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DECOLOURIZATION OF RHODAMINE CONTAINING PAPER MILL WASTE BY FUNGAL CULTURES ISOLATED FROM SOIL

†S.C. KALE* AND A.J. GAVHANE**

**Department of Microbiology & Biotechnology, Faculty of Allied Sciences, Krishna Institute of Medical Sciences "Deemed to be University", (Malkapur) Karad 415 110, (MS), India*

*** Presently working with Department of Biotechnology, Ahmednagar College, Ahmednagar, Dist- Ahmednagar, (MS), India*

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ABSTRACT

In search for the fungi capable of decolourizing Rhodamine containing Paper Mill waste, soil samples from the vicinity and nearby places of ETP plant of Paper Mill were collected and subjected for isolation of fungi by using Sabouraud Glucose Agar plates. In all 9 isolates were obtained. All nine fungal isolates were subjected to primary screening for their decolourizing activity for 'Rhodamine dye containing Paper Mill waste' by spot inoculation method using modified Sabouraud Glucose Agar plates (supplemented with paper mill waste). The four fungal isolates, F - II, F - III, F - IV and D - 1, which showed growth and decolourization were selected and subjected to secondary screening using liquid culture method. Secondary screening revealed that the fungal isolate F – II and D-1were the best strains amongst the four cultures as both showed maximum decolourization at 37 ^{0}C. They showed almost 40% decolourization of Rhodamine containing Paper Mill waste. The F - II and D-1 isolates were further studied for their morphological and cultural characteristics and were identified as a strain of *Aspergillus niger and Rhizopus oryzae*. The identity of the culture was confirmed by referring to the Fungus Identification Service Center.

KEY WORDS : Decolourization, Rhodamine, Paper mill waste, Fungus, *Aspergillus niger, Rhizopus oryzae*

INTRODUCTION

The pulp and paper industry is the largest industrial user of water (Hammer, 1987). A large proportion of this water is discharged, in the form of wastewater into rivers, lakes and oceans, promoting the rise of water pollution. A pulp and paper mill waste characteristically contains very high COD and colour. In India 34 large scale paper mills account for 51% of total capacity and 271 small paper mills account for the remaining 49%. The presence of lignin in the waste is not easily biodegradable, it makes the COD: BOD ratio of waste very high (Rao and Datta, 1979). Colour of process water from paper mill arises from printing ink pigments and dyes used for tinting and shading paper (Patric and Bruno, 2012). Colour removal of effluent from pulp wastes by certain *Aspergillus* sp. has already been demonstrated (Datta *et al.,* 1985; Gupta and Goel, 2004). Fungus species *Phanerochaete chrysosporium*, is being investigated extensively for their potential to remove the colour (Keharia and Madam, 2003). Many other fungi like certain *Rhizopus* spp, *Coriolopsis* spp have been found to have dye or colour decolourizing abilities (Holkar *et al.,* 2016; Chen & Ting, 2015; Nagarathamm and Bajpai, 1999).

Fungi are being investigated for their potential to decolourize coloured effluents, other than *Phanerochaete chrysosporium* (Livernoche *et al.,* 1981; Livernoche *et al.,* 1983) they includes *Trametes versicolor* (Bergebauer *et al.,* 1991), *Tinctosporia* sp.

(Thomas *et al.,* 1981; Royer, 1985; Fukuzumi, 1980), *Schizophyllum commune* (Belsare and Prasad, 1988). It has been found that extent of the decolourization depends on the fungal strain and the origin of effluents (Bergebauer *et al.,* 1991) .

Despite of the lignolytic capacity of the fungi for colour removal, large number of heterogeneous lignin derivatives of the bleachery effluents resists fungal growth. Therefore, systematic screening of larger number of species capable of degrading the effluent lignin is warranted (Bergebauer *et al.,* 1992). Hence, the present study included screening of the fungi which are able to decolourize paper and pulp mill effluent containing commonly used synthetic dyes.

MATERIALS AND METHODS

Collection of effluent from paper mill

The effluent was collected from Indo Afrique Private Ltd. Paper Mill located at Sarola, Tal. Bhor, Dist. Pune. A sterile plastic carboy of 5L capacity was used for collection of paper mill effluent. The sample was brought into the laboratory and stored at 4 °C till study over. The synthetic dye sample of rhodamine also collected from paper mill as commonly in use to prepare coloured papers which is not easily biodegradable. During transportation the carboy was protected from direct sunlight by putting the carboy in bag (Greenberg *et al.,* 1998).

Collection of samples for isolation of Fungi

The soil samples were collected in sterile polythene bags from the nearby area of sludge tank and effluent treatment plant of paper mill and were brought to the laboratory. Both deteriorated paint sample from walls of building and decaying wood sample was collected from the campus of Yashwantrao Chavan College of Science, Karad.

Physiochemical characterization of paper mill effluent

Physiochemical characterization of the paper mill effluent was done for colour, odour, pH, total suspended solids, chemical oxygen demand (COD) by Dichromate reflux method and biological oxygen demand (BOD) Winkler's Alkali-Azide method by the using standard methods (Greenberg *et al.,* 1998).

Isolation of fungi from soil samples

For isolation of fungi, enrichment culture technique followed by spread plate technique was used. Two soils samples, deteriorated paints and decaying wood sample were subjected to serial dilution. Each dilution was then inoculated on modified Sabouraud Glucose Agar (instead of distilled water paper mill waste was used) by Spread plate method (Atlas, 1973; Cappuccino and Sherman, 2005).

All these plates were incubated at room temperature up to seven days. After incubation period was over isolates were subjected to purification. The purified isolates were partially characterized and preserved at 4 °C for further studies. All these isolates were assigned by code numbers.

Characterization of fungal isolates

Morphology of molds was studied by wet mount preparation using lactophenol cotton blue, as the mounting medium (Cappuccino and Sherman, 2005).

Primary screening of fungal isolates for decolourization of paper mill effluent by Agar plate method

Primary screening of all nine fungal isolates for decolourization activity for rhodamine in paper mill waste was done by Agar plate technique. Each of the nine fungal isolates were spot inoculated separately on separate plate of the agar medium (pH 5.4), prepared by using paper mill effluent containing peptone and glucose at the concentration of 1% and 4% respectively with different concentrations of rhodamine (0.1%, 0.01%, 0.001%). One plate of each concentration of dye was kept uninoculated as control. All these plates were incubated at room temperature for five days. After incubation, growth of each isolate was examined for decolourization activity using control for comparison. The fungal isolates showing significant decolourizing activity were selected for the secondary screening.

Secondary screening of fungal isolates for decolourization (Broth dilution method)

Total four fungal isolates were used to inoculate as F-II, F-III, F-IV and D-1 during secondary screening for decolourization ability at 0.01% rhodamine only. The rhodamine dye solution of 0.01 % concentration was taken in a flask and was supplemented with nutrients as; 1% peptone and 4 % glucose and then pH of medium was adjusted to 5.4. This medium was dispensed into the tubes, 10 mL quantity in each tube and then subjected to sterilization. Each of

the fungal isolates was inoculated separately as 10mm diameter disc of agar containing mycelial growth in a separate tube. Control was kept as was kept uninoculated coloured medium and then both the tubes were kept for incubation at room temperature for five days.

After incubation, percent decolourization was determined by using decolourization assay method (Gupta and Goyal, 2004) .

Decolourization study of rhodamine synthetic dye by using F-II and D-1 fungal isolates:

Each of the fungal isolates was inoculated separately as 10 mm diameter disc of agar containing mycelia growth in a separate flask containing rhodamine solution of 0.01 % was supplemented with nutrients; 1% peptone and 4 % glucose and then pH of medium was adjusted to 5.4. Both the flasks with control flask were kept at room temperature for five days of incubation.

During incubation, every day observation for decolourization was carried out and percent decolourization was determined.

Identification of promising fungal isolates

The identification of the promising fungal isolates to the species level was done by studying colonial characteristics, sporulation pattern, spore nature and microscopic observation of the wet mounts (Gilman, 1957; Gupta and Goyal, 2004). Then, identity of the promising culture was confirmed by referring to the Fungus Identification Service Center (National Fungal Culture Collection of India (NFCCI)), Agarkar Research Institute, Pune.

RESULTS AND DISCUSSION

Physicochemical characteristics of paper mill effluent

The results for the characterization of the paper mill effluent for some important parameters are as shown in Table 1

Table 1. Physicochemical characteristics of paper mill effluent

Sr. No.	Parameters	Average Values
1	pH	7.3
2	Odour	obnoxious
3	Colour	White
4	Total Suspended solids	1022.8 mg/L
5	COD	548 mg/L
6	BOD, 5 days at 20^{0}C	80.0 mg/L

Isolation of microorganisms from various sources:

Total nine fungal isolates were obtained from various sources. All these isolates were labeled as A-1, A-4, B-1, B-2 and D-1 (Deteriorated paint) F-I, F-II (from decaying wood), F-III and F-IV (Soil).

Primary screening of fungal isolates:

All the fungal isolates showed visible growth after 48 hrs on rhodamine dye containing modified Sabouraud Glucose Agar (0.001 % and 0.01%) and no growth on 0.1% rhodamine containing media. It is interesting to observe that isolates F-II, F-III, F-IV and D-1 showed maximum growth on 0.01% dye containing modified Sabouraud Glucose Agar exhibited decolourizing activity also after five days incubation. The isolates which showed decolourization only at 0.01% concentration hence same concentration of dye was selected for further studies.

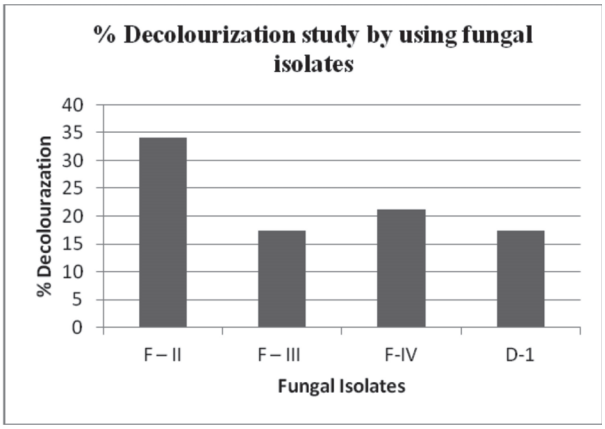

Fig. 1. Secondary screening of Fungal isolates for rhodamine decolourization study

Secondary screening of fungal isolates

Four fungal isolates F-II, F-III, F-IV and D-1 obtained from primary screening for decolourization of rhodamine were subjected to secondary screening showed growth and decolourization after five days incubation at 37 ^{o}C. The percent decolourization that was obtained is shown in Figure-1. It can be seen that F-II, isolate showed maximum decolourization at 37 ^{o}C than other fungal isolates.

Decolourization study of rhodamine synthetic dye by using F-II and D-1 fungal isolates at different incubation time intervals:

Isolate F-II and D-1 were studied further for their

activities to remove rhodamine by 24 hrs interval of incubation period by observing the growth and measuring percent decolourization for upto five days. The results are as shown in Figure 2. Decolourization obtained in case of F-II isolate was as 55.92% while it was 58.99% in case of D-1 isolate after five days at room temperature.

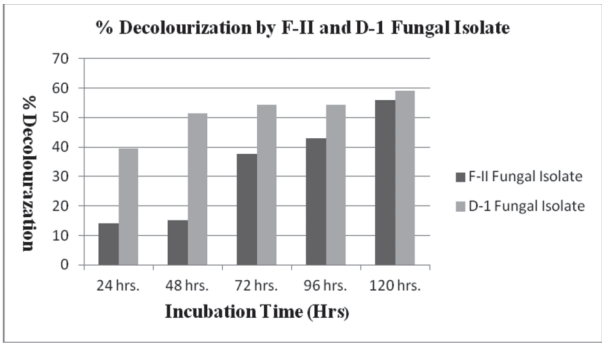

Fig. 2. Rhodamine % decolourization study by F-II and D-1 Fungal isolates

CONCLUSION

Fungal isolates D-1and F-II, seems to be promising strain for decolourization of the rhodamine dye containing paper mill effluents as showed about 58.99% and 55.92 % decolourization respectively within five days at room temperature. In case of D-1, it was found that dye was getting adsorbed to the mycelium and physical adsorption seems to be the principal mechanism of colour removal. Hence, this fungal isolate D-1 was further investigated for its identification and identified as *Rhizopus oryzae*. Fungal isolate F-II is also tentatively identified as *Aspergillus niger*. There is need to provide more attention for its decolourization mechanism study in detail.

Further, there is need to optimize the cultural conditions for decolourization study for each promising isolate and need to study mechanism involved during decolourization and byproducts formed to be evaluated for their toxicity.

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