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Isolation And Screening Of Fungal Species For Decolourization Of Textile Dyeing Mill Effluent

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ABSTRACT

Textile wastes are coloured, highly alkaline, high in BOD and suspended solids and high in temperature. A dye is a substance used to impart colour to fabrics, food and other objects of beautification. Synthetic dyes are used extensively for textile dyeing. Colour is the contaminant to be detected and even small amounts of dyes as low as 5ppm for reactive dyes are clearly visible. These dyes are highly resistant to microbial degradation and hence wastes containing them are less amenable to treatment as far as decolourization is concerned. So, attempts are made to isolate and screen out mold species for decolourization, and this waste can be safely disposed off in natural water bodies. The isolate showing considerable decolourization and reduction in BOD is *Aspergillus* spp. Testing of its efficiency with individual textile dyes, textile waste and optimization conditions these off is in pipeline.

Keywords: *synthetic dyes, decolourization, textile waste, BOD reduction, Aspergillus spp.*

1. INTRODUCTION

Water is one of our natural resources. Each use of water dictates quality need. The highest priority use is man's need for drinking. Besides it, bathing, fishing, domestic use, water recreation, agricultural use as irrigation, commercial uses in various industries. These uses require quality water. One degree of water quality degradation can be called "pollution"- impairment which does not create an actual hazard to public health but does adversely or unreasonably affect the usage of water (Fillos, & Molof, 1972).

Colour is the contaminant to be detected and even small amounts of dyes as low as 5 ppm for reactive dyes are clearly visible (Costa-Ferreira, *et. al.*, 2001). Colour in water may result from the presence of substances of vegetable origin such as tannins, humic acids, peat material, plankton or weeds. Metal ions including iron, manganese, copper and chromium may impart colour (Allen, & Mancy, 1972). Dyes and pigments are usually released into the environment by means of dispersion or a true suspension in industrial effluents.

A dye is a substance used to impart colour to fabrics, food and other objects of beautification. There are various types of dyes based on their chemical composition. Synthetic dyes are classified as azo dyes, nitro dyes, triphenylmethane dyes, indigoid dyes and anthraquinone dyes. Depending upon application and usage, dyes are classified as acidic dyes, basic dyes, reactive dyes, polyaza dyes, vat dyes, azoic or naphthol dyes, disperse dyes (Benkhaya, *et al*., 2020).

Synthetic dyes are used extensively for textile dyeing, paper printing and colour photography and as additives in petroleum products (Venkataraman, 2012). About 10,000 different dyes and pigments exist and over 7x 10^5 tons of these dyes are produced annually. Colour to the water may be contributed by waste from textile mills, pulp and paper mills, tanneries, slaughterhouse, distilleries and other industries and is a good visible indicator of pollution (Gürses, *et.al*., 2016). Any waste even treated possesses organic matter contracting legal standards still is not acceptable for disposal in water bodies aesthetically if it has colour.

2. TEXTILE INDUSTRY

The textile industry is a traditional sector. In India, most of the textile industries are scattered and operated from houses. Therefore, it is necessary to collect and treat the waste in common effluent treatment plant. Amongst various applications of synthetic dyes about 3,00,000 tons of different dyestuffs are used per year, for textile consumers of synthetic dyes. The characteristics of the waste from textile mill depend on the type of fibre used such as cotton, wool, regenerated and synthetics. Generally, textile wastes are coloured, highly alkaline, high in BOD and suspended solids and high in temperature (Table.1)

Table 1. Composition of composite textile mill waste

Characteristics	Values
pH	10
Total alkalinity	17.35 mg/L as $CaCO_3$
BOD	760 mg/L
COD	1418 mg/L
Total solids	6170 mg/L
Total chromium	12.5 mg/L

The decolourization of textile dyes by fungi started from the site of dye effluent discharge was studied in vitro conditions. Textile industries discharge large volumes of waste water containing a variety of pollutants (Mumtaz, *et al.*, 2023). Textile dyes are highly reactive & therefore during processing difficult to treat. During dyeing of cellulosic fibres, about 50 percent dye is washed out into the effluent. The commonly used dyes by the textile industry are azo dyes (orange 3R, Yellow Gr & black RI), anthraquinone (Blue) & Copper phthalocyanine (T- Blue). Therefore, attempts were made to decolorize these dyes by a fungus, isolated from the site where effluents are commonly discharged to soil from textile industry.

During and after dyeing process in textile industry, large amount of dyestuffs are directly lost to the waste water and impart colour to the waste water which in turn imparts into natural water body in which it is disposed off. These dyes are highly resistant to microbial degradation and hence wastes containing them are less amenable to decolourization. Generally, these dyestuffs are designed to resist chemical fading and light induced oxidative fading (Nigam *et al.*, 2000). This mainly makes them more resistant to biodegradation. Amongst the other factors that contribute to reduction in their biodegradability include high water solubility, high molecular weights and fused aromatic ring structures which inhibit penetration through biological membranes (Keharia and Madamwar, 2003).

3. TREATMENT OF TEXTILE WASTE

a) Biological treatment:

A biological stage is usually included as a part of a complete treatment scheme. These methods include: i) Use of percolating filters ii) Activated sludge process.

Both processes can effectively reduce BOD unless inhibitory substances are present. Due to the low biodegradability of many dyes and textile chemicals, biological treatment is not always successful even with the extended treatment available in an activated sludge plant. The work of Porter and Snider (1974,1976) have already revealed that, even after 30 days aeration, COD and colour were not much reduced.

Murakami and Annaka (1978), in Japan, studied the removal of colour from municipal sewage containing about 10% mixed textile waste water by activated sludge process followed by tertiary processes. Activated sludge removed only 30 to 40% of dissolved colours but insoluble colour was almost removed.

Michaels and White (1978) showed that conventional biological treatment plants reduced BOD but may not materially affect COD and colour.

It was found that in the studies on bioelimination of various dyes that all three anionic water-soluble reactive dyes - CI Reactive violet 15; Reactive blue 19 and Reactive black 5 were neither removed nor bio degraded by activated sewage sludge even after 20 days incubation.

Coagulation and sedimentation: (Solanki *et al.*, 2013)

As textile waste may not contain much readily settleable solids. Therefore, in order to improve the removal of settleable solids, coagulants such as lime alum, salts as ferric chloride, ferrous sulphate are used. Coagulation can be an effective means of reducing colour of dye wastes along with COD and BOD. Unfortunately, the coagulant and the dose that suit one waste may be unsuitable for another waste. Further, all coagulant addition produces a significant quantity of waste sludge, which is generally difficult to dewater, and requires disposal.

Singer (1976) investigated 20 dye wastes for the American Dye Manufacturers Institute and reported that, BOD was effectively reduced by biological treatment, colour was not. Dispersed, vat and sulphur dyes were decolourized by coagulation with alum but not by adsorption with activated carbon.

The chemical treatability of secondary treated textile waste water was investigated by coagulation with ferric chloride that reduce COD by 64% and colour by 91% (Solmaz *et al.*, 2006)

Filtration: (Zyłła *et al.*, 2006)

The need for filtration is dependent on the quality required for final discharge or for additional treatment prior to recycles the waste. It would normally only be required to meet a particularly stringent discharge condition. Removal of suspended solids by filtration after chemical coagulation and sedimentation usually has little effect on BOD and COD concentrations.

Adsorption processes:(Bazrafshan, *et al.*, 2016)

Increasing the adsorption of dyes onto suitable material offers a means of removing them from effluents. Various materials can be used for adsorption. Activated carbon adsorption is the most commonly used method. Though carbon is cheaper cannot normally be recovered by regeneration after waste treatment. It is, therefore, wasted and requires disposal, other adsorbents that are, or can be used in effluent treatment includes use of silica gel, activated alumina, wood, clay and synthetic polymers. These alternative adsorbents are unlikely to be widely used, unless they are found both uniquely suitable and available locally and are cheaper.

c] Chemical oxidation: (Hassaan and Nemr, 2017)

Various chemical oxidants have been used in experimental and on full scale. These can destroy colour of dyes and oxidizes another material present in wastewater.

(i) Chlorination: Chlorine and sodium hypochlorite are effective in decolorizing some dyes and have been used for the treatment of effluents from the production dyes. Both chemicals are However, oxidation with chlorine may not be environmentally acceptable especially, if the waste is discharged directly to a river. Excess chlorine can be controlled but the chlorinated product may be as undesirable or their effect as unknown as the dyes themselves.

(ii) Ozonation:

Ozone is one of the strongest of the oxidizing agents that are likely to be used in an effluent treatment plant. As ozone is a toxic and corrosive gas and the residual cannot be safely maintained from the reactors, if there is plant or personnel nearby. The reactor has to be enclosed and provision made either to recycle the gas or to destroy the residual ozone. Fairly high doses of ozone are required for the decolourization of dye wastes. There is only a slight reduction in COD or BOD but colour removal can be complete. Due to high cost, ozone is usually applied as a final treatment after the most potential demand has been reduced.

There are some pilot plant studies carried out on waste from dye industry where ozone was used in the treatment (Ma, *et al.*, 2018).

Use of microorganisms for decolourization of waste:

In the recent past, many workers have reported the abilities of some microorganisms to degrade the waste and to oxidize the dyes. Fungi and white rot fungi have been found to be of interest for their use in decolourization of the waste (Keharia and Madamwar, 2003).

Many of fungi belonging to white rot group have been shown to degrade wide variety of dyes (Banat *et al.*, 1996; Stolz *et al.*, 2001).

The environmental pollution due to dye can be judged by considering following factors: (Katheresan, *et al.*, 2018)

i. Amount of dye discharge.
ii. Toxicity to fish.
iii. Toxicity to microorganisms in nature.
iv. Toxicity to purification plants.
v. Accumulation in nature and food chain.

The major environmental problem of colorants is the removal of dyes from effluents. The concentration of dye may be much less than 1 ppm but the dye is visible even at that or less concentration. As visible pollution often causes more trouble for industries than invisible pollution. Unseen pollution which does not create nuisance will often be tolerated by state agencies, but red and deep brown colours of slaughterhouse waste, the browns of pulp and paper mill waste, various intense colours of textile mill waste and the yellow colour waste from plating mill affects aesthetic merit of streams and other water resources and will focus public indignation directly on those industries.

Different problems caused by the presence of different dyes:

1. Reduction of sunlight penetration of the stream; it affects the photosynthesis and consequently the ecosystem of the stream will be seriously affected.

2. Toxicity to fish and mammalian life.

3. Inhibition of growth of microorganisms particularly in high concentration. Some cationic species such as triphenylmethane affect the flora and fauna even at lesser concentrations (Chung, K. T., *et al.*, 1993).

4. Many of the dyes can cause medical problems as they have carcinogenic and allergenic potential. Several amino substituted azo dyes are mutagenic as well as carcinogenic and their toxicity have been extensively studied along with the risk of occupational cancer associated with their use (Joachim *et al.*, 1985; Gonzales, C. A., *et al.*, 1988).

5. Both aromatic sulphonic acid and azo groups are rare among natural products and thus confer xenobiotic character to sulphonated azo dyes.

From industrial point of view, there is always great value for the effective and low-cost treatment system. Now days biological systems are recognized as the best alternative available for low-cost treatment for effluent treatment and decolourization. Biological systems are recognized by their capacity to reduce biological oxygen demand and chemical oxygen demand, by conventional aerobic digestion. But there is its inability to remove colour from effluent (O'Neill *et al.*, 2000).

There are various problems for treatment of waste containing dyes and its decolourization involves a wide range of pH intervals, salt concentrations and chemicals very often added to complications. Hence, it becomes necessity to attempt for new options for effluent treatment and disposal.

Thus, search for isolation of microbial species, which would have potential for the degradation and decolourization of the waste, has now gained importance. Secondary screening of potential microbial candidates is of most importance as it is likely yield a microbial strain that have potential for exploiting it for degradation and/or decolourization of the waste.

4. MATERIAL & METHODS

Material:-

Following materials were used in the present investigation

1. Waste water sample from textile dyeing mill

2. Soil sample for isolation of microorganisms

Methods:-

I) Collection of samples

1. Collection of waste water sample from textile dyeing mill. The Textile dyeing mill effluent was collected from Vivekananda Co-op Textile ltd. Ichalkaranji. A Plastic bottle of 1 L capacity was used for the collection of the sample. The Textile dyeing mill effluent was collected by the method described in APHA (1998).

2. The collection of soil samples for isolation of microorganisms from site of textile mill.

The soil samples were collected in polythene bags from the area, which was in the near sludge tank & effluent plant of textile mill & were brought to the laboratory.

Table 2. Details of collection of samples used for studies

Sr. No.	Types of samples	Sampling site
1.	Waste Water from textile mill industry	Textile dyeing Effluent outlet
2.	Soil sample	Near sludge tank

II) Primary screening of fungal isolates for decolourization of textile dyeing mill effluent:-

a) Enrichment for fungal isolates: -

The textile dyeing mill effluent itself was used for medium preparation. Different dilutions as 1:1, 1:5 and 1:10 of textile effluent were prepared by using distilled water. Each dilution was prepared in separate flask and each flask supplemented with sabouraud broth medium and same for undiluted textile effluent in a separate flask. All these media were sterilized by autoclaving and inoculated with 1% of sample of soil and incubated at 30^0 C for 8 days on shaker. The fungal isolates were selected and preserved on slants at 4^0 C.

b) Screening of fungal isolates for textile dye decolourization: -

Initially, all fungal isolates, which were to be inoculated, were grown till sporulation occurred on Sabouraud Glucose Agar. For inoculation of these fungal isolates into media (l:1, 1:5, 1:10 and original textile waste agar media) fungal isolates were point inoculated and plates incubated at 30^0 C for week and uninoculated media plates were control plates.

After the incubation period, all these plates were observed for decolourization by comparing with negative control plate of each dilution and undiluted effluent containing plate. The fungal isolate showing maximum zone of clearance was taken as best promising isolate for decolourization.

Result and Discussion:

A) **Table 3.** Morphological & cultural characteristics of the isolates of primary screening of fungi.

Sr no.	Source (Textile waste)	Isolate	Size	Shape	Colour	Margin	Opacity
1	Soil	F I	4 mm	Filamentous	Black	Entire	Opaque
2	Soil	F II	6 mm	Filamentous	White	Entire	Opaque

Aerial mycelium	Elevation	Spore colour
+	Flat	Black
+	Raised	Black

B) Screening of the fungal isolates for decolourization of textile dyeing mill waste using agar plate method: -

Results of the screening of fungal isolates for decolourization of textile dyeing mill waste are as shown in Table no. 4. Fungal isolates designated as F-I, F-II inoculated in undiluted and diluted textile effluent and both isolates don't showed growth on undiluted textile effluent, while, showed growth on 1:1, 1:5 and 1:10 diluted textile effluent containing media. While decolourization zone was observed at 1:5 and 1:10 diluted effluents containing medium by F-I & F-II after 48 hr incubation. In case of 1:1 diluted effluent no decolourization was observed.

Table No. 4.

Decolourisation study for textile dyeing mill effluent using different dilutions of textile effluent by agar plate method.

Sr. No.	Isolate	Growth & decolourisation observed			
		Undiluted effluent	1:1 dilution	1:5 dilution	1:10 dilution
1	F I	-	+	+	+
2	F II	-	+	+	+

- = No growth + = Growth

C) Secondary screening of microorganisms for decolourization of textile dyeing mill effluent:

Secondary screening of fungal isolates for decolourization of textile dyeing mill effluent by using agar plate method

Two fungal isolates showed decolourization at 1:5 & 1:10 diluted textile effluent by agar plate method. Zone of decolourization for each fungal isolate was determined at dilution 1:1, 1:5 & 1:10 within five days incubation at 37°C (table 5).

Among these fungal isolates were F-I showed high efficiency for textile dye decolourization (table 6).

Table No. 5

Secondary screening for decolourisation of textile dyeing mill effluent using fungal isolates by agar plate method.

Sr. No.	Isolate	Decolouration observed at different dilutions of textile mill waste			
		Undiluted effluent	1:1 dilution	1:5 dilution	1:10 dilution
1	F I	-	+	+	+
2	F II	-	-	+	+

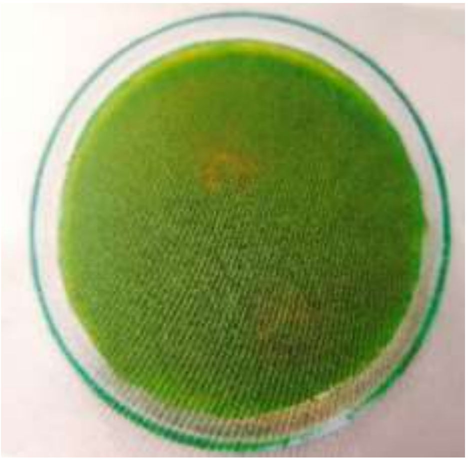
- = No decolouration

+ = Decolouration

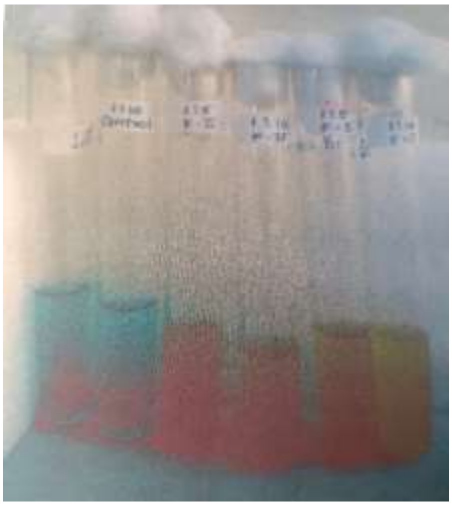
Table no. 6

Decolourization of dyes by fungal isolates

Organisms	O.D. after incubation (days)					Decolourization (%)
	0	2	4	6	8	
F I	0.629	0.462	0.399	0.251	0.215	65.82
F II	0.629	0.590	0.456	0.420	0.360	41.93



Photoplate no.1: Isolate F I showing growth and decolorization in textile dye mill effluent containing medium



Photoplate no.2: Decolorization activity by fungal isolates F I and FII at 1:5 and 1:10 diluted textile dye mill effluent with control for 1:5 and 1:10 dilution.

5. SUMMARY

The effluent from "Vivekananda co-op textile processors LTD Ichalkaranji "was collected & used for decolorization. The characteristics of textile dyeing mill effluent were found to be similar to those reported by other research workers.

The total number of four strains of fungi were isolated by using modified sabouraud glucose agar from the soil sample. All these isolates were screened primarily for their decolourization ability of textile dying mill effluent by agar plate method. According to the results obtained the two fungal isolates were selected for decolourization study, the isolates were designated as F-I & f-II. These two strains show the zone of decolourization on the agar plate. Fungal isolate F-I showed maximum percent decolourization of 1:5 dilution of textile dying mill effluent and also showed growth & decolourization 1:10 dilution of textile dyeing mill effluent.

6. CONCLUSION

Fungal isolate F-I seems to be promising strain for decolourization of textile dyeing mill waste as it brings about 65.82% decolourization within eight days at laboratory flask culture level. Fungal isolate F-I is tentatively identified as a strain of *Aspergillus* species.

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Conflicts of interest:

The authors declare that there are no conflicts of interests.

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