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Research Article

BIODEGRADATION OF TEXTILE DYE CONGO RED BY FUNGUS *MUCOR MUCEDO*

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ABSTRACT
The decolorization of textile dye wastewater is a major aspect of research, to solve the problem of environmental pollution. In the present investigation, textile dye congo red is degraded by fungal spp. *Mucor mucedo*. Modified PDA containing dye was inoculated by the fungus and decolorization was attained after 8 days. Optimisation of this modified PDA was performed by the addition of sucrose and peptone. This caused earlier decolorization of congo red viz. 3 days. Thus this research work shows that *Mucor mucedo* is a potential dye degrader and its rate of growth can be increased by optimisation. The biosorption capacity of this fungus is enhanced by better growth rate and hence early decolorization is visualised.

Key Words: *Mucor mucedo, Biodegradation, Congo red , Decolourization, Textile dyes, Biosorption, Optimisation*

INTRODUCTION
Dyes are in regular usage in the textile printing industries. This is the major cause of environmental pollution, because large amount of dye wastewater is discharged from the printing units. It is estimated that about 10-15% dyes are released into processing water during this procedure (Selvam, et al., 2003). There are different class of organic compounds characterized by the presence of unsaturated groups (chromophores) such as ─C═C─, ─N═N─ and ─C≡N─, which are responsible for the dye colours, and of functional groups responsible for their fixation to fibres, for example, ─NH2, ─OH, ─COOH and ─SO_3H (Molinari, et al., 2004). Dyes may also significantly affect photosynthetic activity in aquatic life by reducing light penetration intensity and may also be toxic to some aquatic fauna and flora due to the presence of aromatics,metals, chlorides, etc. (Dhaneshvar et al., 2007). Physico-chemical methods of dye wastewater treatment are expensive and lead to the formation of large amount of secondary wastes as sludge. Several combined anaerobic and aerobic biological treatments utilising bacteria are in progress to enhance the biodegradation of textile dyes . However, bacterial degradation have some limitations and in recent years, there has been an alternative research on fungal decolourization of dyes present in wastewaters, and it is turning into a promising alternative to replace or supplement for present treatment processes (Ramya et al., 2007). The ability of white rot fungi to degrade synthetic chemicals, such as dyes, is well known, and a white rot fungus,*Phanerocheate chrysosporium* has been reported to decolourize dyes with enzymes involved in lignin degradation, such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Swamy et al., 1999; Robinson et al., 2001). The fungus *Trichoderma harzianum* has also been reported earlier for the degradation of textile dyes (Singh and Singh, 2010). In the present work, we have investigated fungal degradation/decolourization of textile dye, Congo red, using a fungus, *Mucor mucedo*.

MATERIALS AND METHODS
The fungus *Mucor mucedo* was used for the degradation of Congo red, which is extensively used in textile industry.

Preparation of modified PDA containing congo red

Congo red was selected for decolorization by *Mucor mucedo*. During the preparation of 1 liter PDA media, 0.26 gm of dye powder was added in water and solubilized, followed by addition of PDA powder and autoclaving.

Pouring the modified PDA plate

Modified PDA plates were prepared by pouring of media in laminar air flow hood under sterilized conditions. Pouring was done immediately into a sterile, dry petri plate while holding the top carefully above the petri plate bottom in order to avoid contamination. This was done utilizing aseptic technique in a sterile cabinet (laminar air flow cabinet).The top was replaced, allowing the agar to cool and harden, and storing petri plates in laminar air flow. Solidification of media was attained after 30 minutes. Stacking of the plates right side up was done. A petriplate of modified PDA viz. PDA+congo red was prepared to assess decolorization of dye after inoculation of fungus. A control plate containing modified PDA was also prepared to compare with decolorised media plate.

Optimization of modified PDA

Optimisation was carried out by adding 2 gm each of sucrose and peptone powder alongwith 0.26 gm of dye powder in sterilised distilled water and solubilized, followed by addition of PDA powder and autoclaving. A plate of optimised PDA+dye+sucrose+peptone was prepared for inoculation, alongwith a single control.

Inoculation of the fungus

Inoculation of stored pure culture of *Mucor mucedo* was carried out in the modified (PDA+dye) petriplate and optimised (PDA+dye+sucrose+peptone) plate. This was carried out by by radiant streak plate method, using sterilised techniques. The 2 controls viz. modified PDA plate and optimised PDA+dye+sucrose+peptone) were un-inoculated by fungus.

Monitoring for Decolourization

Decolourization of dyes from the *Mucor mucedo* treated petri plates were assessed by the change in original colour viz. red (as compared to control) and by the visual disappearance of colour from the Petri plates. The change in the red colour was observed and the number of days were recorded. The culture plates containing congo red as well as optimised plates were examined for the visual disappearance of colour from the media of the petri plates, when compared to the respective control plates. The decolorization was observed earlier (3 days) in case of optimised PDA containing dye, sucrose and peptone, as compared to that in modified PDA containing only dye (8 days) (Table 1).

RESULTS AND DISCUSSION

In the present study, results for dyes degradation/decolourization by *Mucor mucedo* were positive, and the accumulation of dyes by the fungus also took place. The disappearance of colour and change in original colour in the fungus-treated medium was observed. The evaluation of degradation/decolourization was assessed as the disappearance of colour from the petri plate, during the growth of the fungal mycelium. For the dye congo red, the applied fungus has shown positive result for biodegradation and the red colour of this dye was turned into yellow to pale and finally, a zone of yellow colour was present around the mycelium. A small fraction of dye was also accumulated by the applied fungus, and its mycelium turned into red colour.

DISCUSSION

The present study was carried out to examine the fungal degradation of a single hazardous dye in solid medium PDA, taking single fungus, *Mucor mucedo*, as the experimental organism and textile dye, Congo red as the testing dye. The applied fungus has shown positive results for dye degradation/decolourization, as was indicated by the change and disappearance of colour of the dye from

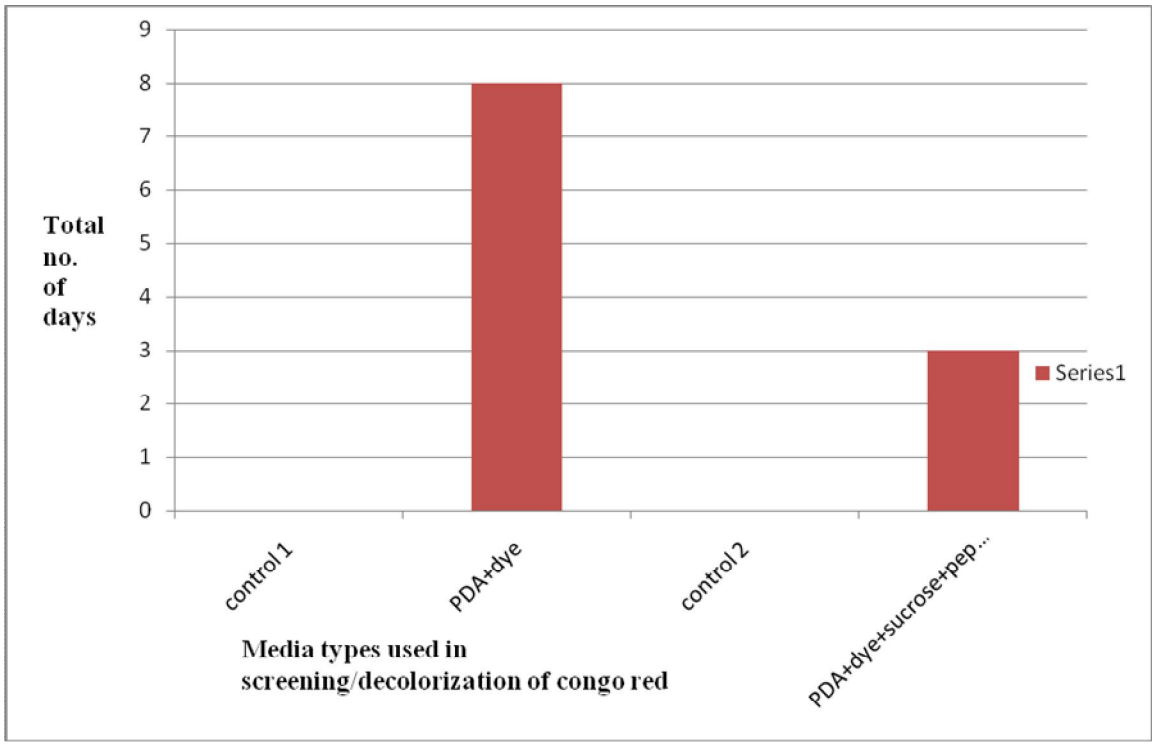

Figure 1: Decolorization period of congo red by *Mucor mucedo* in modified PDA(with dye) and optimised PDA (dye+sucrose+peptone)

Table 1: Visual decolorization of congo red by *Mucor mucedo* in modified PDA and optimised PDA containing sucrose and peptone

S. No.	Time taken for decolorization in modified PDA(containing dye)	Time taken for decolorization in optimised PDA(containing dye+sucrose+peptone)
1.	8 days (192 hours)	3 days (72 hours)

modified and optimised media in petri plates. A zone of different colour (yellow to pale) around the fungal colony was also observed which might be due to the production of extracellular enzymes by the applied fungus, during the biodegradation of tested dyes. The earlier decolorization of congo red in optimised PDA with dye, sucrose and peptone (3 days) may be attributed to the increased nutrient content in the medium for fungal growth. The additional carbon source (sucrose) and nitrogen source (peptone) stimulated increase in growth rate of *Mucor mucedo*. This increased growth rate is attributed to enhanced biosorption capacity and hence earlier decolorization by the fungus. Therefore the modified PDA with congo red took more time for dye decolorization (8 days). (Fig 1). The controls had original dye color and no fungal inoculation showed nil decolorization.

Microbial degradation of Congo red by *Gliocladium virens* (Singh, 2008a), various hazardous dyes likes, Congo red, Acid red, Basic blue and Bromophenol blue, Direct green by the fungus *Trichoderma harzianum* (Singh and Singh, 2010) and biodegradation of plant wastes materials (Singh, 2008b) by using different fungal strains has been investigated earlier.

Cripps et al. (1990) also reported the biodegradation of three azo dyes (Congo red, Orange II and Tropaeolin O) by the fungus *Phanerocheate chrysosporium*. In the present study dyes might be degraded by the production of extracellular enzymes as well as adsorption of dyes by the mycelium of *Mucor mucedo* during its growth in the dye-containing medium (both modified and optimised).

Adsorption of dyes to the microbial cell surface is the primary mechanism of decolourization (Knapp et al., 1995). In our study, the adsorption of congo red by the fungal mycelium was observed, as it was confirmed by the change in the colour of fungal mycelium in tested dye. Adsorption of dye increased by the provision of additional carbon and nitrogen sources, which were the cause of enhancing mycelial growth.

Decolourization of dye is related to the process of extracellular oxidases, particularly manganese peroxidases (Gold *et al.*, 1988). Liginin peroxidise (Lip), manganese dependant peroxidase (MnP) and laccase, all of which are involved in lignin degradation, have been reported to decolourize dyes (Vyas and Molitores, 1995). In the present study, the degradation and decolourization of congo red by *Mucor mucedo* appeared to be due to the production of extracellular enzymes by this fungus in the dye-containing medium. It is quite clear that the change in colour might be due to the biochemical (metabolic) reactions of fungal species.

Mucor mucedo can be considered as responsible for biodegradation/decolourization of textile dyes, and is also responsible for change in dye colour from reddish to a light colour ring around the fungal mycelium. Optimising the medium during screening or decolorization enhances the process.

Further investigations on isolation and purification of enzyme(s), involved in the biodegradation of hazardous dyes, are in progress in the research laboratory of the authors at Mahatma Jyoti Rao Phoole University, Jaipur.

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