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Decolorization of the textile dye (Brown GR) by isolated *Aspergillus* strain from Meerut region

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Abstract

In the present study, an attempt was made to examine the potential of aerobic fungal culture for decolourization of Brown GR textile dye. The effect of carbon source, nitrogen source, pH, temperature, NaCl concentration of dye was studied with an aim to determine the optimal conditions required for maximum decolourization and degradation. The fungal culture exhibited maximum decolourization ability at pH between 6-7 and at 25°C.1% (v/v) inoculums 10^5 spores/ml with fructose sugar were found to be the optimum for decolourization. A maximum of 82% decolourization was observed at 0.05% initial concentration of dye after 5 days of incubation period. The results show that the fungal culture from environment has good potential in removal of Brown GR dye under aerobic conditions.

Keywords: Decolourization, Degradation, Brown GR, Aerobic Conditions, *Aspergillus* strain.

Introduction

The rapid development of dye industry has accelerated dye production along with a series of environmental hazards. Azo dyes are the largest and most diverse group of synthetic dyes, and more than 50% of annually produced dyes are azo compounds[1]. The hazards of azo dyes lie in the high carcinogenicity of their cleavage products and their low bio-degradability[2]. Reactive azo dyes are highly recalcitrant to conventional wastewater treatment processes, with as much as 90% of reactive dyes remaining unchanged after activated sludge treatment[3]. The reductive cleavage of the azo bond under anaerobic conditions usually leads to the formation of aromatic amines, which are highly toxic, mutagenic and carcinogenic[4].

Lots of attempts have been made to remove azo dyes from the dye effluents. Traditional physical and chemical methods have technical and economical limitations, including high costs, low efficiency and ineffective for recalcitrant dyes[5] as well as the production of large amounts of sludge[6]. In contrast, biological treatment provides a better alternative, and many microorganisms have been reported to demonstrate the ability to degrade azo dyes[2]. However, microbial treatment would result in biomass accumulation, which will expand the treatment scale[7] and the decolorization process is usually slow. Hence, the recent focus has shifted towards enzyme based treatment of coloured wastewater. Researchers have isolated azo reductase from bacteria to decompose azo dyes by cleaving their azo bonds[8]. Nevertheless, most reactions need to be conducted under strict anaerobic condition[2]. The degradation of azo dyes by azo reductase was incomplete due to their complex structures, and some of them might even be converted into toxic aromatic amines[9]. An eco-friendly and non-specific enzyme, therefore, is in desperate need for degrading azo dyes.

Laccase (EC 1.10.3.2) has received much attention for its superiority in degrading various recalcitrant pollutants[10]. It is a type of oxidase widely distributed among plant, fungi and bacteria, and can catalyze a variety of aromatic compounds using oxygen as electron acceptor with water as the sole by-product[11]. Some redox mediators can facilitate the catalytic activity of laccase and expand its substrates specificity to a much wider range[12]. Laccase mediator systems (LMS) have been widely used in different fields such as degrading organophosphorus compounds (OPs), insecticide, PHA, pulp bio bleaching and dye decolorization[13-14].

This study aims to investigate the potential of fungal cultures isolated from environmental samples for decolorization of a textile dye, Brown GR. Dye decolorization by fungal cultures was optimized with respect to various nutritional sources (carbon and nitrogen), environmental parameters (temperature, pH & salinity).

Material and Methods

Chemicals and media: All chemicals used in this experiment were of AR grade. The dye Brown GR was collected from a dye industry located at Mohkampur, Meerut, India. Carbon and nitrogen sources used were purchased from Hi-media Laboratories (Mumbai, India).

Isolation, screening and identification of dye degrading fungi: Samples were collected from environmental samples. A Saboraud's dextrose agar (SDA) plate opened into air for 10 to

15 minutes for isolation of dyes degrading microbes and soil, solid waste sample mixed in sterile water and serially diluted from 10^{-1} to 10^{-6} and 0.1 ml of diluted samples spread on Saboraud's dextrose agar (SDA) plates. Plates were incubated at 25^0C for 5 to 7 days till the appearance of fungal colonies. The colonies were further streaked on the respective agar medium to get pure culture and observed under the light microscope for the identification of fungal isolate. All isolates were preserved on SDA slant in refrigerator.

Spore suspension preparation: Total 20-25 mycelium disc of 5 mm diameter obtained from a 5 to 7 days old culture plates of fungus were transferred to 50 ml PDA in a 250 ml conical flask and incubated at 25°C temperature for 5 to 7 days. At the end of the incubation period 30 ml sterile water was added to each culture and the flasks were shaken with shaker. Then the content of each conical flasks were filtered through glass wool. The filtrate contained spores and were used for spore count on PDA. The same spore suspension was used in the experiments described below.

Screening of decolorizing fungi: Screening of 19 isolated fungal strains from the environmental samples was carried out to their ability to degrade the textile dyes by decolorization method [15]. Fungal disc of 5 mm diameter cut from the periphery of the 5 to7 days old culture was placed in flask containing 50 ml mixed textile dyes mineral salt broth separately. After 5 to 7 days, effective decolorization was seen visually. Those isolates showing decolorization of dye were selected for further studies with decolorization of synthetic dye Brown GR. Four fungal strains were found to be potential in dye decolorization and were identified on the basis of their microscopic observations.

Decolorization assay: Decolorization activity in terms of percent decolorization was determined by following method described by Mahbub *et al* [15]. 10 ml of sample was centrifuged at 2000 rpm for 4 minutes. Spectrophotometer was used for absorbance measurement. The decrease in absorbance was monitored at 486nm for Brown GR. Decolorization activity was calculated according to the following formula [15].

$D= [A_o-A_l/ _o] \times 100$

Where, D- decolorization; A_o- initial absorbance; A_l- final absorbance.

Dye decolorization optimization: Decolorization of Brown GR textile dye (0.05g/ 100ml) in MSM broth by all two isolates were optimized with respect to the effect of 1%, carbon sources (glucose, maltose, fructose, sucrose), 1%, organic nitrogen sources (peptone and yeast extract) and inorganic nitrogen sources (ammonium sulphate, ammonium nitrate and ammonium chloride), pH (4-9) and temperature (10, 25, 35 and 45°C). All experiments were carried out with 1% (v/v) inoculums of 10^5 spores/ ml and MSM broth without culture was served as control. All the flasks were incubated at 25°C under shaking conditions for 5 days.

Results and Discussion

Screening of dye decolorization: The obtained spore suspension showed spore count of 10^5 (spores/ml) was obtained. In MSM broth, four fungal isolates showed high decolorization of Brown GR (0.05%, w/v) after 5 days of incubation at 25°C under shaking. Only the rate of decolorization of dye and final percent color removal varied for each isolates. In the present investigation the rate of color removed increased with incubation periods. This was confirmed with the earlier findings of Nehra *et al.* [16], Mahbub *et al.* [15] and Spadaro *et al.* [17].

Optimization of dye decolorization: For the maximization of decolourization of the textile dye, Brown GR by the selected fungal isolates, experiments were conducted for optimization of carbon source, nitrogen source, salinity, pH and temperature.

Effect of carbon sources: All the fungal isolates showed higher percent decolorization than control showing that all the four sugars could be utilized effectively as carbon source by these isolates. The range of activity on decolorization of Brown GR with maltose, glucose, sucrose and fructose was 60%, 74%, 66% and 82% respectively with *Aspergillus sp* (figure 1). From the study *Aspergillus* sp was found to be the most effective decolorizing fungi among all 4 isolates and among the four sugars used. Fructose was the most effective carbon source for maximum decolorization of Brown GR accounting 82% decolorization of the textile dye. *Pleurotus* spp. fungi were able to promote degradation of organic matter present in the effluent resulting in progressive absorbance reduction throughout time of incubation where glucose addition revealed a positive factor; 57 and 76 % of absorbance reduction were achieved after 14 days of incubation in final effluent with glucose, and treated with *P. sajor caju* at 400 and 460 nm, respectively[18]. Wang *et al.*[19] reported a *Citrobacter* sp. decolorized by 96.2% of reactive red 180 dye with 4g/L of glucose as carbon source.

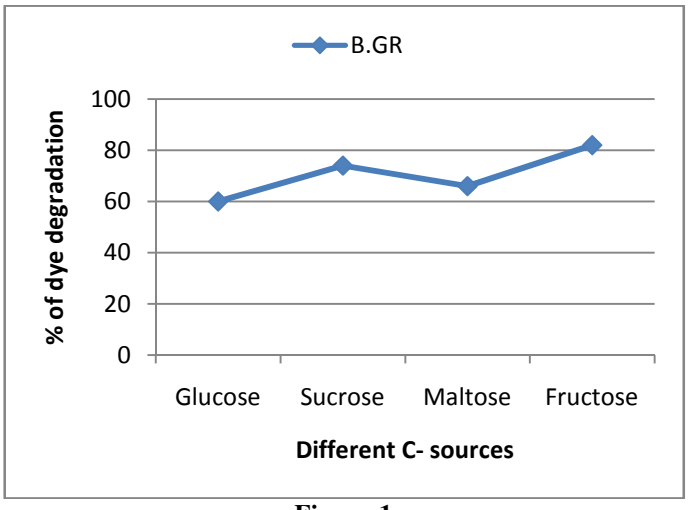

Figure-1
Effect of C- source on % of degradation of Brown GR dye

Effect of nitrogen sources: The range of % decolorization of Brown GR with yeast extract was 76% with *Aspergillus* sp. *Aspergillus* sp was found to be the most effective decolorizer (figure 2). The range of decolorization activity of Brown GR with ammonium chloride, ammonium sulphate, ammonium nitrate, yeast extract and peptone were 72%, 64%, 68%, 68 % and 76% respectively with *Aspergillus* sp. Ponraj *et al.*[20] was found to be 87.33% most effective decolorization of orange 3R for *Pseudomonas* sp with beef extract, 85.29% for *Bacillus* sp. with peptone and 82.81% with yeast extract.

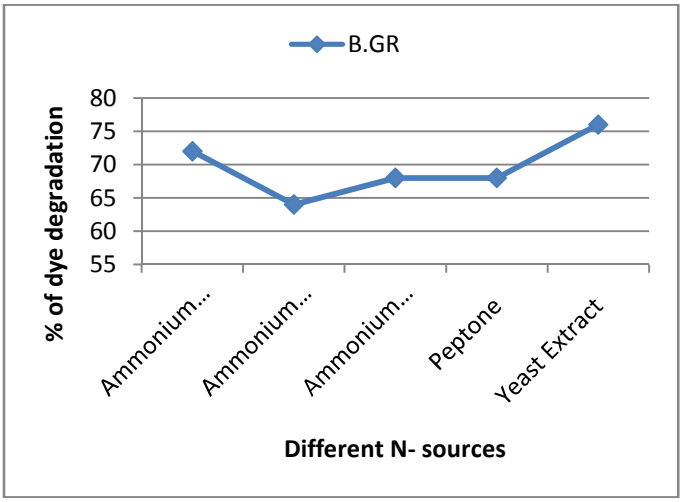

Figure-2
Effect of N- source on % of degradation of Brown GR dye

Effect of pH: The different pH used for the dye decolorization of Brown GR by the four selected fungal isolates. The decolorization observed at pH 4, 7 and 9 is shown in (figure 3). The range of activity on decolorization of Brown GR with pH 4, 7 and 9 were 76%, 74% and 74% respectively with *Aspergillus* sp. and it was found to be the most effective decolorizer at pH 4. Among these three pH used, maximum decolorization of 76% was achieved at pH 4 by *Aspergillus* sp. Wang *et al.*[21] showed maximum decolorization was observed at pH 7.0, with 93.16 and 90.98% color removal in the presence of Ace and Syr, respectively using laccase mediator system. The pH tolerance of decolourizing bacteria is quite important because reactive azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline conditions and at high temperatures[22].

Effect of salinity: The different salinity used for the dye decolorization of Brown GR by the four selected fungal isolates. The decolorization observed in salinity 0.5%, 1.0%, 1.5% and 2.0% are shown in (figure 4). The range of activity on decolorization of Brown GR with salinity 0.5%, 1.0%, 1.5% and 2.0% were 52%, 48%, 52% and 72% respectively with *Aspergillus* sp. *Aspergillus* sp was found to be the most effective decolorizer at salinity 2.0%. Among these four NaCl concentrations were used, maximum decolorization of 72% was achieved at 2.0% NaCl concentration by *Aspergillus* sp. Singh *et al.*[23] found optimum decolorization of dye (56%) was found at 1.0% NaCl concentration, with further decrease 56%, 44% and 42% for 1.0%, 1.5% and 2.0% NaCl concentration respectively. Maximum removal of color was observed at 5th day for all studied NaCl concentrations. Since no significant change in removal of Orange 3R by studied fungus was observed after 5th day.

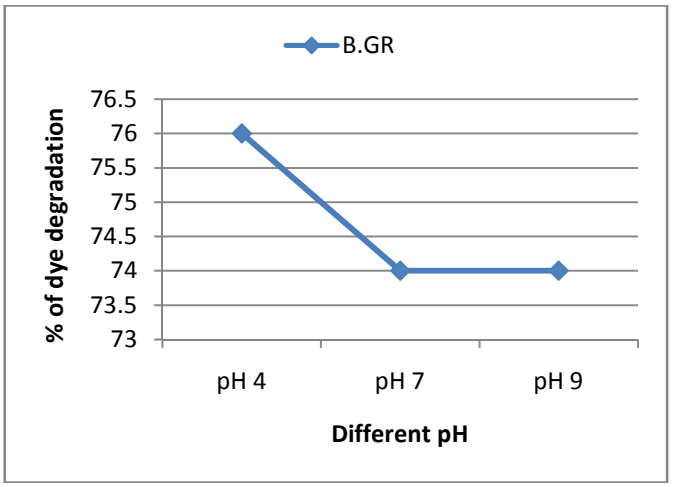

Figure-3
Effect of pH on % of degradation of Brown GR dye

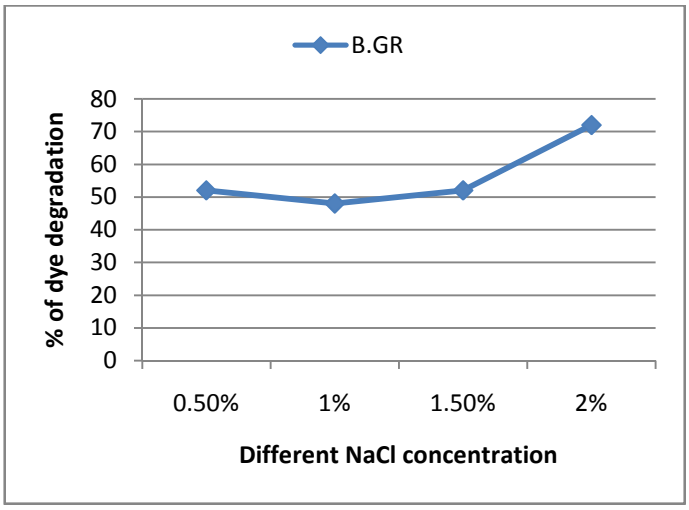

Figure-4
Effect of salinity on % of degradation of Brown GR dye

Effect of temperatures: Different temperatures were used as refrigerator temperature (10°C), room temperature (35°C) and incubator temperature (45°C and 25°C). The maximum decolorization was observed at 25°C (figure 5). The range of decolorization activity of Brown GR with 10°C, 25°C, 35°C and 45°C temperatures were 50%, 76%, 56% and 64% respectively with *Aspergillus* sp. *Aspergillus* species was found to be the most effective decolorizer at 25°C. Overall decolorization efficiency was not temperature dependent but a report showed a

suppressed decolourizing activity at 45°C, this might be due to the loss of cell viability or deactivation of the enzymes responsible for decolourization at higher temperature [24].

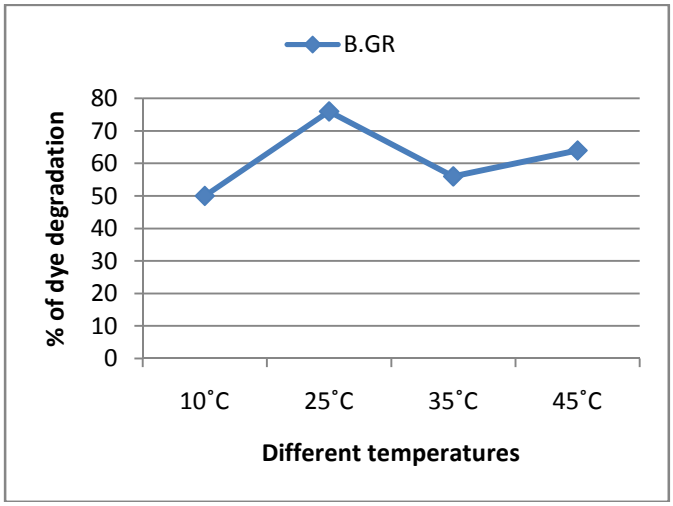

Figure-5
Effect of temperatures on % of degradation of Brown GR dye

The present study is thus an effort to develop a potential fungal isolate as an effective decolorizer of textile dye Brown GR. More research on the decolorization of dye industry effluents and bioremediation of dye contaminated soil using efficient strains of fungal isolates are under progress.

Conclusion

The Brown GR dye is degradable under aerobic conditions with a concerted effort of fungi isolated from environmental samples. Nutrients (carbon and nitrogen sources) and physical parameters (pH, salinity and temperature) had significant effect on dye decolorization. *Aspergillus sp* showed highest decolorization of Brown GR effectively during optimization but predominantly. *Aspergillus sp* showed consistent decolorization of Brown GR dye.

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