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Research Article

DECOLORIZATION OF TRUE BLUE AND CONGO RED USING TWO SOIL BORNE NON-LIGNINOLYTIC FUNGAL SPECIES VIZ. *ASPERGILLUS NIGER* AND *RHIZOPUS NIGRICANS*

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ABSTRACT
Two aerobic fungal isolates viz. *Aspergillus niger* and *Rhizopus nigricans* isolated as pure cultures from the nearby garden soil were utilized for dye decolorization experiments. The maximum decolorization of true blue (20 ppm) and congo red (100 ppm) was shown by these fungi. In PDA media 88% decolorization of true blue and 90% decolorization of congo red was observed by *Aspergillus niger*. However the fungus *Rhizopus nigricans* was not so potent as *Aspergillus* and showed lower percentage of decolorization of true blue (60%) as well as congo red (62.5%). Thus, *Aspergillus niger* is considered to be a utilizable fungus in color degrading experiments and dye wastewater treatments even in large scale.

INTRODUCTION
Wastewater treatment is becoming increasingly important these days. Release of dyes into environment constitutes only a small portion of water pollution. Government legislation is forcing textile industries to treat their waste water effluent. Currently, removal of dyes from effluents is by the physico-chemical means. Such methods are often very costly and though the dyes are removed but accumulation of concentrated sludge creates a disposal problem. There is a need to find alternative methods of treatment that are effective in removing dyes from large volumes of effluents and are low cost such as biological or a combination systems including fungi, bacteria, yeasts, actinomycetes and algae etc.

Textile industries consume large volumes of water and chemicals for wet processing of textiles.

The chemical reagents used are very diverse in chemical composition ranging from inorganic compounds to polymers and organic products (Mishra and Tripathy, 1993; Banat *et al.*, 1996; Juang *et al.,* 1996). Synthetic dyes are used extensively in the biochemical, foodstuff, plastic and textile industries; where it is estimated that 10-14% of the dye is lost in the effluents during the dyeing process. Synthetic dyes share a common feature in that they are not readily biodegradable and when discharged into the environment they are therefore persistent and many of them are also toxic. It is known that a large number of dyes have not been tested for their mutagenic, carcinogenic and toxic potential. The synthetic dye true blue and congo red has seen extensive use in textile printing industries. Wastewater treatment facilities are however unable to completely remove commercial dyestuff from contaminated wastewater, thus contributing to pollution of aquatic habitats. This class of chemicals viz. synthetic dyes is reported to be responsible for the promotion of tumor growth animals. The conventional wastewater treatment systems are unable to remove recalcitrant dyes from the effluents. Some synthetic dyes have been found in soil due to improper waste disposal. In order to minimize the possible damage to humans and environment arising from the production and application of colorants; an International Association "Ecological and Toxicological association of Dyestuff Manufacturing Industry" (ETAD) was established in 1974. Biological processes are getting more attention as they are cost effective, environment friendly and do not produce large amount of sludge. The present study was carried out to study the extent of decolorization performed by 2 fungal species viz. *Aspergillus niger* and *Rhizopus nigricans* utilising True blue and Congo red dyes. This study provides the scope for the potent fungus to be used in dye wastewater treatment systems.

Research Article

Review of Literature

Dye is a substance (generally an organic compound), which is used for imparting permanent color to textiles - silk, wool and other substances. Natural dyes occur in nature e.g. Indigo (a blue dye), alizarin (a red dye). Synthetic dyes are man-made dyes e.g. malachite green (a bluish green dye), azo dye, aniline yellow, orange 1 etc. A colored substance can act as a dye only if it can be fixed to the material being dyed. At the same time it should be resistant to the action of light, water and soap. There are two important conditions for a colored compound to act as a dye.

(a) Presence of chromophore

These are the groups, which are responsible for producing a color to a dye because they are capable of absorbing light in the ultra violet region. Some important chromophores are: - N = O,-N = N-, - C = N, (CH = CH). The compounds bearing chomophores are known aschromogens.

(b) Presence of auxochromes

Dye should be attached to the fibers by means of stable chemical bonds. Some groups form these chemical bonds, which may either be acidic or basic in nature. Such groups are known as auxochromes, some are; - OH. – COOH, - SOH (acidic), NH, NHR, NR (basic). A chromogen without auxochrome can never act as a dye.

About 10,000 dyes and pigments are produced annually worldwide amounting to 7x105 tones which are hazardous and pose serious environmental problems. It is estimated that 10-15% of the dye is lost in the effluent during the dying process.

The recent high profile of color pollution is mainly the result of increasing public awareness and expectations of the environment; coinciding with rising levels of color discharges. One of the more pressing environmental problems that have been facing the textile industry is the removal of the color from dye bath effluent prior to discharge to local sewerage treatment facilities or adjoining watercourses. Considerable efforts have been made on developing suitable treatment systems for these effluents. Only biotechnological solutions can offer complete destruction of the dyestuff with a co-reduction in the biological oxygen demand (BOD) and chemical oxygen demand (COD) (Wilmott *et al*., 1998).

Moreover the synthetic dyes induce a high frequency of chromosomal breakage in number of cell types, indicating that their defects are notlimite4d top one cell line. Under anaerobic conditions bacteria reduce azo leading to the formation of acryl amine derivatives (Chung *et al.,* 1992), which may be methane, dyes have found, have been toxic to experimental animals and cell cultures.

The content ratios of the nucleic acids (RNA/DNA); decreased with the increasing dye concentration. These dyes act more preferentially to lower protein synthesis that inhibits cell division. Due to the inhibitive action; cell shape varied, cells growing under ordinary conditions appeared as small rods and those in the presence of dyes, as filaments. Ogawa *et al.,* 1989 noted that dye inhibits DNA synthesis by stabilizing the double helix and by inhibiting the enzyme activities.

The toxic effects of dyes may cause bone- marrow depression, epileptic anemia and hemorrhagic diseases of the various organs and leukemia in few cases. The greatest tragedy of dyestuff industry has been the occurrence of occupation tumors of urinary bladder (Pappiloma). They are usually malignant. There is a long latent period (20 to 25 years) between the first exposure and ultimate development of tumor

Treatment of dye wastewaters

This is done by 2 basic procedures:

1. Decolorization. Adsorption changing the chromophoric group to the non- chromophoric group
2. Degradation breakdown of the substrate molecule (dye) the biological process.

Presently most of the processes used for the treatment of dye wastewaters are chemical processes, physical or physico-chemical processes which aer generally expensive and of limited applicability. All these methods possess significant differences in color removal, volume, capability, operating speeds

and capital costs. Nowadays Biological treatment methods are getting more attention since it is cheap and offer best alternative with proper analysis and environmental control. Almost all wastewaters can be treated by the use of a number of naturally occurring microorganisms such as bacteria and fungi.

Various effluent treatment methods from dyestuff industries, classified in to three main categories involve three basic stages of treatment namely primary, secondary and tertiary. Primary treatment processes of dye wastewater include equalization neutralization and possible disinfections. Primary stages are mainly physical and include screening, sedimentation, floatation and flocculation. The objective is removal of debris, undissolved chemicals and particulate matter. In the Secondary stage the organic load is reduced. Tertiary method involves adsorption, ion exchange chemical exudation, reverse osmosis etc .

Biological Decolorization by Bacteria

Many organisms are reported to decolorize various triphenlymethane and azo dyes. There are a few reports on the biodegradation of theses dyes by bacteria. It was reported the biodegradation of synthetic dyes by *Pseudomonas pseudomallei13NA*. In general the decolorization of the dyes is not related to their molecular weights and the octanol- water coefficients of the dyes. Yatome *et al.,* (1993) again reported the degradation of Crystal violet, Pararosaniline and Victoria growing cells of *B.subtilis*.Biodegradation of synthetic dyes by bacteria, fungi and yeasts (Azmi *et al*., 1998). They showed the advantages of using biological processes for degradation of dye molecules to carbon dioxide and water and with concomitant formation of less sludge and being eco-friendly.

Biological Decolorization by fungi White – rot fungi are those organisms that are able to degrade lignin, the structura polymer found in woody plants (Barr and Aust, 1994). The most widely studied white-rot fungus is *Phanerochaete chrysosporium*. This fungus is capable of degrading dioxins, polychlorinated biphenyls (PCBs) and other chloro-organics (Chao and Lee, 1994; Reddy, 1995). Davis *et al*., (1994) showed the potential of using *P. sordida* to treat creosote-contaminated soil. It has been has shown that *P. chrysosporium* had the abilityto decolorize artificial textile effluent by 99 % within 7 days. White rot fungi are able to degrade dyes using enzymes, such as lignin peroxidases (LiP), manganese dependent peroxidases (MnP). Other enzymes used for this purpose include H2O2 producing enzymes such as glucose-1-oxidase and glucose-2-oxidase, along with laccase, and a phenol oxidase enzyme (Archibald and Roy, 1992; Thurston, 1994; Schliephake and Lonergan, 1996). These are the same enzymes used for the lignin degradation. Other fungi such as *Hirschioporus larincinus, Inonotus hispidus, Phlebia tremallosa and Cariolus versicolor* have also been shown to decolorize dyecontaining effluent (Banat *et al*., 1996). The ability of white – rot fungi to degrade a diverse array of xenobiotic compounds (Field *et al.,* 1993) is often attributed for use in wide range of dye waste treatments. Das *et al*., (1995) studied the crystal violet decolorization using *P. chrysosporium* in a column bioreactor.

In the present study utilization of soil borne non-ligninolytic fungi is carried out due to its easy availability and ubiquitous nature viz. *Aspergillus niger* and *Rhizopus nigricans*. These have been cultured as pure cultures from the consortium obtained by nearby garden soil. This study proves that non-ligninolytic soil fungi can also be used for degradation processes and are cheap sources, easily available and cost effective saving much time and energy.

MATERIALS AND METHODS

Growth and Maintenance of Fungal Species

Two fungal species *Aspergillus niger* and *Rhizopus nigricans* pure cultures already isolated from the soil were maintained on PDA plates containing dye solution in ppm. The media containing true blue as well as congo red dyes separately were autoclaved at 121^0C for 15 min. Two different dyes used were true blue and congo red, which were procured from Hi-Media were selected for decolorization by fungi. During the preparation of 60 ml PDA +true blue media, 0.02 gm of true blue dye powder was

added in 60 ml distilled water; and also for preparing 60ml PDA+congo red separately, 0.02 gm of congo red dye powder was added in 60 ml distilled water and solubilized, followed by boiling and autoclaving the media (PDA+true blue and PDA+ congo red). Pouring of these media (PDA + Dye) was carried out in petriplates, with solidification. Thus we attain 6 petriplates of PDA+ dye as:

a. 3 of PDA+true blue
b. 3 of PDA+congo red

The single plate of each dye was kept as control. The PDA+true blue 1 petriplate and PDA+congo red 1 petriplate was streaked by inoculation loop by touching pure culture of *Aspergillus niger*. Similarly the PDA+true blue 2 petriplate and PDA+congo red 2 petriplate was streaked by inoculation loop by touching pure culture of *Rhizopus nigricans*. Thus, we have now:

a. PDA+true blue+streaked *Aspergillus niger*; PDA+true blue+streaked *Rhizopus nigricans*; PDA+true blue as control
b. PDA+congo red+streaked *Aspergillus niger*; PDA+congo red+streaked *Rhizopus nigricans*; PDA+congo red as control

The streaked plates as well as controls were incubated at 30^0 C for 10 days. Isolated pure cultures of 2 fungal species were obtained on PDA media containing the 2 types of dyes separately. It was observed that on the 10^{th} day the 2 plates viz. PDA + congo red streaked with *Aspergillus niger* and *Rhizopus nigricans* each attained pure growth covering the entire medium. This growth of hyphae and sporulation was accompanied by decolorization of the congo red dye which was due to biosorption by fungal hyphae. The degradation occurred after biosorption of dye particles and the dye was broken down into simple molecules. This visual decolorization was noted by measuring optical density via colorimeter readings in nm. Similarly, on the 10^{th} day the 2 plates viz. PDA + true blue streaked with *Aspergillus niger* and *Rhizopus nigricans* each attained pure growth covering the entire medium with mycelia matte and spores scattered all over the medium. Here also the colorimeter readings were taken into account in nm.

The present investigation, the fungal isolates viz. *Aspergillus niger* and *Rhizopus nigricans* showed decolorization of dyes upto certain extent. A comparative account was attained by visualization of the 2 fungal isolates microscopically, as well as the dye decolorizing ability. Microscopic examination of fungi showed the biosorption and degradation of dye in fungal mycelium. This degradation occurs by extracellular enzymes secreted by fungi. The dye decolorizing ability was examined by visually noticing the color of media at the lower side of petriplate. The fungal strain found to be the best in dye decolorization was considered as a potent fungus which can be utilised for further research studies.

Measurement of Optical Density by Colorimeter

Each fungal culture mycelium present on the media viz. *Aspergillus niger* and *Rhizopus nigricans* was removed by scalpel. This solid medium was washed with sterilised water and cleaned on the surface by cotton.

The solidified samples used were:

a. PDA+ true blue control
b. PDA + congo red control
c. PDA+ decolorized true blue by *Aspergillus niger*
d. PDA+ decolorized congo red by *Aspergillus niger*
e. PDA+ decolorized true blue by *Rhizopus nigricans*
f. PDA+ decolorized congo red by *Rhizopus nigricans*

For cutting with sterilised knife into small square pieces. These were put in conical flask and heated on hot plate at 100^0C. Each liquified colored medium containing fungus was put in a cuvette and inserted in colorimeter to record the Optical density.

Calculation of Percent Decolorization

Percent decolorization = $\frac{\text{OD initial- OD final x100}}{\text{OD initial}}$

Observations

The following observation table showed that comparatively *Aspergillus niger* was more potent decolorizer than *Rhizopus nigricans*. The OD values attained by colorimeter as OD initial (of control dye color) and OD final (after 10 days decolorization by fungus) were recorded, and the calculation of percent decolorization indicated high potency of aspergillus niger.

Table 1: Percent decolorization of the fungal species obtained from OD values on the 10th day in PDA containing the congo red dye

Name of the fungus	OD value at control (ODi)	Wavelength at ODi	OD value on 10th day (ODf)	Wavelength at ODf	Percent decolorization
Aspergillus niger	0.04	620 nm	0.004	620 nm	90
Rhizopus nigricans	0.04	620 nm	0.015	620 nm	62.5

Table 2: Percent decolorization of the fungal species obtained from OD values on the 10th day in PDA containing the true blue dye

Name of the fungus	OD value at control (ODi)	Wavelength at ODi	OD value on 10th day (ODf)	Wavelength at ODf	Percent decolorization
Aspergillus niger	0.05	585 nm	0.006	585 nm	88
Rhizopus nigricans	0.05	585 nm	0.02	585 nm	60

RESULTS AND DISCUSSION

According to the tables 1 and 2 the optical density of dyes viz. congo red and true blue decolorized by aspergillus niger was less than the decolorized dyes due to rhizopus nigricans. Due to this the calculation of percent decolorzation attained for aspergillus niger was more than *Rhizopus nigricans*. Thus we could resultantly derive that *Aspergillus niger* is a more potent fungus in comparison to *Rhizopus nigricans*. This decolorization efficiency being more can be utilized in large scale wastewater treatment plants and pilot plant projects for treating dye wastewaters. This fungus has a healthy scope in leading water purification industries which are based on biological mode of decolorization which is more ecofriendly and cheap. Although other processes are involved in projects related to dye wastewater treatment but *Aspergillus niger* can give its wonderful contribution, as being an easily available fungus which requires no complicated methods for culture also.

The decolorizing potency of aspergillus niger being high is needed for wastewater treatments and thus this culture can be saved and conserved for future in vitro. The fungal system can be immobilized using alginate beads (a polymer) in the bioreactors in vitro as well as in large scale. So whenever required, the preserved fungus can be utilized in screening of dyes and textile wastewaters.

Conclusion

According to the present study, following conclusions were drawn:

Two aerobic fungal isolates *Aspergillus niger* and *Rhizopus nigricans* isolated showed decolorization of congo red and true blue dyes utilsedin the textile industry.
Aspergillus niger was the most remarkable and potent decolorizer than *Rhizopus nigricans*.
The decolorization by fungi was due to biosorption of dye particles and their degradation in the fungal cell wall into simpler compounds.
Decolorization process is irreversible and the dye cannot be again attained after biodegradation.
Thus, the present investigation has wonderful impact of soil borne fungi to be used in textile dyw wastewater decolorizing industries even in large scale and is a boon for the water recycling and water purification processes.

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