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Fungal decolorization of dye water

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ABSTRACT

The Study was under taken to assess the decolourisation potential of selected microbial species (fungal) to an actural textile dyeing industry effluent. All the microbes tested namely Aspergillus flavus, Aspergillus niger, Candida albicans . polyporus borealis, Pholiota squarrosa, Omphalotus olearius, Armillarria mellea, Pholiota mellea and Trametes vuillosa (fungal) showed greater potential in decolorizing the synthetic dyes. Microbial degradation of textile effluents were critically analysed and discussed. The outcome of the study contributes in taking microbial dye remediation from laboratory to field conditions.

INTRODUCTION

Dye production in India is estimated to be around 64,000 tonnes, which is about 6.6% of the world production. There are around 700 varieties of dyes and dye intermediates produced in India, mainly direct dyes, acid dyes, reactive dyes and pigments. Most of these dyes have not been characterized regarding their chemical nature, purity, possible toxicity or their impact on health and the environment. yet, they are widely used by textile, leathe, paint and even the food industry. The textile industry in India alone consumes upto 80% of the total dyestuffs produced.

The textile industries produce effluents that contain several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes (Cooper, 1995). In many Nigerian cities, the textile factories daily discharge millions of litres of untreated effluents in the forms of wastewater into public drains that eventually empty into rivers (Olayinka and Alo, 2004). This alters the pH, increase the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and gives the rivers intense colourations (Ajayi and Osibanjo, 1980).

Several methods are used in the treatment of textile effluents to achieve decolourization. These include physiochemical methods such as filtration, specific coagulation, use of activated carbon and chemical flocculation. Some of these methods are effective but quite expensive (Do et al., 2002; Maier et al., 2004). Biotreatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluents. The chemical nature of dyes varies, but azo dyes are the most widely used.

A larger proportion of these are azo dyes which can pass through normal water treatment procedures (Stolz, 2001; Pearce et al., 2003; Pandey et al., 2007) resulting in aesthetically unappealing water. In general, complete azo dye mineralization requires both anaerobic and aerobic bacterial process. Azo bonds are reduced under anaerobic conditions leading to generation of aromatic amines. Although laccases can be used for aerobic azo dye reduction,

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they polymerise the aromatic amines leading to secondary colour development and pollutant build-up (Zille et al., 2005).

It is reported that aromatic amines are toxic, carcinogenic and recalcitrant to anaerobic degradation and usually require complete degradation under aerobic conditions (O' Neill et al., 2000; Pearce et al; 2003; I_ik and Sponza, 2007).

Dye is added to the cultures and the loss of color is monitored over a period of days; thereby, color change is an indicator of the rapidity of the process while making comparisons between species. Azo dyes, as the mainly dyeing material in the world (Yuzhu, 2001, PP.251-262) , are often used in the colouring process of several textiles, dyestuff and paper –making products, relatively recently it has been recognized that some azo dyes agents may bring a large amount of waste water, which flow abroad in aqueous solutions. A major source of pollution as 88% of its raw materials are converted into waste and discharged into the water bodies, causing water pollution.

Adequate treatment of textile effluent requires more than one stage as there is need for both colour removal and degradation of aromatic compounds from the decolourisation process. Physico – chemical treatment methods are the least desirable owing to their high costs and generation of secondary pollutants.

The oxidative decolourzations of dyes of several classes have been reported and azo dyes were found to be the most recalcitrant compounds. (Maeir et al., 2004). The decolourisation of azo dyes has been found to be effective under anaerobic conditions. The anaerobic degradation yields aromatic amines which are mutagenic and toxic to humans and cannot be metabolized further under the conditions which generated them (Chung and Stevens, 1993; Do et al., 2002). in activated sludge treatments of dye effluents, reactive azo dyes and aromatic amino derivatives are a non- biodegradable class of compounds which can even inhibit activated sludge organism (maeir et al., 2004)

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MATERIALS AND METHODS:

The present study has been carried out to find the suitable bioremediation measures of textile dye effluent by using Biological and chemical agents.

Sample Collection :

The dye effluent was collected from the Balamalpuram of East Karur. The samples were collected by sing sterile sample bottles and transported to the laboratory within 24 hrs for collection. The collected samples was stored at low temperature for further analysis.

Enumeration of total microbes in the dye effluent sample:

Total microbial counts in the dye effluent sample serially diluted (10^{-1} to 10^{-9}). One ml from the dilution 10^{-3} was plated in Nutrient agar plates, using spread plate method and incubated at 37°C for 24 hrs for fungal counts and PDA agar for fungal counts incubated at 28°C for 48 hrs. Colonies on the plates were counted by using colony counter.

Enumeration of dye degrading microbes:

The dye effluent sample was serially diluted. One µ1 of sample from dilution 10-3 was plated on pre dried mineral salt agar using spread plate technique and PDA agar. A filter paper saturated with sterile dye effluent sample was aseptically placed on the inside fo the inverted

petridishes and the culture plates were incubated for 4 days. Plates yielding 30 to 250 colonies were enumerated for bacterial isolates and 15-105 colonies for fungal colonies.

isolation and Indentification of fungal sample:

The fungal cultures were isolated and identified by pure culture and Biochemical methods. Fungal culture isolated from dye effluent sample, dilution 0.1ml of sample was inoculated on Nutrient Agar plates and kept for incubation at 37° C for 24 hrs.

By the presence of growth away from the line of inoculation whereas non-motile organisms grow only the initial stab line.

Biochemical tests

Catalase test

Catalase test was used to detect the presence of the enzymes Catalase. A capillary tube was dipped into 3% H2O2 and the colony was touched. There was bubble formation, it was positive.

Citrate utilization

The organisms were streaked onto Simmons Citrate agar plate and incubated at 37oC for 48 hours. The results were interpreted based on the change of colour from initial green to deep blue if it was positive.

Indole test

Tryptone broth was inoculated and incubatedat 37oC for 48 hous and added Kovac's reagent and read immediately. The results were interpreted based on the change of colour from yellow to cherry red ring formation.

Methyl red test (MR)

Buffered glucose broth was inoculated and incubated at 37oC for 48 hours. A few drop of methlyl red solution was added to culture and the results were read immediately. The results were interpreted based on the change of colour from yellow to red.

Voges Proskauer test (VP)

The organisms were inoculated in buffered glucose broth and were incubated at 37oC for three days and 3ml of alpha napthol was added followed by 1 ml of 40% KOH. It was mixed well and allowed to stand for 30 min. The results were interpreted based on the change of colour to pink.

ISOLATION AND IDENTIFICATION OF FUNGUS:

Fungus was isolated from dye effluent sample, after serial dilution 0.1 ml of sample was inoculated on Agar plates (Potato Dextrose Agar, Rose Bengal Agar), and kept for incubation at 24oC for 48 hrs. Then the colony appearance was noted, for identification of fungus LCB (Lacto phenol Cotton Blue) staining was performed.

LPCB stain is for examination of fungal material. Phenol kills the fungi, and the lactic acid increases preservation chino (Cotton blue is a stain for chitin and cellulose.

Assay of decolorisation activity

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The fungal stains (aspergillus flavus, Aspergillus niger, Candida albicans polyporus borealis, Pholiota squarrosa, Omphalotus olearius, Armillarria mellea, Pholiota mellea and Trametes vuillosa) and Bacterial strains were grown on PDA agar plates and were streaked on plates containing dyes in media. The plates contained MM2 – Carbon, MM2 – Nitrogen, MM2 – Nitrogen – Carbon. Decolorisation of the dye was visually observed for the extent of zone clearing on the plates.

The extent of dye decolorization by the microbial cultures in broth was determined by spectrophotometer at the maximum absorbance of the respective dyes in the cell free extracts. The percentage of dye decolorisation by the cells was done using the modified method of Yatome et al., (1991).

Cultures were grown in 50ml of broth for overnight at 37oC and 80 rpm to an OD of 1.00 at 600nm. The cultures were centrifuged at 10,000 rpm for 10 min. and washed twice with sterile saline (0.85%) and resuspended in 10 ml of saline solution. 0.1ml of the inoculums was added to the broth containing dye and incubated at 370C, 85 – 100 rpm for 24 hr. The supernatant was collected after centrifugation for absorbance measurement at respective wavelengths. The percentage declolorization was calculated as follows.

$$\% \text{ of Decolorization} = \frac{\text{Initial O.D} - \text{Final O.D x 100}}{\text{Initial O.D}}$$

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Sample Analysis :

Analysing of physic – chemical parameters of the dye effluent sample:

The dye effluent sample was used for various Physico – chemical analysis viz., colour of the sample, Odour by direct smelling of the sample, standard thermometer was used for temperature measurements, The pH of the sample was determined by pH meter.

ADMI color was determined with a Spectrophotometer in accordance with the ADMI Tristimulus method 2120 D detailed in Standard Methods (1998). The spectrophotometer was calibrated before each use with standard platinum cobalt color solution of 100, 200, 300, 400, and 500 ADMI color units. (Edwards, 2000). Biological oxygen demand (BOD), Chemical Oxygen Demand (COD) were analysed.

BIOLOGICAL OXYGEN DEMAND (BOD):

Adjust the pH of water sample to neutrality using 1N acid or 1N alkaline solutions. Fill the water sample in 6 BOD bottles without bubbling. Add 1 ml of Allyl thiourea to each bottle. Determine dissolved oxygen content in 3 of the 6 BOD bottles by titration method. Take the mean of the 3 redings (D1). Incubate the rest of 3 BOD bottles at 27oC in a BOD incubator for 3 days. Estimate the oxygen concerntration in all the 3 incubated samples. Take the mean of 3 readings (D2). Calculate the BOD of water in mg/1 by using the following formula

BOD (mg/1) = D1-D2

Where,

D1 = Initital dissolved oxygen in the sample (mg/1)

D2 = Dissolved oxygen in the water sample (mg/1) after 3 days of incubation.

CHEMICAL OXYGEN DEMAND (COD) :

Take three, 100ml conical flasks and pour 50 ml of water sample in each (i.e., in triplicate). Simultaneously rund distilled water blank standards (also in triplicate). Add 5ml of potassium dichromate solution in each of the 6 flasks. Keep the flasks in water bath at 100oC for 1 hour. Allow the samples to cool for 10 minutes. Add 5 ml potassium iodide solution in each flask. Add 10ml of H2SO4 in each flask. Titrate the contents of each flask with 0.1 M of sodium thiosulphate solution until the appearance of pale yellow colour. Add 1 ml of starch solution to each flask (solution turns blue colour). titrate the again with 0.1 M sodium thiosulphate until the blue colour disappears completely. Find out the COD (mg/l) of the water sample by using the following formula

$$\text{COD of the smaple (mg/l)} = \frac{8 * C * (V_B - V_A) * 1000}{S}$$

Where,

C = Concentration of titrant (0.1 M)

V_B = Volume of titrant used for smaple (ml)\

V_A = Volume of titrant used for blank (ml)

S = Volume of sample taken (ml)

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RESULTS and DISCUSSION :

Sample collection :

The textile dye effluent sample was collected from Sengunthapuram, karur Dt., Tamil Nadu.

Sample : Textile dye effluent

Color : Wine red color

Odour : Alkali odour.

Enumeration of total and dye utilizing microbes in the dye effluent samples:

The count of total fungal counts in dye effluent sample ranged from 1.98×10^3 CFU_g^{-1}

S.No.	Sample	Microbial Counts (CFU g^{-1})
1	Fungal sample	1.98×10^3

Fungal colonies:

Fungus was isolated from dye effluent sample, after serial dilution 0.1ml of sample was inoculated on A gar plates (Potato Dextrose Agar), and kept for incubation at 24oC for 48 hrs. Then the colony appearance was noted, for identification of fungus LCB (lacto phenol Cotton Blue) staining was performed.

LPCB stain is for examination of fungal material. Phenol kills the fungi, and the lactic acid increases preservation chino (Cotton) blue is a stain for chitin and cellulose.

Based on the morphological stuructures they are identified as Aspergillus flavus, Aspergillus niger, Candida albicans, polyporus borealis, Pholiota squarrosa, Omphalotus olearius, Armillarria mellea, Pholiota mellea and Trametes vuillosa

DEGRADATION ANALYZING :

For Fungi :

The isolated fungal samples Aspsergillus flavus, Aspergillus niger, Candida albicans, polyporus borealis, Pholiota squarrosa, Omphalotus olearius, Armillarria mellea, Pholiota mellea and Trametes vuillosa were checked for the extent of dye degradation both in solid media plates as well as in liquid media. All the isolates gave maximum degradation. Visual degradation indicated that degradation was higher in case of broth as compared to minimal media.

The overnight broth cultures (Aspergillus flavus, Aspergillus niger, Candida albicans. polyporus borealis, Pholiota squarrosa, Omphalotus olearius, Armillarria mellea, Pholiota mellea and Trametes vuillosa) with dye effluent were centrifuged and resuspended in 10ml of saline solution and 0.1 ml of the inoculums was added to the broth containing crude oil and incubatedat 24oC, 85-110 rpm for 48-96 hrs. The supernatant was collected after centrifugation for absorbance measurement at 650 nm wavelengths. Among the three isolated fngal strains Aspergillus flavus, Aspergillus niger candida albicans showed higher degradation than other six isolates. The percentage degradation was calculated given in Table-1.

After inoculation of isolalated fungal species, the pH, COD and BOD were analyzed for 2 weeks.

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The BOD variations, COD variations and decolorization % were analyzed by isolated 9 fungal species and reported in the tables 1,2&3.

Tabel - 1 Fungal Decolorization

Sl.No	Fungal cultures	Percentage degradation (%)
1	Aspergillus flavus	74
2	Aspergillus niger	89
3	Candida albicans	67
4	polyporus borealis	52
5	Pholiota squarrosa	65
6	Omphalotus olearius	42
7	Armillarria mellea	35
8	Pholiota mellea	45

9	Trametes vuillosa	37

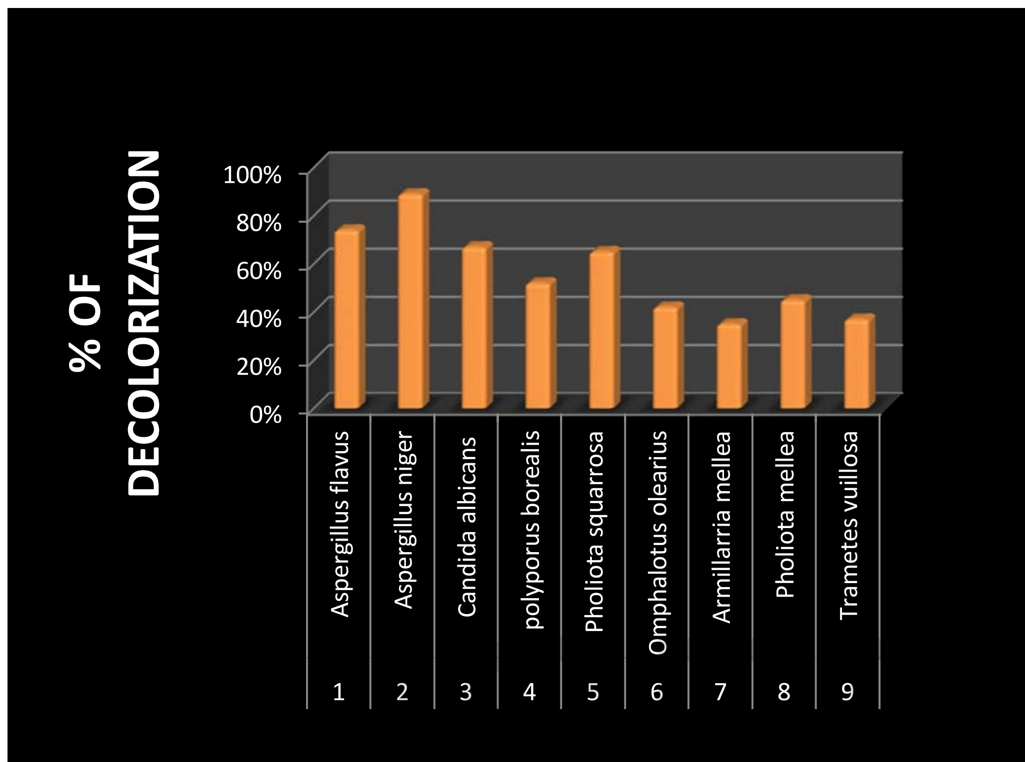

Fig-1 Fungal decolorization

Table-2 COD REMOVAL BY FUNGAL STRAINS

S.NO	Fungal strain	COD mg perllitre					
		FIRST DAY	3 DAYS	6 DAYS	9 DAYS	12 DAYS	%OF COD REMOVAL
1	Aspergillus flavus	938	620	522	354	252	73.13
2	Aspergillus niger	938	595	498	327	225	76.01
3	Candida albicans	938	692	574	425	312	66.73
4	polyporus borealis	938	774	692	617	554	40.93
5	Pholiota squarrosa	938	803	757	698	632	32.62
6	Omphalotus olearius	938	749	664	532	454	51.59
7	Armillarria mellea	938	891	754	676	641	31.66
8	Pholiota mellea	938	774	689	616	532	43.28

9	Trametes vuillosa	938	835	760	669	578	38.37

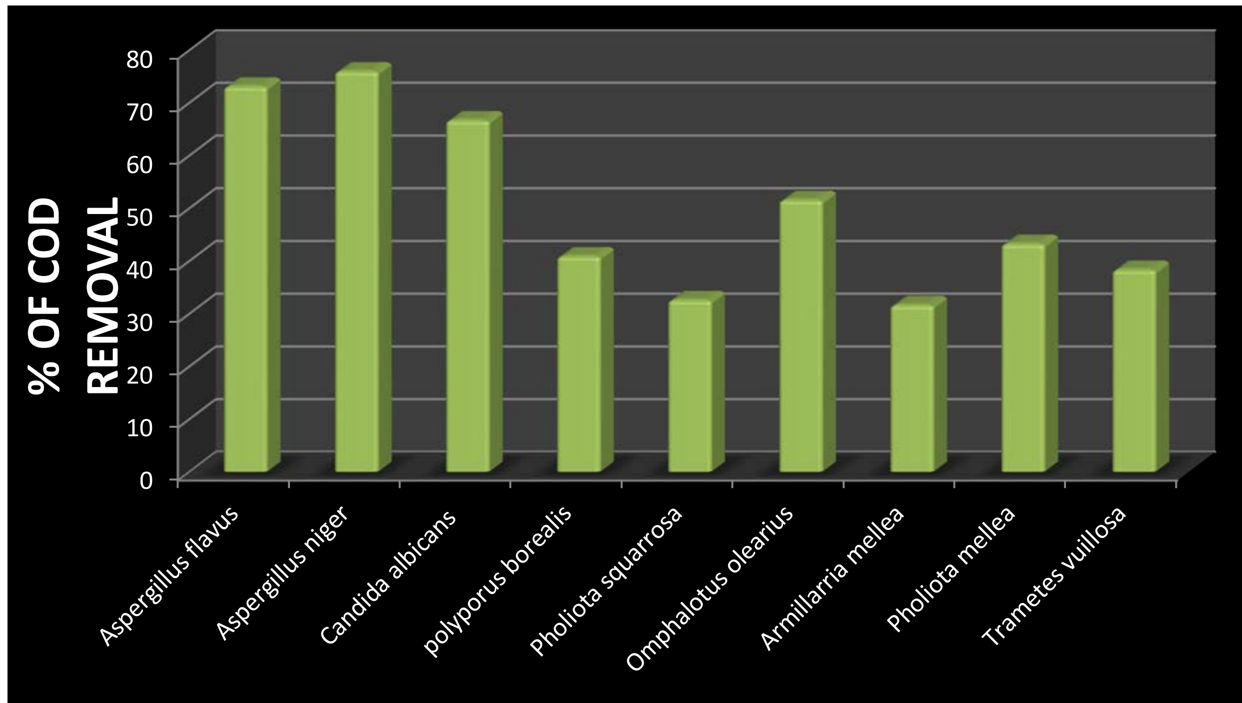

Fig-2 COD Removal By Fungal Strain

Table -2 BOD Removal by Fungal strain

S.NO	Fungal strain	BOD mg perlitre					
		FIRST DAY	3 DAYS	6 DAYS	9 DAYS	12 DAYS	%OF BOD REMOVAL (after 12days)
1	Aspergillus flavus	47	37	36	28	22	53.19
2	Aspergillus niger	47	35	33	24	18	61.7
3	Candida albicans	47	39	37	29	20	57.44
4	polyporus borealis	47	38	35	31	27	48.93
5	Pholiota squarrosa	47	41	36	32	29	38.29
6	Omphalotus olearius	47	42	39	34	26	44.68
7	Armillarria mellea	47	39	34	28	24	48.93
8	Pholiota mellea	47	45	39	31	28	40.42

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9	Trametes vuillosa	47	43	35	30	25	46.8

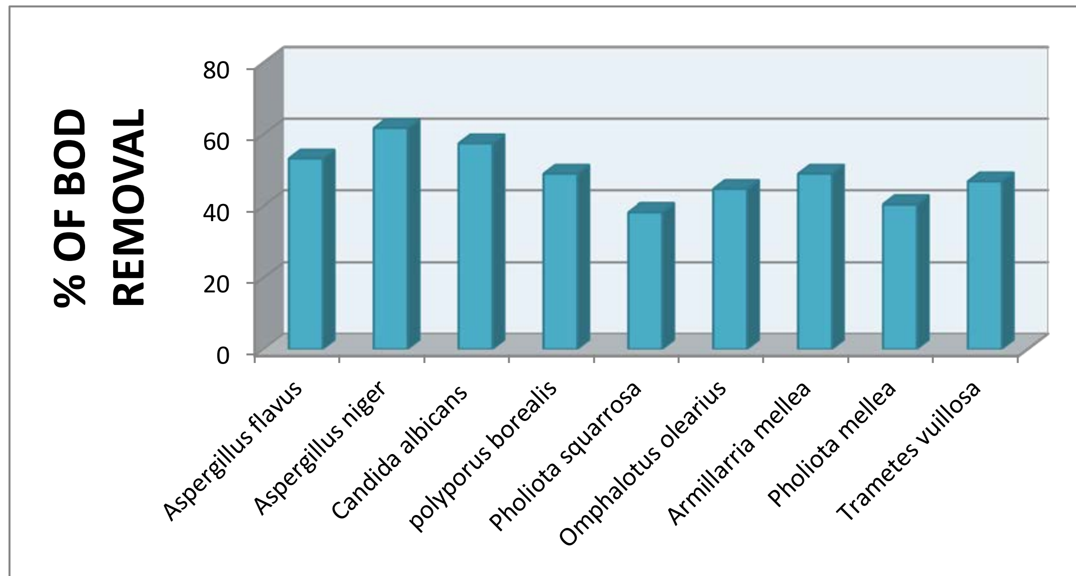

Fig -2 BOD REMOVAL BY FUNGAL STRAINS

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DISCUSSION ;

For Fungi:

The isolated nine fungal samples, Aspsergillus flavus, Aspergillus niger, *Candida albicans,* were checked for the extent of dye degradation both in solid media plates as well as in liquid media. All the isolates gave maximum degradation. Visual degradation indicated that degradation was higher in case of broth as compared to minimal media.

The overnight broth cultures (*Aspergillus flavus, Aspergillus niger, Candida albicans* polyporus borealis, Pholiota squarrosa, Omphalotus olearius, Armillaria mellea, Pholiota mellea and Trametes vuillosa) with dye effluent were centrifuged and resuspended in 10 ml of saline solution and 0.1ml of the inoculum was added to the broth containing crude oil and incubated at 24°C, 85 – 110 rpm for 48-96 hrs. The supernatant was collected after centrifugation for absorbance measurement at 650 nm wavelengths. Among the nine isolated fungal strains, Aspergillus niger(89%) showed higher decolorization than other eight isolates.

Hence in order to obtainin an efficient dye removal, microorganisms may be better obtained from similar effluent as these are more accustomed to the type of dyes present and extreme conditions of effluent like alkalinity, high temperature, BOD, TDS etc., Kilic et al. Degradation of azo dyes by micro-organisms has been extensively documented.

Azo dyes can be degraded by ligning degrading fungi, white rot fungi, bacterial peroxidases, aerobic and anaerobic bacteria possessing azoreductases, and anaerobic bacteria capable of producing reduced flavins and hydroquinones (Stolz 2001 ; Cheng 2006). Bacterial biodegradation of azo dyes is often initiated by cleavage of azo bonds by azoreductases which

are followed by the aerobic degradation of the resulting amines (Stolz 2001). Fungal degradation of azo dyes mainly occurs from the ligning peroxidase activity under aerobic conditions (Cheng 2006).

Aspergillus niger had higher COD removal % of 76.01 and BOD removal % 61.07

Than other eight fungal strains.

CONCLUSION:

Although decolorization is challenging process to textile dye wastewater, the result of this findings and literature suggest a great potential for fungal to be used to remove color from dye effluent. This investigation had established that the fungal strains were adaptive in nature and can decolorize.

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