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Research Article

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# Identification and Evaluation of Azo Dye Decolorizing Fungal Species from Industry Effluent for the Development of Consortia Bio Inoculants

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***Abstract***

*Azo dyes are the largest class dye used in textile and other industries. Beside of its large benefits it is also toxic, carcinogenic, mutagenic and teratogenic, that constitutes a significant burden to the environment, human as well as animal health. Physicochemical treatment was employed to degrade and detoxify these azo dyes. However this method has limitation in releasing toxic degradant and not economical. It is timely important to search alternative biological ecofriendly treatment methods. The aim of this study was to screen, identify and evaluate potential fungal species having the ability to decolorize and degrade the Azo dyes for the development of consortia bio inoculants.150 industry effluent waste water was collected and fungal species were isolated & identified using Biolog Microstaton identification technology. The fungal azodye decolorization activities were qualitatively evaluated on Remazol yellow, Navey blue, Procion red MX-5B dyes using solid agar plate and broth assay. The result revealed that the percentage of frequency of fungi in waste water was 63.88 % were filamentous fungi and 38.22% non-filamentous fungi. Aspergillus species were dominant (32%). Thirteen fungal species was identified using Biolog MicroStaion reading machine. These are Trichosporon begilli, Rodotrula auranticaA, Candida palmioleophila, Cryptococus terrus, Yarrowia lipolytica, Cryptococus albidus, Aspergillus ochraceus, Aspergillus restrictus, Aspergillus carneus, Penicillium brevicompactum, Penicillium roqueforti, Penicillium digitatum, Fusarium javanicum. Among the fungal isolates tested, only 6 fungi species showed 72-92% decolorization potential measured by Biolog turbidimeter, these are T. begilli, R. aurantica A, P. roqueforti, P. digitatum, A. ochraceus F. javanicum four species, C. palmioleophila, C. terrus, Y. lipolytica, C. albidus, 34-71% decolorizing ability of Navy blue. 3 species, P. brevicompactum, A. carneus, A. restrictus showed 16-29% decolorizing ability. Trichosporon begilli was superior in clear zone formation on solid agar plate test (5.4mm). Through supporting this preliminary study by HPLC, &spectrophotometer analysis for enzymes and secondary metabolite study, it is possible to formulate and develop fungal bioinnoculant consortia for mycoremediation service.*

**Keywords:** Azo Dye, Biolog, Carcinogenic Micro Station, Mutagenic, Turbidimeter Teratogenic

## Introduction

Worldwide over 10,000 types of dyestuff and pigments are produced with their annual production over 7 × $10^5$ tons and used in dyeing and printing activities in textile, tannery, paper, plastic, detergent, food, cosmetic, bleaching and pharmaceutical industries [1]. (The total world colorant dyestuff production is estimated to be 8,00, 000 tons per year and approximately 10–15% dyes are discharged in the wastewater during the dyeing process and enters into the environment as a waste. [2-5]. In the last decade, consumption of synthetic dyes has been rapidly increasing particularly in the textile industry. It has been estimated that more than 2 × $10^5$ tons per year of textile dyes may be discharged worldwide [6]. According to CSA survey of the Ethiopian manufacturing sector in 2012/13, there were 109 firms engaged in manufacturing of textile and apparel. In Ethiopia, there are more than fourteen major textile and garment factories [7]. Ethiopian textile processing units consumed about 14,250,406 kg of various types of dyes and chemicals in 2011 with their current production capacity [8]. Due to expansion and growth industry activities in Ethiopia, dyestuff utilization in textile, tannary, paint, paper, and other industry also growing rapidly.

Dyes can be grouped on the basis of their origin (natural and synthetic), chemical structures (acridine, anthraquinone, chromophoric, azin, and nitroso dyes), and applications (vat dyes, dispersive dyes, and azoic colors). Broadly dyes may be nonionic (disperse

dyes), anionic (direct, acid and reactive dyes), and cationic forms (basic dyes). The chromophoric groups in anionic and nonionic dyes generally consist of azo groups or anthraquinone types. Of all the different types of dyes, azo dyes are the most useful and widely used colorants which account more than50% of the global industrial demand [9]. Owing to their genotoxic/carcinogenic potential, the annual disposal of ~4,500,000 tons of dyes and/or degraded products is an environmental and socio-economic concern [10]. Due to the presence of azo bond (N=N) bearing aromatic rings and sulfonate groups, and exist as sodium salts the azo-dyes are highly recalcitrant to biodegradation processes [11]. Azo dyes (monoazo, diazo, triazo and polyazo) are toxic, carcinogenic, mutagenic and teratogenic, that constitute a significant burden to the environment and human as well as animal health [12]. A prolonged intake of azo dyes can result in the formation of tumors, allergies, respiratory problems and birth defects and complication. Dye wastewater from textile and dyestuff industries is characterized by high alkalinity, biological oxidation demand (BOD), chemical oxidation demand (COD), total dissolved solids with dye concentrations generally below 1 g/dm [13]. Due to high COD level. The colored effluents are not only aesthetically unacceptable, but also prevent the passage of sunlight through contaminated waterways. This reduces the photosynthetic activity of aquatic flora, which causes depletion of dissolved oxygen, ultimately leading to death and putrefaction of aquatic fauna and the ecological equilibrium of water affected [13, 14]. Its color in water body is unpleasant aesthetically. In developing countries discharge 90% of their wastewater into the water bodies without any treatment [15]. For many decade physical and chemical treatment methods for dye removal were employed such as membrane filtration, electro kinetic coagulation, electrochemical destruction, ion-exchange, irradiation, precipitation, ozonation, and katox treatment but these methods are too expensive, less efficient, generate secondary waste and not ecofriendly. Alternative and optional ecofriendly waste removal method is timely important to reduce and avoid environmental pollution. Microbial bioremediation of dye waste water treatment is cost-effective and eco-friendly [16].

Mycoremediation is an optional and a modern concept for environmental pollution management highly involved in degradation, eradication, immobilization, or detoxification diverse chemical wastes and physical hazardous materials from the surrounding environment through the action of fungi and using their by product. Currently, fungi have been proven to be effective in degrading and mineralizing recalcitrant textile dyes due to their powerful enzymatic machinery that is extracellular ligninolytic enzyme system like laccase, manganese peroxidase, and lignin peroxidase), low substrate specificity, its robust morphology and diverse metabolic capacity [17, 18]. Due to the presence of azo bond (N=N) bearing aromatic rings and sulfonate groups, the azo-dyes are highly recalcitrant to biodegradation processes. The microbial degradation of textile dyes is more effective under anaerobic condition. However, toxic aromatic amines are formed at the end of anaerobic process. A sequenced anaerobic/ aerobic biological treatment of textile dye effluents by microbial consortia is suggested to be potential [19]. The mechanism of microbial degradation of azo dyesinvolves the reductive cleavage of azo bonds (–N=N–) with the help of azo-reductase [20]. Fungal biomass is considered as a good biosorbent for textile dyes because fungi can be cultivated economically in substantial amounts employing simple fermentation techniques and cheap growth media [21]. Dyes and pigments are being removed by these fungi either in living or dead form through bio-sorption, bio-degradation, bioaccumulation and enzymatic mineralization, using lignin peroxidase, manganese peroxidase, manganese independent peroxidase and laccase [11,22,23]. Amongst fungi, some ascomycete ones such as *Penicillium, Trichoderma, Pichia, Candida and Magnusiomyces*, have demonstrated the ability to degrade or even mineralize dyes [24].

For instance, various strains of fungi, *Penicillium oxalicum, Candida and Magnusiomyces,* were also identified for their potential to completely mineralize and detoxify azo dyes [9]. *Aserpgillus niger, Aspergillus flavus, Aspergillusfumigatus, Trichoderma viride, Fusarium oxysporum, Penicillium chrysogenum Scheffersomyces spartinae, Dichomitussqualens, Irpex flavus and Phlebia spp. Pichia occidentalis, and Mucor sp.* which were responsible for the degradation of a wide range of textile dyes [25]. *Aspergillus foetidus, Rhizophus arrhizus, Phanerochaete chrysosporium, Trametes villosa, Fusarium solani, Pleurotus spp. Thermomucorindicae seudaticae* degrade wide variety of dye [14]. Microorganisms use contaminant as a food and source of energy [26]. Thus the biological mode of treatment of dye bath effluents offers distinct advantages over the conventional modes of treatment. Therefore, the main objective of this research is selecting potential ecofriendly azo dye decolorizing fungi species for effective bio inoculant consortia formulation from textile waste water for mycoremdiation service.

## Statement of the Problem

Physico-chemical treatment processes, can generate a large volume of sludge, and the degradation of metal complex azo dyes produce toxic products like 1, 4-phenylenediamine, o-tolidine, sulphide, anilines, arylamine and heavy metals which are proved to be carcinogenic & mutagenic to human being and great environmental problem due to recalcitrant nature. These problem need ecofriendly biological treatment methods.

## Material and Methods

### Sampling Area Description

The sample was collected at Kombolcha textile industry. It is situated 24 km of south-west of Dessie town and 380 km north of Addis Ababa. Kombolcha is a city and woreda in north-central Ethiopia. Located in the South Wollo zone of the Amhara regional state, it has a latitude and longitude of 11°5′N 39°44′E with an elevation between 1842 and 1915 meters above sea level. The average annual temperature is 23 °C.Sampling was done at Kombolcha textile industry zone and three adjust rivers, these are Borkena, eyoul, & Worka rivers where waste water is released to the rivers. Borkena river crosses the town from east to west directions. Its tributaries are Worka and Eyoule rivers.

### Sampling Strategy

Stratified sampling strategy were employed depending on sampling area it is stratified as treatment plant area, non-treatment plant area, Influent, effluent, adjacent rivers, upstream and downstream sample was collected from primary tank, equalizer tank, anaerobic tank, aerobic tank, sludge tank, final effluent tank, rivers upstream area, river downstream area.

## Sample Collection

From 30 sampling site a total of 150 waste water each about 15mLin 45 mL size plastic sampling tube containing PDA broth was collected aseptically from kombolcha textile industry zone and the 3 rivers upstream and downstream zone adjacent to the industry. Waste water samples immediately transferred into ice box and transported to microbial biodiversity directorate laboratory at Ethiopian Biodiversity Institute at 2011E.C.

## Source of Azo dye and Media preparation

Remazol yellow, Navey blue, Procion red MX-5B kindly obtained from kombolcha textile industry where currently used for dying and printing in the textile processing. The fungal growth is taken place on potato dextrose agar where 39 gm of commercial PDA powder mixed in 1 liter of distilled water. It is autoclaved at 121°C for 20 min. The pH value of the medium was adjusted to 6.5. All reagents were analytical grade and solutions were prepared in distilled water.

## Isolation and Screening

150 samples were merged/pooled into 15 samples depending on sample similar property like downstream area, upstream area, primary tank area, equalizer tank area, inlet area, outlet area etc collected samples. 1 mL of waste water will be taken from each pooled sample and diluted serially up to $10^{-6}$ mL. About 0.1mL sample will be transferred on PDA growth media aseptically, & incubated for 48 hrs at 28°C. A single yeast colony and filamentous fungi will be sub cultured on growth media until the purified cultures will be maintained and kept at 4ºC in cryo preservative vial until further analysis employed.

## Fungi screening for dye decolorization test

## Solid- Agar Plate Dye Decolorization Test

Remazol yellow, Navey blue, Procion red MX-5B kindly obtained from kombolcha textile industry and used. Isolates were screened for decolorization ability on solid agar media containing 3 types of azo dye. 100ppm each dye was added to PDA media and loopful of fungal inoculant transferred into solid media at three spot point. Decolorizing and clearing zone around colony were measured at 3days' intervals. Decolorization index was measured & recorded using following formula. All decolorization experiments were performed in triplicates and control.

DI=Colony diameter +Halozone diameter/Colony diameter

## Decolorization at Different Temperatures

To study the temperature requirement for the best decolorization activity of the fungal species, different temperatures ranges 28ºC, 37ºC and 45ºC were checked up 9 days incubated. Clearance zone diameter was measured on solid agar plate test. All decolorization experiments were performed in triplicates and control.

## Decolorization at Different pH Ranges

To study the medium condition for the best decolorization pH. PDA medium having different pH ranges, 6, 9, 12 prepared and fungal growth and clearing zone recorded. Clearance zone formation around colony was measured. All decolorization experiments were performed in triplicates and control.

## Decolorization at Different Dye Concentration

To study fungi decolorization ability at different dye concentration at 3 day intervals. Dye in solid media added at 100ppm, 200ppm, 300ppm, 400 ppm screened &evaluated. All decolorization experiments were performed in triplicates.

## Azo Dye Decolorization in Liquid PDA Broth Media Test

Positive fungus for dye decolorization on solid agar media screening was further investigated for its ability to decolorize azo dyes in PDA liquid media. 100ppm Azo dye immersed in 10 mL sterile distilled water until it is adjusted to 0% transmittance using electronic Biolog turbidimeter and 10% turbid fungal cell mass inoculum was prepared in 10 mL distilled water. Finally, fungal inoculum and azo dye was transferred into to 10 mL potato dextrose agar containing growth media in 45 mL sterile test tube and incubated in an incubator shaker at room temperature with agitation of 130 rpm for 10 days. 13 fungal species decolorizing potential were determined using absorbance-transmittance percentage using Biolog turbidimeter. The efficiency of color removal (decolorization) was expressed as the percentage ratio of the decolorized dye concentration to that of initial one based on the following equation. All decolorization experiments were performed in triplicates.

Decolorization/Removal%= Ci-Cf ×100 where (Ci) and (Cf) are the initial and final dye concentrations (mg/l), respectively.

## Fungal Identification

## Morphological Identification

Colony morphology like color, size, margin, shape, elevation and others were recorded depending on the methods of and doctor fungi on line reference [27]. Examination also supported by relevant literatures for identification of fungi [28-30]. Cellular morphology was identified using Lacto phenol cotton blue staining and observed in compound microscope. Hypha shape, conidiospor, Characteristic of spore, reproductive spore was recorded depending on the methods of and doctor fungi on line reference [27]. Examination also supported by relevant literatures for identification of fungi [31-33].

## Fungi Identification using Biolog Microstation

Strong fungi in decolorization ability screened on solid agar test and broth test selected and subculture to Biolog universal growth agar and incubated at 26 °C for 48h for species identification. Pure fungi colony suspension was prepared in 9ml sterile distilled water and adjusted to 47/75T using Biolog turbidimeter for yeast/filamentous fungi (YT/FF) respectively. 100 µ L of inoculums was dispensed to each well of the Biolog YT/ FF Micro plate tagged with 71 carbon source utilization assays and 23 chemical sensitivity assays Microplate. Tetrazolium redox dyes are used to calorimetrically indicate utilization of the carbon sources or resistance to inhibitory chemicals. Microplate. was incubated at 26 °C 24-72h.The YT/FF/ Micro plate measures both metabolic reactions as well as turbidity growth to produce identifications. YT/FF Micro Plate will be read by the Micro Station Reader at 24 h, 48 h, and 96h at a single wavelength of 590 nm. The Biolog software micro log3 ver. 4.20.05 compared the results obtained with the test strain to the database and provided identification based on distance value of match and separation score produces similarity index value and

probability. Above 0.5 similarity index and >75 % probability result is an accepted species identification [34].

## Statistical Analysis

The data analysis involved various descriptive statistics such as means and percentages frequency was calculated. SPSS Ver 24 was employed to analyzed the data.

## Result and Discussion

Percentage Frequency of Pure Isolated Fungi from Mixed Culture

A total of 145 fungal pure isolates colony were screened from primary mixed culture (Figure.1). Pure colonies having similar morphology were clustered in order to detect percentage frequencies of the fungi in the textile industry waste water samples. 63.88 % were filamentous fungi and 38.22% were non-filamentous fungi. From filamentous fungi, *Aspergillus* species were dominant (32%), *Penicillium* species (30%), *Trichoderma* species (17%), *Fusarium* species (13%). From yeast species *Cryptococcus* and *Trichosporon* spp were dominant. (Figure. 2). This result is supported by report that fungal genera such as *Acremonium, Rhodotorula, Candida, Geotrichum, Cladosporium, Sporothrix, Geotrichum candidum, Penicillium, Trichophyton and Scopulariopsis* were frequently occurring in activated sludge and waste water [35]. reported taxonomic assignment indicated that Basidiomycota and Ascomycota were the two most dominant phyla, accounting for 48.38% and 38.36% in waste water. According to *Aspergillus* species was the most occurring genera from textile effluent [36]. According to report the genus *Rhodotorula* are frequently found in polluted environments [37, 38]. reported that yeasts genera frequently isolated from forested wetlands are *Cryptococcus, Candida, Rhodotorula, Pichia, Trichosporon* are also abundant in wastewaters, and capable of decolorizing azo dyes by biodegradation mechanism [39]. reported the fungi species were isolated from effluent contaminated plant rhizosphere near textile dyeing industrial area and identified as *Trichoderma viride, Trichoderma koningii, Trichoderma harzianum, Aspergillus niger, Aspergillus flavus, and Fusarium oxysporum.*

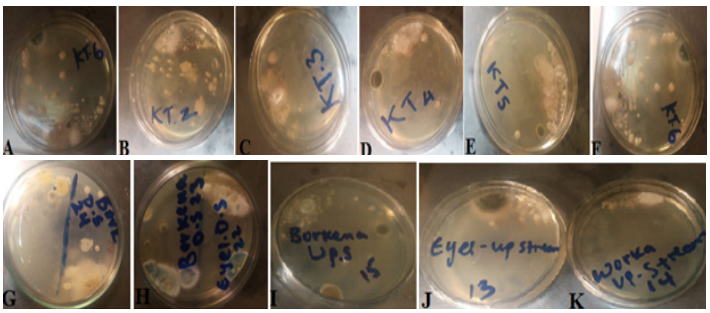


**Figure 1:** Primary fungi mixed culture grown on PDA and the sample taken from kombolcha textile treatment plant (A- F) and the three adjacent rivers to textile industry where effluent is released (Borkena, Eyuel, Worka downstream and upstream rivers area(G-K).

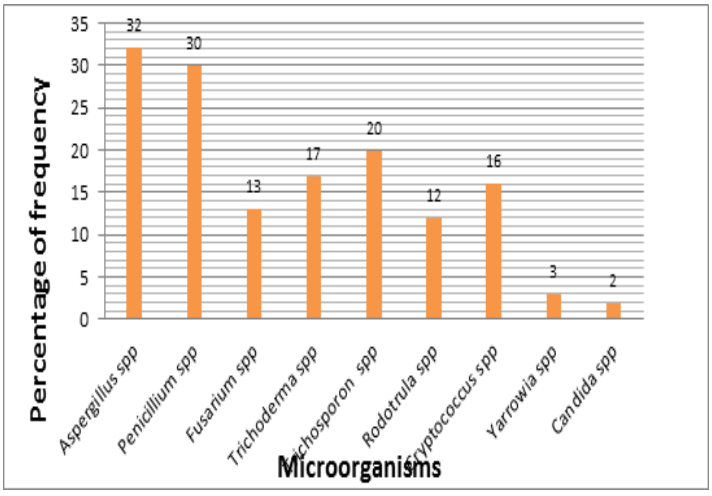


**Figure 2:** Percentage frequency of fungi isolated from industry effluent waste water Solid-plate dye decolorization test result

All fungal isolates were tested on PDA solid agar media containing Remazol yellow, Navey blue, Procion red MX-5B dye each at 100ppm dye concentration. The result indicates that 13 fungi specie were positive for Navey blue, Procion red MX-5B dye and their clearing or decolorize the dye around their colony. The highest clearing zone is recorded in Navey blue by *Trichosporon begilli* 5.4mm followed by *R .auranticaA* (5.1mm) and lowest recorded is *Aspergillus ristrictus* (1.2mm) (Figure.3&4C). The highest clearing zone in Procion Red MX-5B dye also recorded by *Trichosporon* begilli 2.2 mm followed by *Pencilium.digitatum* (2.4mm).Where as in Remazol yellow azo dye there is no significant clearing zone is recorded. In general the species of fungi positive for solid-plate dye decolorization test for primary screening were *T.begilli, R .auranticaA, Candida palmioleophila, Cryptococus terrus, Yarrowia lipolytica, Cryptococcus albidus,Aspergillus ochraceus, Aspergillus restrictus, Aspergillus carneus, Penicillium brevicompactum Penicillium roqueforti, Penicillium digitatum, Fusarium javanicum.* This result also supported by that *T. beigelii* could eliminate almost completely the color with significant reduction in TOC. TLC, HPLC and FTIR analysis confirmed the biodegradation of Navy blue. *T. beigelii* gave the better performance on the decolorization along with a 95% TOC reduction within 24 h [40]. One study indicates that clearing zone in azodye by *Aspergillus fumigatus* and *Candida albican* decolorization on carbon fuchsine clearance of 5mm and 20mm in diameter respectively recorded [41]. (The lignin-degrading enzymes in *Aspergillus spp.* are directly involved not in the degradation of lignin in their natural ligninocellulose substrate but also in their degradation of various xenobiotic compounds, inclusive of dye [42,43]. who reported that A. niger, *A. flavus and Penicillium sp.* are active azo dyes-degrading fungi.

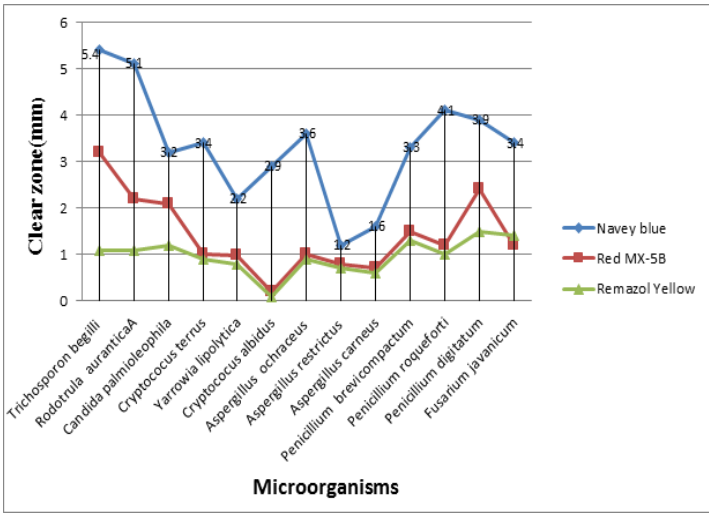


**Figure 3:** Solid plat agar dye decolorization or clearing index

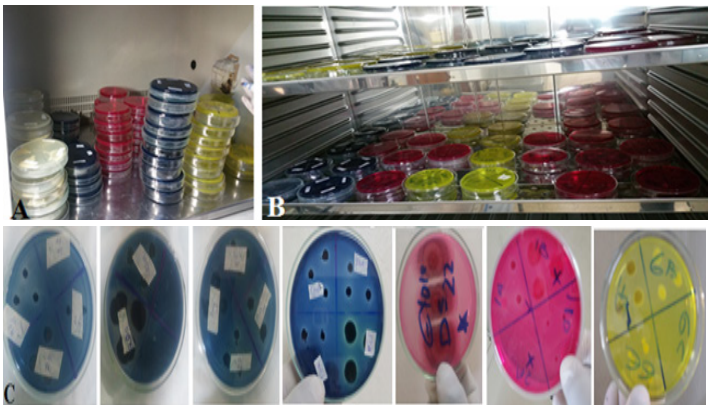


**Figure 4:** A:-Prepared solid agar dye plate and pure fungal inoculant in the biosafety cabinets. B:- inoculated solid agar plate during incubation time in laboratory incubator. C:-Clearing zone formation pattern around fungi colony in the Navey blue, Procion red MX-5B, Remazol yellow dye containing plate agar.

## Decolorization at Different Temperatures Test Result

To study temperature requirement for the best decolorization activities of the fungal species, different temperatures 28ºC, 37ºC and 45ºC were checked on solid agar plate decolorizing test. The clearing zone in diameter was recorded at by *Trichosporon begilli (5.4mm), Penicillium roqueforti(4.1mm), Penicillium digitatum(3.9mm), Aspergillus ochraceus (3.6mm), Cryptococus terrus(3.4mm), Cryptococus albidus(2.9mm)* at 37oC. The maximum clearing diameter 5.4mm recorded by *Trichosporon begilli* on Navey blue azo dye and Red MX-5B (3.2mm) at 37ºC.where as *Rhodotrula aurantica A, Yarrowia lipolytica, Penicillium brevicompactum, Fusarium javanicum* best decolorize at 28$^0$C where the higher clearing diameter on Navy blue was recorded by *Rhodotrula aurantica* A,(5.1mm) at 3 days interval measure of viability and decolorizing ability on solid plate agar test. Two species *Candida palmioleophila & Aspergillus carneus* able to decolorize 3.6mm and 1.6mm diameter Navy blue at 45ºC (Figure.5). In this study Navy blue preferentially degraded by fungi than Remazol yellow& Procion red MX-5B dye and fungi require different range of temperature for degradation (Table.1).

Maximum degradation was seen at 37ºC by *Trichosporon begilli* .This may be owing to a greater production of enzymes and optimal growth conditions of the isolate for its dye decolorizing ability. The temperature also plays a crucial role for the physiological performance of microbial cultures there by affecting the rate of dye decolorization [14]. The temperature exerts a major effect on the efficiency of dye decolorization and the optimum condition varies between 30-40$^0$C However, a few studies have reported that thermophiles can degrade azo dyes at high temperatures [44-46]. report the growth of *A. carneus* is moderate from 24-26 °C and optimal from 41-42°C [47]. also report *Candida palmioleophila* can grow at 42$^0$C [48]. had reported maximum Navey color reduction with *Aspergillus niger* after 4 days of incubation period at temperatures 28 ºC and 35 ºC. The majority of microbial species degrade dyes at faster rate in a temperature range of 30–40 °C. According to decolorization of Navy blue was studied at various temperatures (30–50 °C), faster decolorization was observed at 37 °C within 24 h incubation. reported similar result with this study that *Trichosporon begilli* is a good candidate for 100% decolorization and degradation of Navy blue and this results in conclusion suggest the potential of T. beigelii for future application towards treatment of real dye bearing wastewaters by using appropriate bioreactor [40,49]. performed study on decolorization of Remazol brilliant blue R azo dye using crude laccase which was obtained from white rot fungus *Ganoderma lucidum*. They achieved about 50.3% decolorization at temperature and pH 35 °C, 4.0 [50]. work indicates that optimal temperature of 27°C was best suited for the efficient decolorization by fungi.in this study also *Rhodotrula aurantica A, Yarrowia lipolytica, Penicillium brevicompactum, Fusarium javanicum* show clearing zone around their colony and decolorize at 280C [51]. report the range of decolorization activity of different fungi for true blue with room temperature was 91.02%, 97.26%, 80.71%, 64.22% and 91.21% with *A.flavus, A.niger,Helminthosporium, Pencilium spp,* Mucor spp where *A.niger* and *Mucor sp.* were found to be the most effective decolorizer at 27 0C and 37 $^0$C respectively.

**Table 1: Fungi clearing zone diameter on 3 types of azodye at different temperature range**

| | Mean decolorizing zone in diameter on 3types of Azo dye | | | Highest clearing performance temperature(0C) | | |
|---|---|---|---|---|---|---|
| *Species* | Navey blue(mm) | Red MX-5B (mm) | Remazol Yellow(mm) | 28°C | 37°C | 45°C |
| *Trichosporon begilli* | 5.4 | 3.2 | 1.1 | | 37 | |
| *Rodotrula auranticaA* | 5.1 | 2.2 | 1.1 | 28 | | |
| *Candida palmioleophila* | 3.2 | 2.1 | 1.2 | | | 45 |
| *Cryptococus terrus* | 3.4 | 1 | 0.9 | | 37 | |
| *Yarrowia lipolytica* | 2.2 | 0.99 | 0.8 | 28 | | |
| *Cryptococus albidus* | 2.9 | 0.2 | 0.1 | | 37 | |
| *Aspergillus ochraceus* | 3.6 | 1 | 0.9 | | 37 | |
| *Aspergillus restrictus* | 1.2 | 0.8 | 0.7 | 28 | | |
| *Aspergillus carneus* | 1.6 | 0.7 | 0.6 | | | 45 |
| *Penicillium brevicompactum* | 3.3 | 1.5 | 1.3 | 28 | | |
| *Penicillium roqueforti* | 4.1 | 1.2 | 1 | | 37 | |
| *Penicillium digitatum* | 3.9 | 2.4 | 1.5 | | 37 | |
| *Fusarium javanicum* | 3.4 | 1.2 | 1.4 | 28 | | |

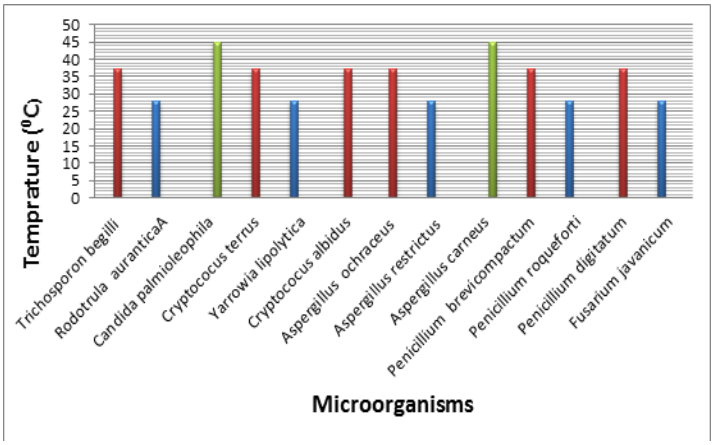


**Figure 5:** Fungi species showed clearing zone around their colony on solid agar media at different temperature range for Navy blue dye

## Decolorization at Different pH

Fungal isolates at three pH ranges 6, 9, 12 were evaluate the performance of decolonization activities. The result revealed that 11 fungi species were show best decolorization at pH 6 where as one fungi that is *Aspergillus carneus* decolorizing pH 9 and *Fusarium javanicum* decolorize at pH 12 (Figur.6) [52]. reported that the optimum pH for dye removal by using fungi was 6.0 to 8.0. Supporting evidence indicates microbial ability to decolorize Navey blue dyes in terms of percentage reduction of color was observed effectively in the pH range 4-7. Maximum dye decolorization was reported to be 93.73% and 78.4%, at pH 4.5 with *Aspergillus niger* and *Penicillium spp* respectively. These results suggested that better fungal growth usually occurs at low pH values [53]. According to pH has an effect on the activity of the fungal enzymes which is used in the decolorization of different dyes present in the effluent [54]. Above or below the optimal pH range the activity of the fungal enzymes reduces which reduced the bioremediation activity of the organisms involved.

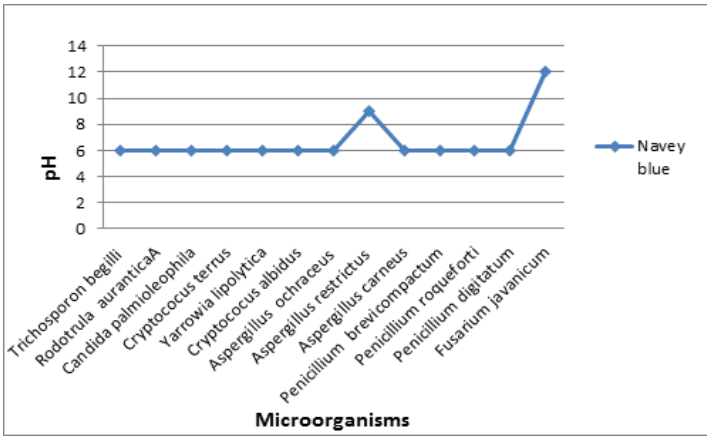


**Figure 6:** Fungi growth pH during Navey blue decolorization

## Decolorization at Different Dye Concentration

Different concentration of dye was evaluated at 100ppm, 200ppm, 300ppm, 400ppm screened &evaluated. The rate and extent of decolourization was affected by the addition of dye ranging from 100 to 400 ppm. Navy blue concentration 100 ppm gets decolorized up to 93.25% followed by 200 ppm (54.66%), 300ppm (35.50%) and 400 ppm (10.90%) The result revealed that *Trichosporon begilli, Aspergillus ochraceus, Cryptococus albidus, Penicillium roqueforti, Penicillium digitatum* were able to decolorize up to 400ppm where as the rest decolorize up to 300ppm on solid agar screening methods and dye broth assay measured by biolog turbidiometer measured. Reported the effective decolorization of Navy

blue dye by utilizing Exiguobacterium sp. when the dye concentration increase percentage of decolorizing is decreasing. 50 mg $L^{-1}$ Navey blue gets decolorized up to 91.25% followed by 100 mg $L^{-1}$ (56.66%). The percentage of decolorization of Navey blue decreased for the dye concentration beyond 100 mg l−1, as only 40 and 20% decolorization was observed after 48 h at 150 and 200 mg $L^{-1}$ dye concentration, respectively by T.begilli. The rate of decolorization decreased with increasing dye concentration [40]. Because of nutrient depletion and fungal cell death could be the reason [55]. reported the decolorization efficiency of the Aspergillus allhabadii fungus was found to be higher (90.57 ± 0.33%) with 150 mg/L under shaking condition at 30°C with glucose used as carbon source.

## Decolorization of Azo Dyes in Liquid PDA Broth Media

Thirteen positive fungus for dye decolorization on solid media was further investigated for its ability to decolorize synthetic dyes in liquid broth media. 15mL PDA broth containing each dye 100ppm/L were prepared in 25mL test tube and autoclaved at 121°C for 15 min. The result revealed that 13 fungus species their percentage of decolorization was reported based on absorbance and transmittance result of Biolog turbidimeter evaluation. Transmittance is simply [[the percentage of light impinging on a solution that passes through the solution and emerges to be detected by the instrument. It is zero for a completely opaque solution and 100% when all the light is transmitted. Six fungi strains, i.e., *Trichosporon begilli, Rhodotrula aurantica A, Penicillium roqueforti, Penicillium digitatum, Fusarium javanicum Aspergillus ochraceus* 72-92% transmittance effective in decolorizing of Navey blue, four species, *Candida palmioleophila, Cryptococus terrus, Yarrowia lipolytica, Cryptococus albidus*, 34-71% decolorizing ability of Navy blue. 3 species, *Penicillium brevicompactum, Aspergillus carneus,, Aspergillus restrictus* showed 16-29% decolorizing ability Navy blue where all species did not show significant decolorization on Remazol yellow & Procion red MX-5B.(Figure,7,A,B,C) [56]. report among different fungal strains, *Aspergillus foetidus* was found to be effective (> 95% within 48 h) in the decolorization of azo reactive dyes. also reported that *Aspergillus flavus* and *Penicillium canescens* were found to be the best isolated fungi for decolorization of direct blue dye [57]. It was found that A. flavus exhibited the highest decolorization activity 92%, followed by *P. canescens* 89% respectively [58]. report that lignolytic *P. ostreatus, P. sapidus* and *P. florida* to 20 ppm azo dye concentration shows 88, 92 and 98 % decolorization respectively. This study is corresponds with the report of that Navy blue was decolorized completely within 24 h by *T. beigelii.* Decolorization performance of Navy blue was 100% under static condition and 30% under shaking condition [40]. Decolorization performance of Navy blue by using five different microorganisms: *T. beigelii* (100%), *Y. lipolytica* (42%), *B. megatarium* (38%), *C. blayota* (40%), *Acinetobacter sp.* (38%), were reported by [40]. In this study among the three azodye, the Navy blue potentially decolorized up to 92% by *T. beigelii*. There for *T. beigelii.* could be a good candidate for mycoremediation of Navey blue pollution (Figure 8 D& E). Different fungi species do have ability to degrade different groups of azodye [59]. performed studies on decolorization of disperse red 3B dyes. They used a consortium of fungus and microalgae. Fungus used was *Aspergillus sp*. XJ-2 and micro algae used was *Chlorella sorokiniana* XJK. The consortium of fungus and algae showed better result for decolorization of azodye.

Their experiment showed 98.09% decolorization under optimized conditions for the use of consortium of fungus and algae. A study was performed by on decolorization of a textile dyes, namely, Remazol brilliant blue royal. using Coriolus versicolor and Pleurotus ostreatus and they found the decolorization of remazol brilliant blue royal as 80.42% and 70.42% for fungi Coriolus versicolor and Pleurotus ostreatus, respectively at optimized level [60,61]. performed a decolorization study on azo dye degradation by isolate Aspergillus niger D2-1. Results of maximum decolorization by Aspergillus niger D2-1 using 100 ppm concentrations of two azo dyes, namely, reactive yellow and reactive red for decolorization in the presence of glucose (as carbon source) and yeast extract (as nitrogen source) in seven days at pH 9.0 were 98.62 % and 92.42 %, respectively. They found that fungus decolorized textile waste water effluent in promising way up to 59%. Recently isolated Hypocrea koningii was found to be most potent decolorizer of five textile azo dyes (Red HE7B, Reactive Violet 5, Red Black B, Light Navy Blue HEG, Dark Navy Blue H2GP) [39].


**Figure7:** Azo dye degradation at the first day A:-Decolorization of Navey blue, Procion red MX-5B, Remazol yellow dye at the first day. B: - Decolorization of Navey blue, at 5th day.C:- Decolorization of Navey blue, at the end of 9th day

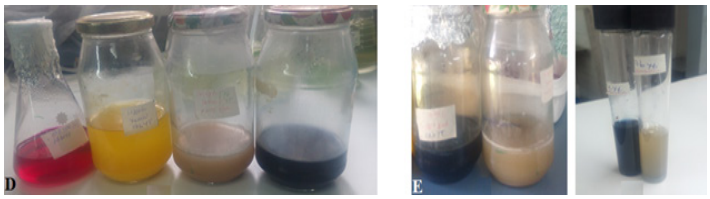


**Figure 8:** Azo dye degradation at the end of eight day with control group. D. Decolorization of Navey blue, Procion red MX-5B, Remazol yellow at the end 9 th days in 100mL broth by the top

*Trichosporon begilli,* showing high clearing diameter on solid media E. Complete decolorization of Navey blue by. *Trichosporon begilli* in100mLbroth & in 15mLtest tube at the end of eight dyes with control group.

## Macro and Micromorphology Characteristics of Selected Dye Decolorizer Fungi

Fungi show different colony morphology and color depending on the types of growth media. Their shape, size, elevation, reverse side, mycelium color, margin and color of the colony were observed on PDA growth agar media. Macro morphological results were noted for azo dye degrader fungi down in summarized table 2& 3 (Figure. 9). The cellular morphology were stained by using lacto phenol blue and observed by microscopic observation. Its conidia shape, conidia color, hyphae morphology were noted. Table 4 Summarize the result.

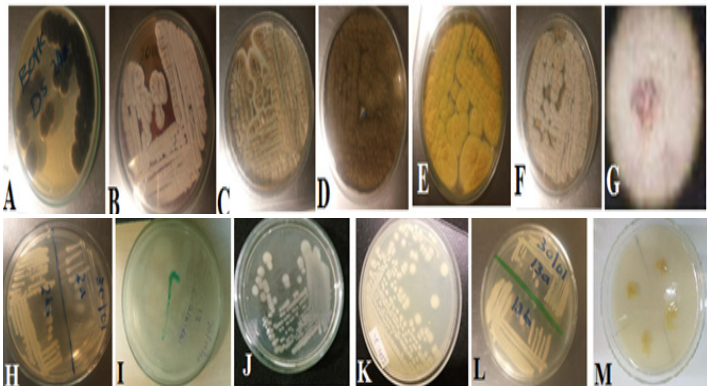


**Figure 9:** Cultural characteristics of fungi Screened fungi **A**. *Penicillium roqueforti* **B**, *Aspergillus carneus* **C**. *Penicillium digitatum* **D**. *Aspergillus restrictus* **E**. *Aspergillus ochraceus* **F**. *Penicillium brevicompactum,* **G**. *Fusarium javanicum* **H** *Yarrowia lipolytica* **I**, *Cryptococus albidus* **J** *Rodotrula auranticaA*,**K**., *Cryptococus terrus,* **L**.,*Candida palmioleophila*, **M**., *Trichosporion begilli.*

**Table 2: Macro morphology of yeast**

| Species | Shape | Elevation | Size | Margin | Texture | Color |
|---|---|---|---|---|---|---|
| *Trichosporion begilli* | Irregular | Flat | Large | Lobate | Concentric | White yellow |
| *Cryptococus albidus* | Round | Pulvinate | Large | Entire | Radiate | White yellow |
| | | | | | | |
| *Rodotrula auranticaA* | Round | Flat | Large | Undulate | Radiate | White |
| *Cryptococus terrus* | Round | Flat | Large | Undulate | smooth | white |
| *Yarrowia lipolytica* | heavily convoluted | uneven mounds | Large | ridges | wrinkles | white to cream-coloured |
| *Candida palmioleophila* | | | large | lobate | Smoth | White yellow creamy |

**Table3. Macro morphology of filamentous fungi**

| Species | Surface color | Marigin | Revers side | Elevation |
|---|---|---|---|---|
| *Aspergillus ochraceus* | yellow to pale orange | Velvety smooth | gray gold | Flat |
| *Aspergillus restrictus* | Blackish brown gray | Velvety smooth | dark gray | Flat |
| *Aspergillus carneus* | White brown yellow | wrinkled | Dull yellow brown to victoria lake | Flat |
| *Penicillium brevicompactum* | White green | wrinkled | White Brown | Flat |
| *Penicillium roqueforti* | Blackish green | Smooth velvety | White green | Flat |
| *Penicillium digitatum* | yellow-green floccose texture | Smooth velvety | olive green | Flat |
| *Fusarium javanicum* | White pink | Smooth velvety | white | Flat |

**Table 4: Micromorphology of fungi**

| Species | Hyphae | Seration | Condia Heads color | Condia Heads/shape |
|---|---|---|---|---|
| *Trichosporion begilli* | - | - | - | - |
| *Cryptococus albidus* | - | - | - | - |
| *Rodotrula auranticaA* | - | - | - | - |
| *Cryptococus terrus* | - | - | - | - |
| *Yarrowia lipolytica* | - | - | - | - |
| *Candida palmioleophila* | - | - | - | - |
| *Aspergillus ochraceus* | unsepted conidiophores | biseriate and hyaline | globous pale yellow conidia | Columnar conidial heads |
| *Aspergillus restrictus* | Septated | Mono seretted | Covered in slime, blackish green-gray | Conida are nearly spherical to elliptical and are rough and spinulose |
| *Aspergillus carneus* | Septated | Biserated globese elongated | Cream color, pinkish buff or near dark olivebuff | Distinct globose conidia head , Radiate |
| *Penicillium brevicompactum* | Septated | Biserated | White color | Apically inflated |
| *Penicillium roqueforti* | Septated | Mono seretted | White green | Round shape |
| *Penicillium digitatum* | Septated | flask-shaped to cylindrical | White brown conidia | chains of single-celled conidia |
| *Fusarium javanicum* | Non Septated | Sereted | White brown | Selender macro conidia |

## Identification of Fungi Species Using Biology MicroStation

Morphologically identified and evaluated fungi for their dye decolorization finally goes to species identification using Biolog Microstation(Figure.10) FF/YT Micro Plate was read by the Micro Station Reader at 24 h, 48 h, and 72 h at a single wavelength of 590 nm, results were recorded by micro plate reader and processed for identification by micro log3 software ver. 4.20.05 (Biolog, Hayward, CA). A similarity index calculated based on the reaction profiles [34]. By comparing with the fungi database (MicroLogTM-SystemRelease 4.2 User Guide 2001, Biolog). The result revealed that 13 fungi species were identified associated with textile effluent waste water. using Biolog Microstation where representative fungi consisting of 7 filamentous fungi and 6 yeast species All identified fungi summarized in table 5. The similarity index is value is greater than 0.5 and probability >75 is an accepted result (Table. 5). According to the report of Ascomycota was the largest phyla followed by Basidiomycota [55]. *Hygrocybe sp., Sporobolomyces sp., Rhodotorula sp., Stemphylium sp., Parascedosporium sp*., and Cylindrocarpon sp., were found to have statistically significant in waste water. *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* were dominant species in all wastewater. According to report fungal strains isolated from waste water from textile industry samples revealed the presence of *Rhizopus spp, Aspergillus spp., Penicillium spp., Candida spp., Drechslera spp., and Rhodotorula spp., Aspergillusspp., and Rhizopus spp* [56,36]. report that fungi species identified from textile industries are *Fusarium camptocera, Gibberella baccata, Aspergillus fumigatus, Aspergillus niger, Aspergillus phoenicis, Aspergillus terreus, Penicillium digitatum, Penicillium implicatum, enicillium lenosum, Penicillium oxalicum and Penicillium verrucosum.*


**Figure 10:** Biology Microstation identification equipment

**Table 5: Biolog micro station species identification result**

| Species | Similarity | Probability | Species identified site/sampled area |
|---|---|---|---|
| *Trichosporion begilli* | 0.65 | 100 | Kombolcha textile treatment plant |
| *Cryptococus albidus* | 0.64 | 100 | Eyoule river down stream |
| *Rodotrula auranticaA* | 0.54 | 99 | Borkena river down stream |
| *Cryptococus terrus* | 0.55 | 98 | Worka river down stream |
| *Yarrowia lipolytica* | 0.57 | 100 | Kombolcha textile treatment plant |
| *Candida palmioleophila* | 0.55 | 100 | Kombolcha textile treatment plant |
| *Aspergillus ochraceus* | 0.5 | 92 | Kombolcha textile treatment plant |
| *Aspergillus restrictus* | 0.56 | 91 | Kombolcha textile treatment plant |
| *Aspergillus carneus* | 0.6 | 92 | Kombolcha textile treatment plant |
| *Penicillium brevicompactum* | 0.51 | 97 | Kombolcha textile treatment plant |
| *Penicillium roqueforti* | 0.57 | 91 | Kombolcha textile treatment plant |
| *Penicillium digitatum* | 0.62 | 94 | Kombolcha textile treatment plant |
| *Fusarium javanicum* | 0.59 | 95 | Kombolcha textile treatment plant |

## Conclusion

These results indicate that Azo dyes are decolorized easier and faster by fungi within 9 days. Among the 13 fungal strains tested depending on qualitative evaluation on solid agar plate test and degradation percentage depending on biolog turbidiometer measure *Trichosporon begilli, Rhodotrula auranticaA,, Penicillium roqueforti, Penicillium digitatum, Fusarium javanicum* 72-92% showed promising result in Azodye decolorization. Among the three azodye Remazol yellow, Navey blue, Procion red MX-5B,the Navey blue showed good degradation by *Trichosporon begilli, Rhodotrula auranticaA,, Penicillium roqueforti, Penicillium digitatum, Fusarium javanicum. However Trichosporon begilli* performance in Navey blue was superior. These could be a good candidate for mycoremedition service after further degrediant toxicity analysis. The biolog microstation able to identify 13 fungi from industry waste effluent at species level *Trichosporon begilli, Aspergillus ochraceus, Cryptococus terrus, Cryptococus albidus, Penicillium roqueforti, Penicillium digitatum, Candida palmioleophila, Cryptococus terrus, Yarrowia lipolytica, , Penicillium brevicompactum, Aspergillus carneus,, Aspergillus restrictus, Fusarium javanicum.* Fungal based decolorization may be performed in two ways. In first way, a fungi or group of fungi may be applied directly for the decolorization of dyes and second way, there enzymatic liquid culture medium may also applied to dyes.

## Recommendation

This result is depending on qualitative evaluation test on solid agar plate decolorization and biolog turbidiometer turbidity measure for identification purpose. Further degradation ability for other types of azodye could be done evaluated and supported by UV-spectrophotometer analysis. Optimized with other parameters at large scale. Some extremophile fungi must be isolated and evaluated from extreme environment because of their enzyme capacity for degradation.


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