drastically affected dye decolorization potential of fungal isolate. The maximum decolorization ((76.45%) was observed at 30°C (Fig.3.5). Suppressed decolourization at 45°C might be due to the loss of viability or deactivation of the enzymes responsible for decolourization at higher temperature [21].

3.6 Optimization of medium pH

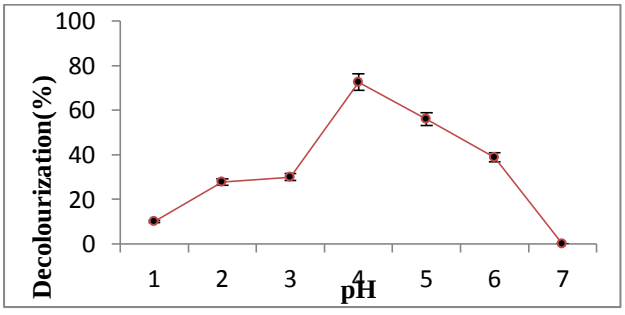

Fig 3.6 Effect of medium pH on dye decolourization

Fungus has a strong capability to grow on wide range of pH. pH affects not only the biosorption capacity but also the colour of the dye solution and the solubility of some dyes. Therefore pH is an important factor in colour removal (Fu and Viraraghavan, 2001[22]. The pH range of the medium varied from 3.0 to 9.0 and highest dye decolourization was observed with pH 6.0.The decolourization (%) was 72.58 %(Fig 3.6), the percentage of decolourization declined as pH was altered [23, 22] reported that initial pH of the dye solution significantly influenced the chemistry of both dye molecules and fungal biomass. They found the effective initial pH for the decolourization of Basic blue 9 and Acid blue 29 was 6.0 and 4.0 respectively.

3.7 Optimization of inoculum size

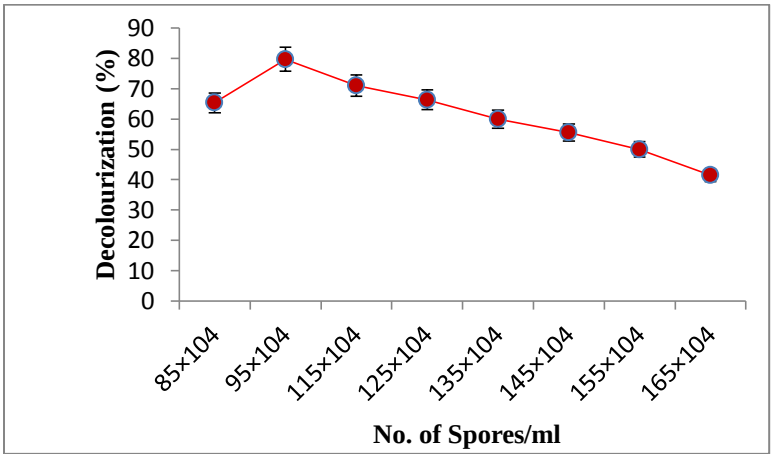

Fig 3.7 Effect of inoculum size on dye decolourization

Inoculum size was varied and highest decolourization of the dye was recorded with an inoculum size of 9.5×10^5 spores/ml and decolourization % was 79.66%(Fig.6) Influence of the volume of inoculum on decolorization of the Red 3BN by *P.chrysogenum*, *A. niger* and *Cladosporium sp.* was studied by Kumar et al.,(2012). *A.niger and P.chrysogenum* were equally effective in the decolorizing Red 3BN and showed more than 95% decolorization of the dye. The ideal volume of inoculum was found to be 2% for *P.chrysogenum* and 10% for *A.niger.*

3.8 Optimization of decolourization profile of methyl red using *Acremonium sclerotigenums*

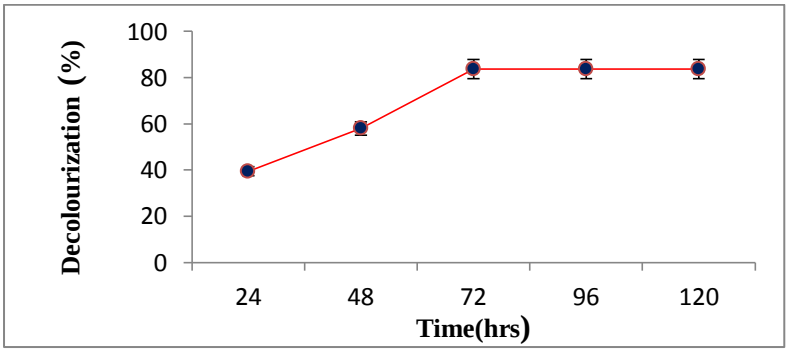

Fig3.8 Decolourization profile of methyl red

The dye decolourization profile was studied for 5 days, samples were taken at the interval of 24hrs and the highest dye decolourization was observed after 72 hrs and was 83.65 %(Fig.3.8). In contrast to our results Kumar et al., 2012 [14] reported the time course of decolorization of red 3BN under optimum conditions by *P.chrysogenum*, *A.niger* and *Cladosporium sp.* and results indicated that both *P. chrysogenum* and *A.niger* were capable of executing nearly 100% decolorization of red 3 BN under their respective optimal conditions

while *Cladosporium sp.* exhibited slightly lower level of decolorization activity after 6 days. The difference may be due to the structure of the dye and growth characteristics of the organism.

3.9 Optimization of dye concentration for decolourization of dye

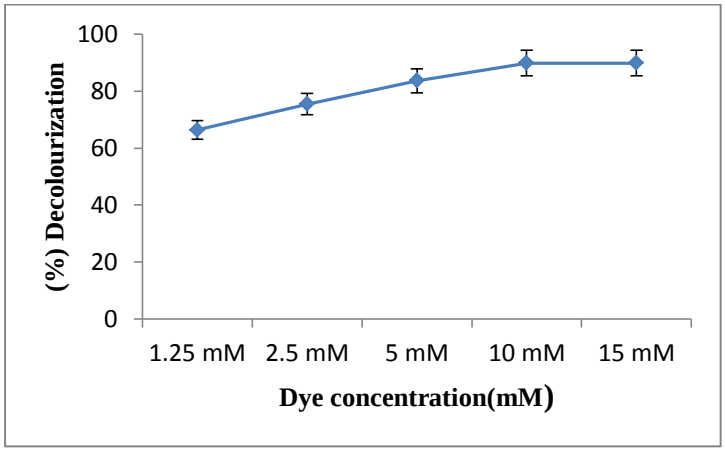

Fig.3.9 Effect of dye concentration

Concentration of dye influences the decolourization rate. Dye conc. was varied from 1.25 mM – 25 mM and highest decolourization was observed when dye was used at a conc. of 10 mM for methyl red. The decolourization was 89.85 % (Fig. 3.9).The rate of decolourization declined with increased concentration of methyl red. The Increased dye concentrations had an adverse effect on dye decolorizing efficiency of the fungi. Also, the class of the dye which defines its structure also influences the dye degradation [4, 6, and 24]. This might be attributed to the toxicity of dye to bacterial cells through the inhibition of metabolic activity, saturation of the cells with dye products, inactivation of transport system by the dye or the blockage of active site of azo reductase enzymes by the dye molecules [25]. For industrial applications, the microorganisms should be able to bear high concentrations of the dye since the dye concentration in a typical industrial effluent can vary between 10 and 50 mg [26, 27].

4. UV-Spectrophotometric analysis

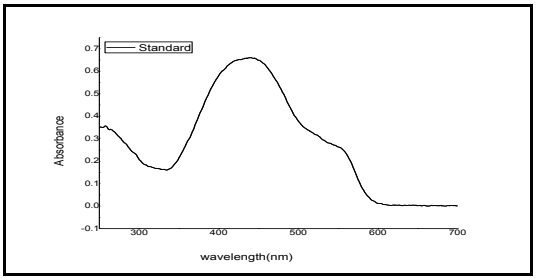

Fig.4.1 U.V Spectrum of methyl red

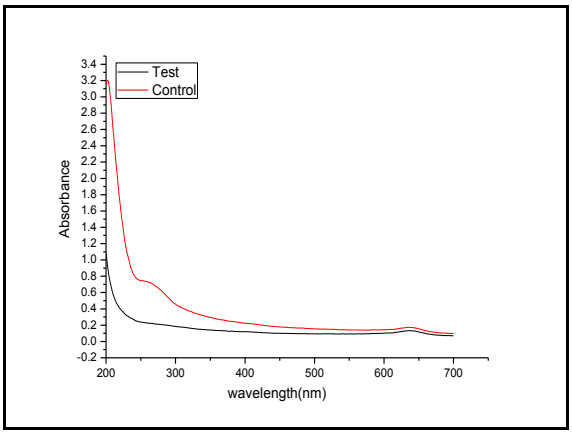

Fig. 4.2 U.V Spectrum of dye after decolourization

After the optimization of various parameters, degradation of dye by fungus *Acremonium sclerotigenum* was further confirmed by UV-spectroscopy (Fig.4.1). The spectrum of the dye degradation by fungus was plotted in shimadzu UV-Spectrophotometer (Fig.4.2). Decrease in absorbance of test in comparison to control confirms the decolourization of methyl red.

5. CONCLUSION

Current study proved the dye decolorization potential of *Acremonium sclerotigenum* against methyl red. Temperature and pH were found to be important parameters. Removal of dye may be due to biodegradation of chromophore in dye molecule because of extracellular enzyme production by fungi.

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Conflict of interest

Authors claim that there is no conflict of interest.

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