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Research Paper

Isolation and optimization of culture conditions for decolorization of textile waste effluent using screened fungi

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Abstract

Isolation and identification of dye decolorizing fungal isolate from textile dye effluent was carried out. The isolates of *Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Aspergillus oryzae,* and *Penicillium sp. Penicillium notatum* were isolated from the textile effluent samples collected from Sanagner area, Jaipur. Different parameters were used for optimizing. Conditions for maximum decolorization effect by the fungal isolates. The optimization conditions used to decolorize textile effluent were different pH, temperature, aeration carbon, and nitrogen. Among the different pH used, *A. niger* and *A. oryzae* showed maximum dye decolorization (79.09±0.29% and 80±0.31%) with pH 5. Among the different temperatures used. *A. niger* and *A. oryzae* showed maximum dye decolorization (87±0.34%, 85±0.24%) with 30°C temperature. Among the carbon sources, *A. niger* and *A. oryzae* showed maximum dye decolorization (83.9±0.33% and 85.09±0.32%) with glucose as a carbon source. Among the nitrogen sources used, *A. niger* and *A. oryzae* showed maximum dye decolorization (83.9±0.25% and 85.09±0.31%) with Ammonium Sulphate as a nitrogen source. It is clearly evident from the results that fungal isolates were able to decolorizing the textile dye in effluent efficiently. Dye decolorization with microorganisms is low cost effective and environmentally friendly and the only way for ultimate controlling of pollution generated by textile and dyestuff industries. However, more and more research and development works are needed to develop a viable alternative process for the treatment of textile effluent.

Keywords: optimization, decolorization, textile effluent, decolorizing fungi

Introduction

Dyes are intensely colored organic compounds which have widespread application. Amongst various applications of synthetic dyes about 30,000 tons of different dye stuffs are used per year for textile dyeing operations, thus dye houses are the major consumers of synthetic dyes and consequently are the main cause of water pollution and imposes severe damage to the quality of the soil[1]. The textile industry in India alone consumes up to 80% of the total dyestuffs produced. Some of the dyes or their breakdown products also have a strong toxic and mutagenic influence on the living organisms[2]. Textile industries generate waste water with different characteristics. The waste water characteristics vary according to the process employed[3]. Various waste liquors coming out of the operations in wet processing such as desizing, scouring, bleaching, mercerizing, dyeing, printing and finishing[4]. Many dyes and pigments are hazardous and toxic at the concentration discharged to receiving water for human as well as aquatic life. The water pollution caused by the textile mill effluent in hazardous for aquatic Eco system[2]. The high concentration of dyes causes many water born diseases and increase BOD of the receiving water because of their complex structure and largest molecular size. Dyes used in the textile industry are difficult to remove by conventional waste water treatment methods since they are stable to light and oxidizing agents and are resistant to aerobic digestion. Parameters that affect water quality are temperature, turbitidity, pH, alkalinity, acidity, BOD, COD and color[3]. Most of

the dyes are toxic in nature and their high concentration causes many water borne diseases and increases the problem. The presence of carcinogens has also being reported in combined waste water of dyeing and printing units[2]. As the dyes present in waste water decompose very slowly in normal conditions, a treatment method to remove the dyes has to the employed[5]. The solution to the environmental problems caused by the textile dye effluent is being sought by physical, chemical and biological treatment processes. The physicochemical methods include adsorption, chemical precipitation, flocculation, electro floatation, oxidation via chlorine, peroxide, electrolysis and ozone treatment, reduction, electrochemical destruction and ion-pair extraction[6]. Biological methods of removal involve the use of microorganism such as bacteria and fungi to turn these pollutants into non-toxic harmless substances. Biological processes convert organic compounds completely into water and carbon dioxide, have low cost and are easy to use[7]. Various bacteria and fungi are effective in decolorization; and in many cases, adsorption of dyes to the microbial cell surface is the primary mechanism for decolorization[8].

The microbial degradation and decolorization of dyes have received considerable attention from the viewpoint of treating industrial wastewater containing dyes. However aromatic amines produced by all these anaerobic microorganisms may be toxic and carcinogenic[9]. Wastewater treatment facilities are often unable to completely remove commercial dyestuffs, thus contributing to the pollution of aqueous habitats[10].

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

Chemicals and media

All chemicals used in this experiment were of AR grade. The dye true blue was collected from a dye industry located at Sanaganer area, Jaipur. Carbon and nitrogen sources used were purchased from Himedia Laboratories (Mumbai, India).

Isolation, screening and identification of dye decolorizing fungi

The dyeing industry effluent sample was collected from a dyeing industry located at Sanaganer area, Jaipur. The effluent temperature and other physical characteristics were examined at site and in laboratory, respectively. One ml of effluent was transferred into 9 ml of distilled water in sterile test tubes. Serial dilution was done up to 10 by thorough mixing. 0.1 ml of sample from each dilution was spread on potato dextrose agar (PDA) plates containing chloramphenicol with the help of L-rod. The petridishes were incubated at room temperature for 5 days[11]. A plug of mycelium of the fungal isolate was placed on a clean slide containing a drop of Lactophenol Cotton Blue (LCB) solution[12]. The mycelium was spread using a sterile needle and a clean cover slip was placed above the preparation and observed under the light microscope for the identification of fungal isolate[13].

Preservation and maintenance

Pure fungal isolates were obtained on the PDA plates; these isolates were further sub-cultured on PDA slants and incubated at room temperature. After sufficient growth was obtained, the slants were stored in refrigerator and served as stock cultures. Subcultures were routinely made every 30 to 60 days.

Screening of decolorizing fungi

Screening of decolorizing fungi by plate assay

The PDA plates were prepared containing Reactive Red dye at a concentration of 75mg/l. For each plate, a sterilized filter was placed in centre and was inoculated with fungal isolate broth culture; un-inoculated PDA plates were maintained and used as control. All the plates were incubated at 28^{o}C for 7days and observed for clearance zone which depicts decolorization. The experiments were performed in duplicate for each fungal isolate. Fungi isolated from effluent and soil samples were used to check the decolorization potential using a standard dye, Reactive Red. *Phanerochaete chrysosporium* was also used for decolorization studies[14].

Screening of decolorizing fungi by broth assay

In a 250 ml flask**,** 100 ml of sterile enrichment broth medium containing Reactive Red dye (100 ppm concentration) was inoculated with mycelial gel disk (8mm) obtained from PDA plates of selected

fungal isolate and incubated at 28^{o}C. After 15 days of incubation, withdrew 2ml of sample from each flask, in the test tubes under aseptic condition and diluted to 10 ml with distilled water (1:4 ratio) then centrifuged at 2,000-3,000 rpm for 20 minutes, to remove any particulate matter. Finally decolorization was analyzed by determining the absorbance at 695 nm and is expressed as relative percentage taking the non-inoculated control as 100% absorbance. Optical density was measured by UV-VIS spectrophotometer (Hitachi U2800, Tokyo, Japan). The experiments were carried out in triplicates and the mean values were taken. The % decolorization was calculated according to the following equation[15]:

% Decolorization= $(A_i-A_f/A_i) \times 100$

Where, A_i= Initial Absorbance and A_f= Final Absorbance.

Decolorization assay

To 100 ml of sterile enrichment broth medium in 250 ml flask added 2 ml waste effluent and inoculated it with fungal mycelia gel disk 8mm which was obtained from 5 day old culture and incubated at 28^{o}C After 15 days of incubation, withdrew 2ml of sample from each flask in the test tubes under aseptic condition as in case of standard Reactive Red.

Optimization of Enrichment medium

Decolorization of textile dye effluent (1ml) in enrichment broth by all five isolates was optimized with respect to the effect of pH (4-8) and temperature (20^{o}C, 25^{o}C, 28^{o}C and 30^{o}C) and and static vs. Shaking (120rpm). Optimization of carbon sources of enrichment medium different carbon sources**;** 1%, (glucose, maltose, fructose, sucrose), 0.25%, nitrogen sources (sodium nitrate, peptone, yeast extract, ammonium sulphate)[16]. All experiments were carried out with 1%, (v/v) inoculum of 1ml and enrichment medium broth without culture was served as control. The flasks were incubated for 15 days and then the decolorization ability was measured spectrophotometrically at 690nm[17].

Statistical analysis

The data were analyzed as mean of triplicates ± standard deviation (SD). Duncan's multiple range test (DMRT) was employed to test the level of significance at $p<0.05$[18].

Results and Discussion

Microbial decolorization of true blue has not been investigated so far. Decolorization of textile dye effluent is serious environmental problem, which is evident from the magnitude of research done in this field in the last decade.

Treatment of textile dye effluent by physical and chemical methods have a high cost potential and a high sludge problem, whereas biological process convert organic compounds completely into water and carbon dioxide, have low cost and are easy to use[18]. In the present study fungal decolorization of textile dye effluent was carried out using the fungal isolates obtained from the textile dye effluent. Textile dye effluent samples were collected from the disposal site of effluent for screening efficient microorganisms, (fungi)[19].

Screening of dye decolorization

This study showed that selected fungal isolates were capable of removing the color of dye from the solid agar medium. Clear zones around the fungal colonies were visible on agar plates, which show the ability of selected fungal isolates to remove dyes from solid medium. Appearance of decolorization zones around the fungal colonies resulted in screening of fungi capable of decolorizing dye[20].

The fungal isolates which were able to form the decolorization zones were LCJ 1,LCJ 2, LCJ 3, LCJ 4 and LCJ 4A. Isolates LCJ 1A, LCJ 2A, LCJ 5 and LCJ 5B did not form the decolorization zone in the dye containing plate. The isolates which were not able to decolorize the dye were omitted from further screening procedures.

Dye decolorization ability of the fungal isolates was also checked using liquid enrichment medium with Reactive Red dye. Decolorization was analyzed by determining the absorbance at 695 nm wave length and is expressed as relative percentage taking the non-inoculated control as 100% absorbance.

Later, when the isolates were identified we found that the decolorizing ability percentage shown by *Phanerochaete chyrsosporium* was maximum (82±0.33%). *A. niger* showed 78±0.28% and *A. oryzae* showed a better decolorizing ability 80±0.22% than *A. niger*, *A. fumigatus* (LCJ 2) showed 63.9±0.29% decolorizing ability but *A. flavus* (LCJ 1) showed 64±0.37% activity and *Penicillium notatum* (LCJ 4A) 55±0.21%, had average decolorizing ability. Comparison of decolorization ability by different fungi is shown in Figure 1.

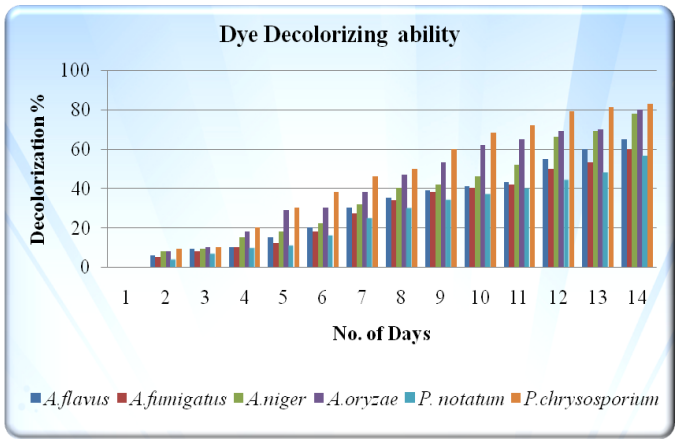


Figure 1: Decolorization of the Reactive Red dye by different species of fungi in enrichment broth medium

Specifically above results suggests that *A.oryzae* and *Aniger* showed decolorization equivalent to *P.chrysosporium*. But, *A.fumigatus, A.flavus* and *P.notatum* decolorized the Reactive Red but not to such an extent as *A.niger* and *A.oryzae*.

Identification

Fungal isolates which gave positive results for dye decolorization were identified as strains of *Aspergillus sp.* and *Penicillium sp.*, based on their macroscopic and microscopical characteristics.

Macroscopic characteristics observed for *LCJ 1* were green colony, reverse of the colony was creamish, colony texture was cottony and granular. *LCJ2 colonies* appeared to have dark-green color, smoky gray-green with a slight yellow reverse. *LCJ3* had dark - brown colony yellow reverse. *LCJ4* had light-brown colony, pale greenish-yellow, oliveyellow or with different shades of green were identified. *LCJ4A* appeared as green – blue colony, The colonies are initially white and become blue green in time.

Microscopical observations of the isolates, *LCJ1, LCJ2, LCJ3* and *LCJ4* showed septate and dichotomous hyphae. Conidiophores were coarsely roughened, uncoloured, vesicles spherical, metulae covering nearly the entire vesicle in biseriate species. Conidial heads were radiate, uni- and biseriate and were confirmed as *Aspergillus sp.* which were then identified as *A. flavus, A. fumigatus, A. niger and A. oryzae* based on taxonomical characteristic[21].

The fungal isolate, *LCJ4A* showed terverticillate hypahe and conidia were spherical to elliptical in shape, conidia were smooth and had green color reflection in the mass and so it was identified as *Penicllium sp.* It was confirmed as *Penicillium notatum* based on taxonomical characteristic.

Hence the five fungal isolates which were screened using decolorization studies were identified as *LCJ 1 Aspergillus flavus*, *LCJ 2 A.fumigatus*, *LCJ 3 A.niger*, *LCJ 4 A.oryzae*, *LCJ 4A Penicillium notatum*.

Decolorization assay

From the selected fungi, five identified isolates *A. flavus, A. fumigatus, A. niger, A. oryzae and P.notatum* were found to be efficient in reducing the effluent upto 70% in liquid media.

The decolorization percentage of *Aspergillus oryzae (LCJ 4)* it was 82.60±0.29%, for *A. niger (LCJ 3)* 80±0.28% and for *A. flavus (LCJ 1)* 69±0.31% *A.fumigatus (LCJ 2)* showed 67±0.33% *P. notatum*

(LCJ 4A) 62±0.37% (Figure 2). The two fungal species *A. oryzae* and *A. niger* showed good decolorization percentage of the effluent.

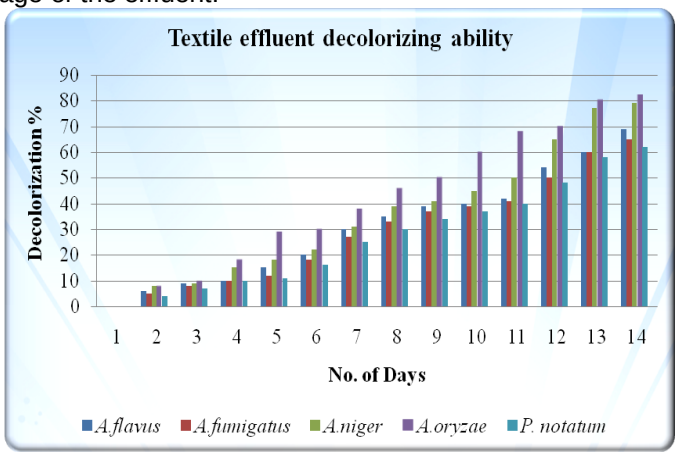
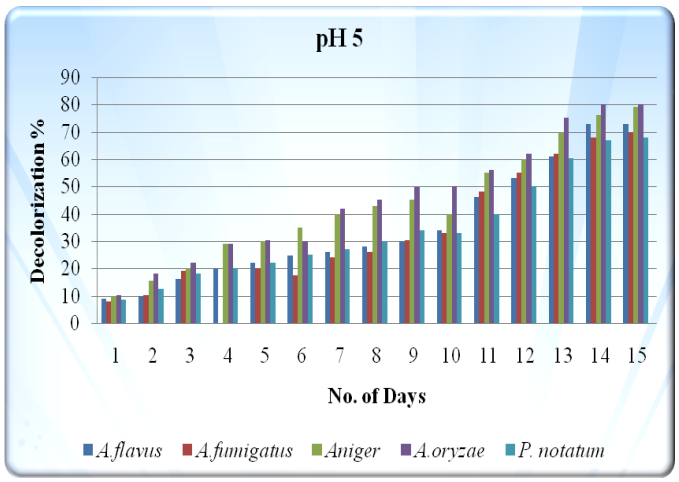


Figure 2: Decolorization of the textile effluent by different species of fungi

A.fumigatus, A.flavus and *P.notatum* decolorized the effluent but not to such an extent as *A.niger* and *A.oryzae*. *A.niger* and *A.oryzae* showed significant results in decolorizing the effluent.

Effect of pH

The different pH used for the dye decolorization of true blue by the five selected fungal isolates was pH 4-8 respectively. The maximum decolorization observed at pH 5 and 6 is shown in (Figure 3). The range of activity on decolorization of effluent with pH 4 was *A. oryzae* 65±0.35%, *A.niger* 62±0.22%. *A.flavus* showed the decolorization percentage equal to 64±0.29%. *A.fumigatus* 55 ± 0.31% and *P.notatum* 57.04± 0.32% showed the minimum decolorization%(Table 1). The range of decolorization activity of effluent with pH 5 was *A. oryzae* 80±0.31% *and A.niger 79.09*±0.29% showed maximum decolorization on 15th day of incubation*. A. flavus 72*±0.26% *A.fumigatus 70*±0.33% *and P.notatum 69.08*± 0.24% were found as an average level of decolorization(Figure 3) (Table 1). The range of decolorization activity of effluent with pH 6 was *A. oryzae* 75±0.32% *and A.niger 74.09*±0.33% but, it was not found to be lesser than the level showed with pH 5. *A. flavus showed 60*±0.27%, *A. fumigatus 63* ± 0.41% *and P. notatum 61*± 0.31%; these values were found to be average level of decolorization(Figure 3) (Table 1).



The range of decolorization activity of effluent with pH 7 was *A. oryzae* 58±0.23% and *A. niger 54.7*±0.36% showed lesser decolorization as compared to that obtained at pH 5 and 6. *A. flavus 44*±0.30%, *A. fumigatus* 41±0.37% *and P. notatum* 43±0.35% were observed lesser% decolorization as compared to above mentioned fungi(Table 1). Whereas at pH 8 decolorization activity of effluent was *A. oryzae* 50±0.32%, *A. niger* 49±0.31%, *A. flavus 39*±0.28%, *A. fumigatus* 30 ± 0.34% *and P. notatum 28*± 0.31%(Table 1). Decolorization percentage values found at this pH were very less as

compared to those found at pH 5 (Table 1) reported optimum pH 3 for decolorization of reactive blue 4 using immobilized polyphenol oxidase[22].

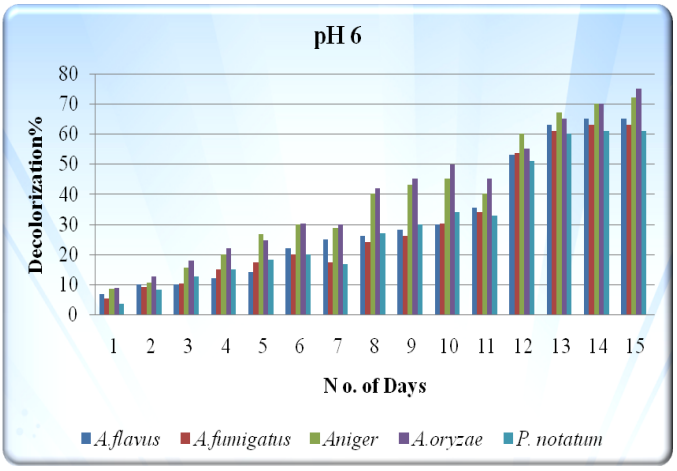


Figure 3: The graph depicts decolorization percentage, using different fungal isolates, when culture medium was adjusted at pH 5 and 6

Table 1: Showing decolorizing percentage from pH (4-8) at pH 5, the decolorization was statistically significant by isolated fungi at p<0.05 level

	Decolorization Percentage				
Fungus	**pH 4**	**pH 5**	**pH 6**	**pH 7**	**pH 8**
A. flavus	64±0.29%	**72.9±0.26%**	60±0.27%	44±0.30%	39±0.28%
A. fumigatus	55±0.31%	**70.08±0.33%**	63±0.41%	41±0.37%	30±0.34%
A. niger	62±0.22%	**79.09±0.29%**	74.09±0.33%	54.7±0.36%	49±0.31%
A. oryzae	65±0.35%	**80±0.31%**	75±0.32%	58±0.23%	50±0.32%
P. notatum	57.04±0.3%	**69.08±0.24%**	61±0.31%	43±0.35%	28±0.31%

Effect of temperatures

Different temperatures used were as incubator temperature (20°C, 25°C, 28°C and 30°C). The maximum decolorization was observed at 30°C. The range of decolorization activity of effluent with 30°C with *Aspergillus oryzae* 87±0.34%, *A. niger* 85±0.24%. *A. flavus 78*±0.26%, *A. fumigatus* 75±0.33% *and P. notatum* 72± 0.27%. *P. notatum* showed minimum decolorization as compared to *A. niger* and *A. Oryzae* (Figure 4) (Table 2). The range of decolorization activity of effluent with 28°C, the % decolorization observed with *Aspergillus oryzae* 80±0.31%, and *A. niger* 80±0.21%. *A. flavus* 69±0.25%, *A. fumigatus* 65± 0.33% *and P. notatum* 60± 0.24% (Table 2).

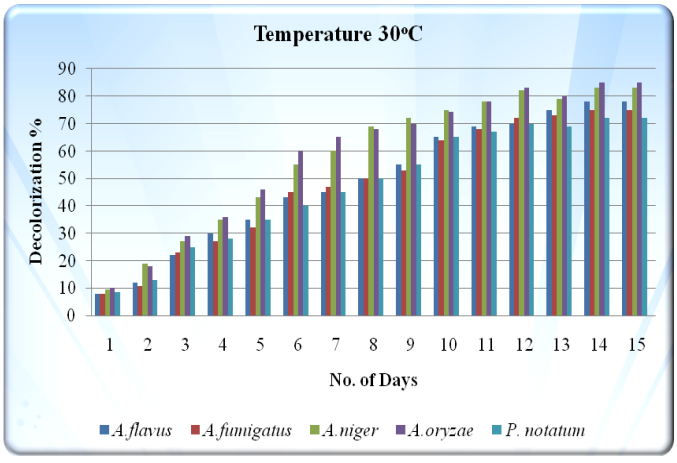


Figure 4: The graph depicts decolorization percentage, using different fungi, when culture flasks were incubated at 30 °C

The range of decolorization activity of effleunt with 25°C, *Aspergillus oryzae* 73.02±0.31% and *A. niger* 74.09±0.29% showed greater percentage of decolorization. *A. flavus* 62.98±0.26%, *A. fumigatus* 64.02 ± 0.33% *and P. notatum 61.99*± 0.24% showed lesser decolorization % (Table 2). The range of decolorization activity of effluent with 20°C temperature, *Aspergillus oryzae* 62.09±0.34% *and A. niger 64.02*±0.26% decolorizing the effluent. *A. flavus* showed 59.28±0.27%, *A. fumigatus* 59.02 ± 0.34% *and P. notatum* 54.32± 0.31% showed minimum decolorization percentage as shown in (Table 2).

Table 2: Decolorization percentgae by fungi at different incubation temperature There was significant effect of percentage of decolorization at variant Temperature 30°C, by isolated fungi at p<0.05 level

Fungus	Decolorization Percentage			
	30°C	28°C	25°C	20°C
	Temperature			
A. flavus	78±0.26%	69±0.25%	62.98±0.26%	59.28±0.27%
A. fumigatus	75±0.33%	65±0.33%	64.02±0.33%	59.02±0.34%
A. niger	85±0.24%	80±0.21%	74.09±0.29%	64.02±0.26%
A. oryzae	87±0.34%	80±0.31%	73.02±0.31%	62.09±0.34%
P. notatum	72±0.27%	60±0.24%	61.99±0.24%	54.32±0.31%

Effluent was decolorized with mix culture with an optimum decolorizing temperature of 30°C and 28°C. This may be owing to a greater production of enzymes and optimal growth conditions of the isolate for its dye decolorizing ability[23]. Hence pH and temperature optimum for dye effluent decolorization was found to be pH 5 and 30°C (Figure 3 and 4, Table 1 and 2). Successfully decolorize four different azo dyes under (decolourization percentage >97%) using strain Phanerochaete chrysosporium[25].

Effect of shaking vs. static mode

It was found that the fungus was more efficient in decolorization in shaking condition[24], *Aspergillus oryzae* 89±0.29%, *A. niger* 87±0.30% ,*A. flavus* 79±0.26%, *A. fumigatus* 78±0.32% and *P. notatum 75*± 0.29% (Figure 5). In static mode *Aspergillus oryzae* 82.98±0.33%, *A. niger 80.99*±0.25%, *A. flavus* 72.28±0.27%, *A. fumigatus* 70.76 ±0.31% and *P. notatum* 69.76±0.34% (Figure 5).

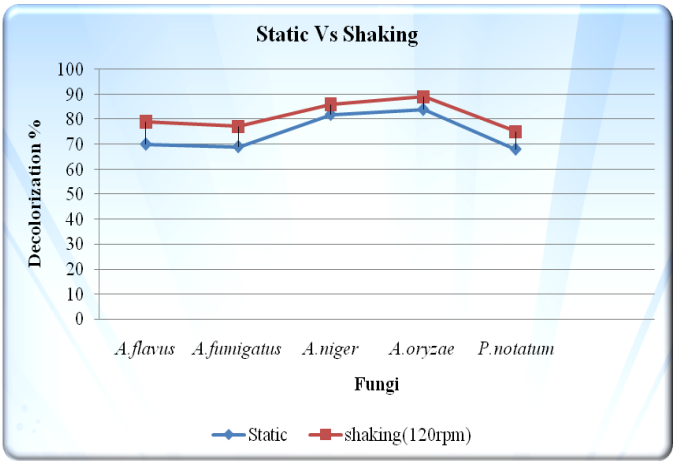


Figure 5: Decolorization percentage in cultures using static or shaking mode during incubation

Effect of carbon sources

All the fungal isolates showed higher percent decolorization than control showing that Glucose sugar could be utilized effectively as carbon source by these isolates The range of activity on decolorization of effluent with glucose was the highest with *Aspergillus oryzae* 85.09±0.32%, *A. niger* 83.9±0.33%, *A. flavus* 69±0.27%, *A. fumigatus* 67±0.41% *and P. notatum* 66±0.31% (Figure 6) (Table 3). When fructose was used as sole carbon source, decolorization percentages obtained with different fungi- *Aspergillus oryzae* 73±0.24%, *A. niger* 71±0.32% *and A. flavus* 69±0.29% *A. fumigatus* 67±0.23% *and P. notatum 63*± 0.35%(Table 3). In culture medium when sucrose was used, values showed maximum

decolorization with *Aspergillus oryzae* 62.03±0.25%, *A. niger 60.8*±0.31% *and A. flavus* 58±0.32% *A. fumigatus* 56±0.33% *and P. notatum* 53.9± 0.36%(Table 3). With maltose as carbon source, fungi showed comparatively less decolorization as compared to glucose. *Aspergillus oryzae* 76±0.32% showed maximum decolorization among isolated fungi. *A. niger* 77±0.31%, *A. flavus* 70±0.38% *A. fumigatus* 68±0.37% and *P. notatum* 65±0.25% (Table 3)

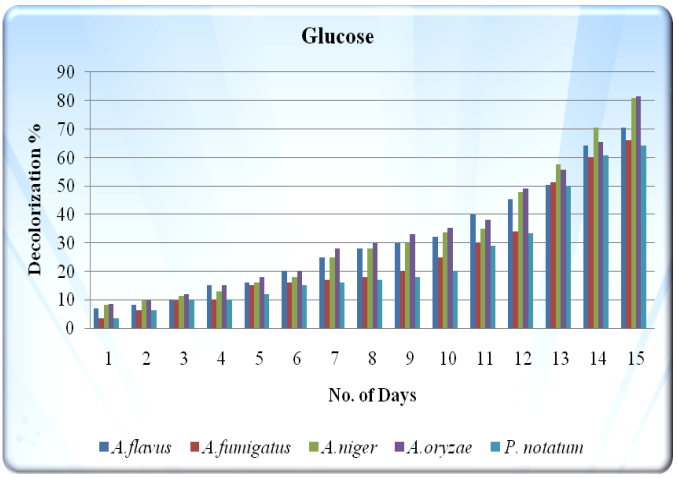


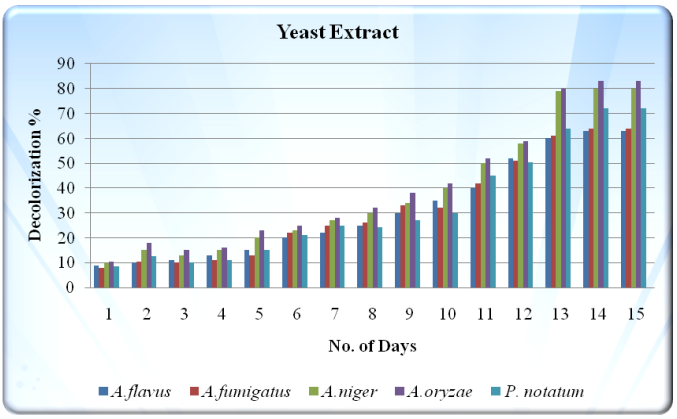
Figure 6: Decolorization percentage study using glucose as the sole carbon source in culture medium

Table 3: Decolorization % when culture medium has different carbon sources There was a significant effect p<0.05 shown by glucose as compared to the other carbon sources

Fungus	Glucose	Fructose	Sucrose	Maltose
Decolorization percentage				
A. flavus	69±0.27%	69±0.29%	58±0.32%	70±0.38%
A. fumigates	67±0.41%	67± 0.23%	56±0.33%	68±0.37%
A. niger	83.9±0.33%	71±0.32%	60.8±0.31%	77±0.31%
A. oryzae	85.09±0.32%	73±0.24%	62.03±0.25%	76±0.32%
P. notatum	66±0.31%	63±0.35%	53.9±0.36%	65±0.25%

Effect of nitrogen sources

The range of percentage decolorization of effluent with Yeast extract was the highest for *Aspergillus oryzae* 83.09±0.32%, *A. niger* 80±0.33% *and P. notatum* 74±0.31 % (The minimum decolorization was shown by *A. flavus* 67±0.27% *A. fumigatus* 65±0.41% (Figure 7) (Table 4). Similarly decolorization percentage in presence of Ammonium sulphate as sole nitrogen source were as follows: *Aspergillus oryzae* 85.09±0.31%, *A. niger* 83.9±0.25%. *P. notatum* 80± 0.34%, *A. flavus* 67±0.29% and *A. fumigatus* showed minimum decolorization 65 ± 0.33%(Figure 7)(Table 4). With peptone, decolorization percentages observed were such, *A. niger 62*±0.33%, *Aspergillus oryzae* 61.9±0.32%, *P. notatum* 59± 0.34% *A. fumigatus* 57±0.36% *and A.flavus 55*±0.29%(Table 4).



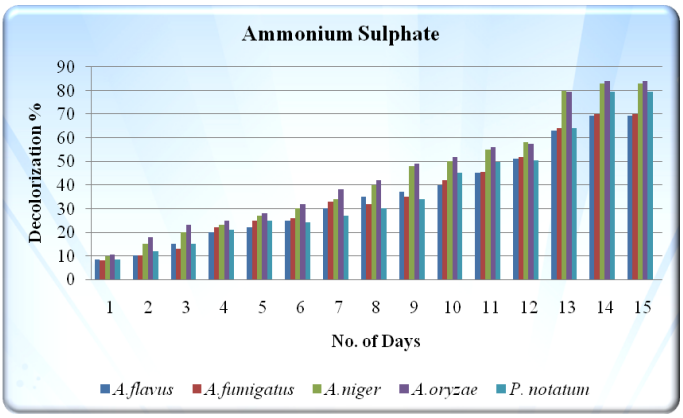


Figure 7: Decolorization percentage study using yeast extract and Ammoniium Sulphate as the sole nitrogen source in culture medium

Lowest decolorization percentages were observed using ammonium nitrate as nitrogen source as, *Aspergillus oryzae* 49.08±0.32%, *A. niger* 43.7±0.33% *A. flavus* 39.9±0.27% *A. fumigatus* 37±0.41% *and P. notatum* 39± 0.31% after 15th days of incubation(Table 4).

Table 4: Decolorization% of effluent using different nitrogen sources in culture medium. There was a significant effect p<0.05 shown by Yeast extract and Ammonium Sulphate as compared to the other nitrogen sources

Fungi	Yeast extract	Ammonium Sulphate	Peptone	Ammonium Nitrate
A. flavus	67±0.27%	67±0.29%	55±0.29%	39.9±0.27%
A. fumigatus	65±0.41%	65±0.33%	57±0.36%	37±0.41%
A. niger	80±0.33%	83.9±0.25%	62±0.33%	43.7±0.33%
A. oryzae	83.09±0.32%	85.09±0.31%	61.9±0.32%	49.08±0.32%
P. notatum	74±0.31%	80±0.34%	59±0.34%	39±0.31%

The present study is thus an effort to develop a potential fungal isolate as an effective decolorizer of textile dye true blue. 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 effluent is degradable under aerobic conditions with a concerted effort of fungi isolated from textile dye effluent. Physical parameters (pH, temperature and aeration) and nutrients (carbon and nitrogen sources) had a significant effect on dye decolorization. Showed highest decolorization of true blue effectively during optimization, but predominantly showed consistent decolorization of dye in effluent.

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