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Research Article

A Preliminary Study on Mycodegradation Azo dye

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Abstract

The present investigation was carried out to find out the various industrial used azo dyes degradation by different groups of fungi. The soil samples were collected from two different systems like rhizosphere and industrial effluent. Soil samples were subjected to serial dilution and plating techniques to isolate the fungi. The isolated fungi were identified by lactophenol cotton blue technique. Azo dyes like Crystal violet, malachite green and methyl red were selected in the study. Dye degradation studies were carried out by two different methods such as solid medium and decolorization by liquid medium and Dye decolourization were calculated. Totally 23 different fungi were isolated from both soil samples. In this, *Aspergillus* was the common genera among the fungal isolated. Species like *Penicillium, cladosporium, cuvularia, Drechslera, Fusarium* were also isolated. From the rhizosphere soil, total of 13 fungi and 15 species of fungi were isolated from industrial effluent soil.

Keywords: Azo dyes; rhizosphere; industrial effeluent ; Crystal violet; malachite green; methyl red ; Dye degradation studies.

Introduction

Ever since the beginning of mankind, people have been using colorants for painting and dyeing their surroundings, their skins and their clothes. The first evidence of the use of colorant materials by man goes as far as 15000-9000 BC, in the walls of the Altamira cave in Spain. The drawings were performed with inorganic pigments like soot, manganese oxide, hematite and ochre. Historically there is a dye, derived from animal sources (molluscs), that is very important, although presently has no relevance and it's not commercially available. It is Tyrian Purple (Figure 1) and the pigment itself is not in the mollusc; however, when the precursor is extracted it can be converted to the dye by air or light. The presence of this dye goes as far as 1400 BC in the Late Bronze Age as found recently in Lebanon. It has always been rare and costly being used by Roman emperors and high ranking ecclesiastics (Clark *et al.* 1993).

Another ancient dye that is still in use, although not from natural origin nowadays, is indigo. It was extracted from *Indigofera tinctoria* by fermentation and had a characteristic blue colour. It was used as a pigment by the Romans because it had to be

chemically reduced to become water soluble. It was firstly synthetically produced by Adolf von Baeyer in 1880, and actually is used to dye denim (Clark *et al.* 1993).

Up to the end of the nineteenth century natural dyes, obtained mainly from plants (roots, stems, leaves, flowers, fruits, seeds and lichens – Ingamells 1993), were the main colorants available for textile dyeing procedures. The main disadvantages of the use of natural dyes are the need for several steps in the dyeing process, the diversity of sources and related application procedures, the rapid change in trends and the demand for good fastness properties on different substrates that would require a complete database describing possible applications (Bechtold *et al.* 2003).

The pioneering synthesis of mauveine by W. H. Perkins started the era of synthetic dyes, with chemical and physical properties better suited to contemporary demands, better level of quality and more reproducible techniques of application. It also allowed the development and extension of the use of particular products. For example, the development of synthetic fibres such as polyester and cellulose triacetate would have been severely hindered without the design and synthesis of dyes with appropriate properties (Ingamells 1993).

Since then thousands of dyes have been synthesised, and dye manufacture has become a significant part of the chemical industry. Nowadays, when care of the environment is a major issue, it is tempting to assume that the use of natural colours is an environmental friendly alternative to present-day practice. There are several groups studying the use of natural dyes in modern dyeing industry (Tsatsaroni and Liakopoulos-Kyriakides 1995, Angelini *et al.* 1997, Ishigami and Suzuki 1997, Angelini *et al.* 2003, Bermejo *et al.* 2003, Kim *et al.* 2004b, Paul *et al.* 2004, Kamel *et al.* 2005, Singh *et al.* 2005). Some of the advantages of the use of this type of compounds are the absence of toxicity upon humans, the use of sustainable sources and the fit into the natural pathways of biodegradation of the released dyebaths.

Biological treatments

The fate of environment pollutants is largely determined by abiotic processes, such as photooxidation, and by the metabolic activities of microorganisms. Since catabolic enzymes are more or less specific, they can act on more than their natural substrate. This explains why the majority of xenobiotics are subject to fortuitous metabolism (cometabolism) (Knackmuss 1996) and several groups explore these microbial capacities for the bioremediation of dyes.

The limitations of biological processes are mainly caused by limited biodegradability of primarily xenobiotic compounds like dyes, by toxic or inhibitory effects of pollutants for the microbial population and by the slow rate of biodegradation of particular pollutants (Jeworski and Heinzle 2000).

Enzymes

In the studies of biological degradation of dyes an effort as been made in order to identify, isolate and test the enzymes responsible for the decolourisation. In the case of extracellular fungal enzymes, like manganese and lignin peroxidases and laccases (Figure 2), or cytosolic azoreductases from bacteria, this has been achieved by several groups (Dass and Reddy 1990, Gosh *et al.* 1992, Spadaro and Renganathan 1994, Heinfling *et al.* 1998a, Rafii and Coleman 1999, Schliephake *et al.* 2000, Campos *et al.* 2001, Suzuki *et al.* 2001, Nyanhongo *et al.* 2002, Blümel and Stolz 2003, Ryan *et al.* 2003, Maier *et al.* 2004).

The application of enzyme preparations shows considerable benefits over the use of microorganisms. Commercial preparations can be easily standardized, facilitating accurate dosage. The application is simple and can be rapidly modified according to the character of the dye or dyes to be removed (Forgacs *et al.* 2004). Nevertheless the use of whole cells rather than

Figure 1 A - Chemical structure of tyrian purple; B - Sea shells from which tyrian purple was extracted; C - A purple-dyed fabric.

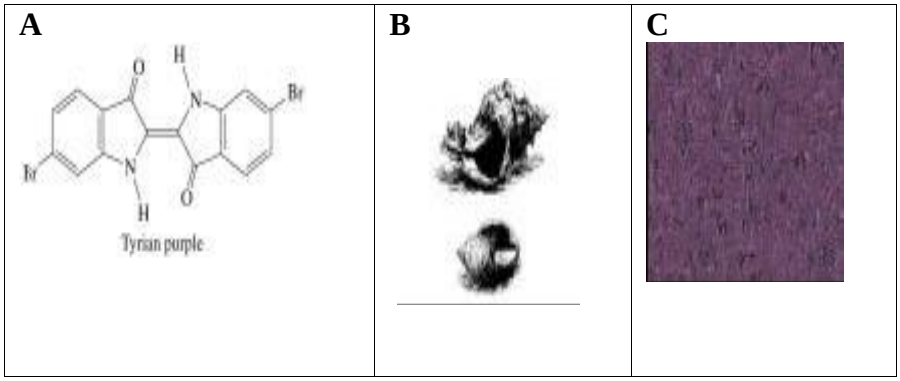
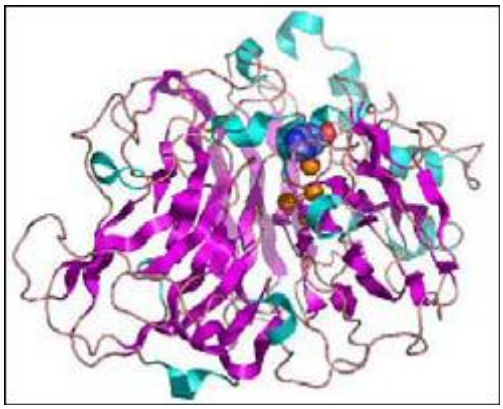


Figure.2 Laccase from *Trametes versicolor*.



isolated enzymes is advantageous, because costs of purification are extremely high and the cell offers protection from the harsh process environment to the enzymes. Also, degrading is often carried out by a number of enzymes working sequentially and not by one single enzyme (Pearce *et al.* 2003).

Fungi

The most widely studied dye-decolourising microorganisms are the white-rot fungi like *Phanerochaete chrysosporium.* (Glenn and Gold 1983, Bumpus and Brock 1988, Pasti-Grigsby *et al.* 1992, Chao and Lee 1994, Conneely *et al.* 1999, Chagas and Durrant 2001, Kunz *et al.* 2001, Martins *et al.* 2002, Mielgo *et al.* 2002), *Trametes versicolor* (Field *et al.* 1992, Borchert and Libra 2001, Tekere *et al.* 2001), *Coriolus versicolor* (Kapdan *et al.* 2000, Kapdan and Kargi 2002) and *Bjerkandera adusta* (Field *et al.* 1992, Heinfling-Weidtmann *et al.* 2001).

Yeasts

In literature the ability to degrade azo dyes by yeasts was only described in a few reports. The first two reports use the ascomycete yeast *Candida zeylanoides* isolated from contaminated soil to reduce model azo dyes (Martins *et al.* 1999, Ramalho *et al.* 2002). The characterisation of an enzymatic activity is described in further studies with the yeast *Issatchenkia occidentalis* (Ramalho *et al.* 2004), and the enzymatic system involved is presented in a work with *Saccharomyces cerevisiae* (Ramalho *et al.* 2005).

Aim and objectives

The present study was initiated to find out the Azo dye degradation by fungi with the following objectives Isolation of fungi from the soil samples of agricultural and industrial effluent. Identification of fungi using Lactophenol cotton blue staining. Azo dye degradation by fungi using agar plate method and Decolorization activity of fungi.

Materials and Methods

Collection soil samples

Soil samples were collected from the rhizozphere and dye effluent. Normally 10g of sample was collected in each station in sterilized glass container and then transferred to sterilized polythene bags and properly sealed.

Dyes used

Dyes like crystal violet, malachite green and methyl red were used for decolorization studies.

Isolation of fungi from water sample by platting technique

After sampling, within 24 hours, the water samples from each station were subjected to appropriate dilutions (10^{-2} to 10^{-5}) and transferred 0.1 ml of sample aseptically into the agar containing plates like Potato dextrose agar/ Rose Bengal agar (Hi-Media) with addition of mixture antibiotics, Tetracycline and ampicillim (Spread plate method) The plates were incubated in room temperature at 28° C for 4-5 days. Control plates were also maintained. Sterilization of glasswares, preparation of media were carried out as per the methods described by Booth 1971.

Dye degradation studies

Dye degradation in broth culture was done by following the method described by Jothimani and Prabakaran (2003).

The fungal cultures were inoculated into the Potato Dextrose broth and incubated at 27C for 3 days. After 3 days, 1ml of the culture suspension was transferred to 50 ml of Czepex-Dox broth in 250 ml Erlrnmeyer flasks. These flaskes were incubated in a shaker at 200rpm at room temperature for 4 – 8 days. On the fourth day an aniquot of one of the aforementioned dye (20mg) was added in to the broth culture and incubated for 5 days. Uninoculated flasks served as control to assess the aboitic decolourization. OD values were meatured spectrophotometrically at 594nm to estimated the decolourization process. The rate of decolourization was calculated using the following the formula as described by Sani andBanarjee [1999].

$$\text{Decolourization}(\%) = \frac{\text{Initial absorbance- after decolourization absorbance}}{\text{Initial absorbance}} \times 100$$

Dye degradation studies on Solid medium

Dye degradation in solid medium was done by adopting the method of swarming and Ramsay [1999] solid mineral salt media (g L-1 sucrose 30. sodium nitrate2, KH_2PO4 1 Mgso4 0.5, Kcl 0.5 Feso4 0.01, Thiamine hydrochloride 0.01, Distilled water 1000ml and micronutrients solution 1.0ml). (mgl-1 B4O7 Na2 100, Mnso4 10, (NH4)6 Mo7O24 10, Cuso4 50 and distilled water). Wasd prepared and 20mg of filter sterilized dye was added after sterilization of the medium. Fungal strains were inoculated into the plates and incubated at 28*C for 8 days control plates. The extent of clear zone formation around the colonies were absorbed and recorded. For biomass estimation Mycelial mat recovered from the whatman No.1 filter paper was washed with distilled water and dried at 70*C for 48 hours and weighed (Yesilada and Ozcan 1998).

Results and Discussion

Isolation of fungi from Rhizosphere soil

Totally 13 fungi were isolated from the agriculture soil. In this Aspergillus was common genus which is followed by *Aspergillus flavipus, Aspergillus ochrarea, Aspergillus niger, Aspergillus flavus,*

Aspergillus fumigatus fallowed by *Cladosporium tenuissimum, Cladosporium uredinicola* which is fallowed by one species *Penicillum jantinallum, Curuvalaria lunata, Rhizopus nigrieans, Trichoderma viride, Fusarium semitectum* were also isolated in the Rhizosphere soil.

Isolation of fungi from industrial effluent soil

Totally 15 fungi were isolated from the industrial effluent soil. In this Aspergillus was common genus which is followed by *Aspergillus niger, Aspergillus fumigatus, Aspergillus oryzae, Aspergillus funiculosis, Aspergillus sulpheres* which is followed by *cladosporium uredinicola, Cladosporium nigriellum* followed by *Alterneria triticicola, Alterneria dennisii* then followed by one species *curuvalaria tritica, Fusarium semitectum, Penicillum citrinum,Bipolaris sorokiniana, Drechslera tripogonis, Parapericonia angusii*.

Degradation studies.

crystal violet

Decolourization in Rhizosphere soil:

Maximum decolourization activity was observed in *Aspergillus flavus* which is 47.90% followed by *Aspergillus niger* which is 47.82%, *Cladosporium uredinicola* 41.83%, *Curuvalaria lunata* 41.71% then followed by *Aspergillus ochrarea* 38.46%, *Drechslaera tripogonis* 31.46%, then *Penicillum jantinallum* 28.15% then *Trichoderma viride* 21.52%, *Fusarium semitectum* 18.75% then Aspergillus flavipes 12.03%, *Cladosporium tenuissimum* 11.0% followed by minimum decolourization activity was observer in *Rhizopus nigricans* 2.48%, *Aspergillus fumigatus* 4.06%.

Zone formation in Rhizosphere soil:

Maximum zone formation was observed in *Rhizopus nigrieans* 32mm, *Penicillum jantinallum* 25mm, *Fusarium semitectum* 22mm, *Drechslera tripogonis* 20mm, *Aspergillus fumigatus* 20mm, than *Aspergillus flavus* 15mm, *Trichoderma viride* 15mm, than *Cladosporium tenuissimum* 14mm Followed by minimum zone formation was observed in *Curuvalaria lunata* 13mm, *Aspergillus ochrarea* 13mm, Aspergillus flavipes 20mm.

Industrial effulent

Degradation of Industrial effluent:

Maximum decolourization activity was observed in *Aspergillus oryzae* 74.74% followed by *Aspergillus funiculosis* 56.83% than *Alterneria triticicola* 53.57%, *Curuvalaria tritici* 53.29%, *Drechslera tripogonis* 39.16%, *Fusarium semitectum* 38.94%, *Aspergillus sulphureus* 38.77%, *Aspergillus fumigatus* 33.58%, *Cladosporium uridinicola* 22.68%, *Alterneria dennisii* 22.29%, *Parapericonia angusii* 17.72%, *Penicillum citrimum* 13.04% followed by minimum decolourization activity was observed in Bipolaris sorokiniana 1.51%, *Aspergillus niger* 1.21%.

Zone formation in industrial effluent soil:

Maximum zone formation was observed in *Fusarium semitectum* followed by *Drechslera tripogonis* 21mm, *Aspergillus oryzae* 15mm, *Cladosporium nigrellum* 14mm, *Alternaria triticicola* 12mm, *penicillum citrimum* 11mm, *Alternaria dennisii* 7mm, *Parapericonia angusii* 7mm, *Bipolaris sorokiniana* 10mm, Aspergillus fumiculosis 10mm followed by minimum zone formation was observed in *Curuvalaria tirtici* 5mm.

Malachite green

Decolourization in rhizosphere soil:

Maximum decolourization actrivity was observed in *Fusarium semitectum* 22.68%, *Cladosporium uredinicola* 18.75%, *Aspergillus fumigatus* 17.76%, *Aspergillus flavus* 12.56%, *Drechslera tripogonis* 11.5%, *Cladosporium tenuissimum* 8.33%, *penecillum jantinallum* 6.25%, *Aspergillus ochrarea* 5.37%, Aspergillus flavipes 5.23%, *curuvalaria lunata* 5.20% followed by minimum decolourization

Table.1 Total number of fungi isolated from Rhizosphere soil

S.No	Name of the fungi
1	*Aspergillus flavipes*
2	*Aspergillus ochraceous*
3	*Aspergillus niger*
4	*Aspergillus flavus*
5	*Aspergillus fumigatus*
6	*Penicillum janthinellum*
7	*Cladosporium tenuissimum*
8	*Cladosporium uredinicola*
9	*Rhizopus nigricans*
10	*Fusarium semitectum*
11	*Curuvalaria lunata*
12	*Drechslera tripogonis*
13	*Trichoderma viride*

Table.2 Total number of fungi isolated from dye effluent soil

S.no	Name of the fungi
1	*Aspergillus niger*
2	*Aspergilus fumigatus*
3	*Aspergillus funiculosis*
4	*Aspergillus sulphureus*
5	*Aspergillus oryzae*
6	*Cladosporium uredinicola*
7	*Cladosporium nigrellum*
8	*Curuvalaria tritici*
9	*Fusarium semitectum*
10	*Penicillum citrinum*
11	*Bipolaris sorokiniana*
12	*Alternaria dennisii*
13	*Alternaria triticicola*
14	*Drechslera tripogonis*
15	*Parapericonia agusii*

Table .3 Total number of fungi isolated from crystal violet (decoloration)

S.No	Name of the fungi	**decoloration in %**
1	*Aspergillus flavus*	47.90
2	*Aspergillus flavipes*	12.03
3	*Aspergillus fumigatus*	4.06
4	*Aspergillus ochraceous*	38.46
5	*Aspergillus niger*	47.82
6	*Curuvalaria lunata*	41.71
7	*Cladosporium uredinicola*	41.83
8	*Cladosporium tenuissimum*	11.00

9	*Drechslera tripogonis*	31.46
10	*Penicillum janthinellum*	28.15
11	*Rhizopus nigricans*	2.48
12	*Fusarium semitectum*	18.75
13	*Trichoderma viride*	21.50

Table .4 Total number of fungi isolated from dye effluent soil (crystal violet decoloration)

S.no	**Name of the fungi**	**%**
1	*Aspergillus niger*	1.21
2	*Aspergilus fumigatus*	33.58
3	*Aspergillus funiculosis*	56.83
4	*Aspergillus sulphureus*	38.77
5	*Aspergillus oryzae*	74.74
6	*Cladosporium uredinicola*	22.68
7	*Cladosporium nigrellum*	7.59
8	*Curuvalaria tritici*	53.29
9	*Fusarium semitectum*	38.94
10	*Penicillum citrinum*	13.04
11	*Bipolaris sorokiniana*	1.51
12	*Alternaria dennisii*	22.29
13	*Alternaria triticicola*	53.57
14	*Drechslera tripogonis*	39.16
15	*Parapericonia agusii*	17.72

Table .5 Total number of fungi isolated from crystal violet r.soil (zone)

S.No	**Name of the fungi**	**decoloration in %**
1	*Aspergillus flavus*	15
2	*Aspergillus flavipes*	12
3	*Aspergillus fumigatus*	20
4	*Aspergillus ochraceous*	13
5	*Aspergillus niger*	25
6	*Curuvalaria lunata*	13
7	*Cladosporium uredinicola*	-
8	*Cladosporium tenuissimum*	14
9	*Drechslera tripogonis*	20
10	*Penicillum janthinellum*	25
11	*Rhizopus nigricans*	32
12	*Fusarium semitectum*	22
13	*Trichoderma viride*	15

Table .6 Total number of fungi isolated from dye effluent soil (crystal violet zone)

S.no	Name of the fungi	%
1	*Aspergillus niger*	-
2	*Aspergilus fumigatus*	-
3	*Aspergillus funiculosis*	10
4	*Aspergillus sulphureus*	15
5	*Aspergillus oryzae*	-
6	*Cladosporium uredinicola*	5
7	*Cladosporium nigrellum*	22
8	*Curuvalaria tritici*	11
9	*Fusarium semitectum*	10
10	*Penicillum citrinum*	21
11	*Bipolaris sorokiniana*	12
12	*Alternaria dennisii*	7
13	*Alternaria triticicola*	-
14	*Drechslera tripogonis*	14
15	*Parapericonia agusii*	7

Table .7 Total number of fungi isolated from malachite r.soil (decoloration)

S.No	Name of the fungi	decoloration in %
1	*Aspergillus flavus*	12.56
2	*Aspergillus flavipes*	5.23
3	*Aspergillus fumigatus*	17.76
4	*Aspergillus ochraceous*	5.37
5	*Aspergillus niger*	2.74
6	*Curuvalaria lunata*	5.20
7	*Cladosporium uredinicola*	18.75
8	*Cladosporium tenuissimum*	8.33
9	*Drechslera tripogonis*	11.17
10	*Penicillum janthinellum*	6.25
11	*Rhizopus nigricans*	2.74
12	*Fusarium semitectum*	22.68
13	*Trichoderma viride*	2.71

Table .8 Total number of fungi isolated from dye effluent soil (malachite green decoloration)

S.no	Name of the fungi	%
1	*Aspergillus niger*	8.29
2	*Aspergilus fumigatus*	8.72
3	*Aspergillus funiculosis*	13.33
4	*Aspergillus sulphureus*	18.35
5	*Aspergillus oryzae*	4.39
6	*Cladosporium uredinicola*	12.26
7	*Cladosporium nigrellum*	5.80
8	*Curuvalaria tritici*	13.84

9	*Fusarium semitectum*	15.38
10	*Penicillum citrinum*	13.75
11	*Bipolaris sorokiniana*	7.23
12	*Alternaria dennisii*	17.94
13	*Alternaria triticicola*	6.17
14	*Drechslera tripogonis*	21.60
15	*Parapericonia agusii*	19.50

Table .8 Total number of fungi isolated from malachite green r.soil (zone)

S.No	**Name of the fungi**	**decoloration in %**
1	*Aspergillus flavus*	13
2	*Aspergillus flavipes*	-
3	*Aspergillus fumigatus*	27
4	*Aspergillus ochraceous*	23
5	*Aspergillus niger*	-
6	*Curuvalaria lunata*	12
7	*Cladosporium uredinicola*	-
8	*Cladosporium tenuissimum*	22
9	*Drechslera tripogonis*	-
10	*Penicillum janthinellum*	12
11	*Rhizopus nigricans*	-
12	*Fusarium semitectum*	17
13	*Trichoderma viride*	15

Table .9 Total number of fungi isolated from dye effluent soil (malachite green zone)

S.no	Name of the fungi	%
1	*Aspergillus niger*	-
2	*Aspergilus fumigatus*	11
3	*Aspergillus funiculosis*	11
4	*Aspergillus sulphureus*	-
5	*Aspergillus oryzae*	-
6	*Cladosporium uredinicola*	7
7	*Cladosporium nigrellum*	6
8	*Curuvalaria tritici*	-
9	*Fusarium semitectum*	12
10	*Penicillum citrinum*	9
11	*Bipolaris sorokiniana*	6
12	*Alternaria dennisii*	-
13	*Alternaria triticicola*	-
14	*Drechslera tripogonis*	10
15	*Parapericonia agusii*	-

Table .10 Total number of fungi isolated from methyl red R. soil (decoloration)

S.No	Name of the fungi	decoloration in %
1	*Aspergillus flavus*	72.28
2	*Aspergillus flavipes*	64.51
3	*Aspergillus fumigatus*	8.52
4	*Aspergillus ochraceous*	27.38
5	*Aspergillus niger*	17.50
6	*Curuvalaria lunata*	20.55
7	*Cladosporium uredinicola*	13.33
8	*Cladosporium tenuissimum*	8.32
9	*Drechslera tripogonis*	16.76
10	*Penicillum janthinellum*	70.83
11	*Rhizopus nigricans*	32.48
12	*Fusarium semitectum*	55.55
13	*Trichoderma viride*	3.27

Table .11 Total number of fungi isolated from dye effluent soil (methyl red decoloration)

S.no	Name of the fungi	%
1	*Aspergillus niger*	8.93
2	*Aspergilus fumigatus*	21.98
3	*Aspergillus funiculosis*	69.53
4	*Aspergillus sulphureus*	24.26
5	*Aspergillus oryzae*	51.35
6	*Cladosporium uredinicola*	10.45
7	*Cladosporium nigrellum*	60.23
8	*Curuvalaria tritici*	7.14
9	*Fusarium semitectum*	14.40
10	*Penicillum citrinum*	44.63
11	*Bipolaris sorokiniana*	40.13
12	*Alternaria dennisii*	71.12
13	*Alternaria triticicola*	12.84
14	*Drechslera tripogonis*	79.87
15	*Parapericonia agusii*	51.95

Table .12 Total number of fungi isolated from methyl red r. soil (zone)

S.No	Name of the fungi	decoloration in %
1	*Aspergillus flavus*	12
2	*Aspergillus flavipes*	18
3	*Aspergillus fumigatus*	10
4	*Aspergillus ochraceous*	10
5	*Aspergillus niger*	13
6	*Curuvalaria lunata*	15
7	*Cladosporium uredinicola*	20

8	*Cladosporium tenuissimum*	17
9	*Drechslera tripogonis*	23
10	*Penicillum janthinellum*	15
11	*Rhizopus nigricans*	23
12	*Fusarium semitectum*	25
13	*Trichoderma viride*	21

Table .13 Total number of fungi isolated from dye effluent soil (crystal violet zone)

S.no	**Name of the fungi**	**%**
1	*Aspergillus niger*	19
2	*Aspergilus fumigatus*	27
3	*Aspergillus funiculosis*	29
4	*Aspergillus sulphureus*	25
5	*Aspergillus oryzae*	22
6	*Cladosporium uredinicola*	25
7	*Cladosporium nigrellum*	28
8	*Curuvalaria tritici*	16
9	*Fusarium semitectum*	25
10	*Penicillum citrinum*	28
11	*Bipolaris sorokiniana*	25
12	*Alternaria dennisii*	28
13	*Alternaria triticicola*	23
14	*Drechslera tripogonis*	21
15	*Parapericonia agusii*	19

activity was observed in *Aspergillus niger* 2.74%, *Rhizopus nigrieans* 2.74%.

Zone formation in Rhizopus soil:

Maximum zone formation was observed in *Aspergillus niger* 27% followed by Aspergillus fumigatus 23%, *Penicillum janthinallum* 22%, *Rhizopus nigricans* 17%, *TRichoderma viride* 15%, *Aspergillus flavipes* 13%, *Fusarium semetectum* 12%, *Cladosporium tenuissimum* 12%.

Degradation in Industrial effluent:

Maximum decolourization activity was observed in *Drechslera tripogonis* 21.6%, *Parapericonia angusii* 19.5%, *Aspergillus sulphureus* 18.35%, *Alternaria dennisii* 17.94%, *Fusarium semitectum* 15.13%, *Curuvalaria tirtici* 13.84%, *Penicillum citrinum* 13.75%, *Aspergillus funiculosis* 13.33%, *Cladosporium uredinicola* 12.26%, *Aspergillus fumigatus* 8.72% *Aspergillus niger* 8.29%, *Bipolaris sorokiniana* 7.23% followed by minimum decolourization activity was observed by *Alternaria triticicola* 6.17%, Cladosporium nigrellum 5.80%, *Aspergillus oryzae* 4.39%.

Zone formation in Industrial effluent soil:

Maximum zone formation was observed in *Fusarium semetectum* 12mm, followed by *Aspergillus fumigatus* 11mm, *Aspergillus funiculasis* 11mm, *Alternaria triticicola* 10mm, Penicillum citrinum 112mm, *Cladosporium uredinicone* 7mm, *Cladosporium nigrellum* 7mm than followed by *BIpolarise sookiniana* 6mm.

Methyl red
Decolourization activity in Rhizosphere soil

Maximum decolourization was observed in *Aspergillus flavus* 72.28%, *Penicillum jantinallum* 70.83%, *Aspergillus flavipes* 64.51%,

Fusarium semitectum, 55.55%, *Rhizopus nigricans* 32.48%, *Aspergillus ochrarea* 27.38% , *Curuvalaria lunata* 20. 55%, *Aspergillus niger* 17.5%, *Drechslera tripogonis* 16.76%, *Cladosporioum uredinicola* 13.33%, *Aspergillus fumigatus* 8.52%, *Cladosporium tenuissimum* 8.32% maximum value was observed in *Trichoderma viride* 3.27%.

Zone formation in Rhizosphere soil

Maximum zone formation was noted in the *Fusarium semitectum* 25mm, then followed by *Rhizopus nigricans* 23mm, *Trichoderma viride* 21mm, *Cladosporium uredinicola* 20mm, *Aspergillus flavipes* 18mm, *penicillum jantinallum* 15mm, *Curuvalaria lunata* 15mm,Aspergillus ochrarea 13mm, followed by minimum zone formation was noted in *Aspergillus flavus* 12, *Aspergillus fumigatus* 10mm, *Aspergillus niger* 10mm.

Decolourization activity in Industrial effluent:

Maximum decolourization activity was observed in *Drechslera tripogonis* 79.87% followed by *Alterneria dennisii* 71.12%, *Aspergillus funiculosis* 69.53% , *Cladosporium nigrellum* 60.23%, Para *periconia angusii* 51.95% , *Aspergillus oryzae* 51.35% *penicillum citrinum* 44.63%, *Bipolaris sorokiniana* 40.15% *Aspergillus sulphureus* 24.26%, *Aspergillus fumigatus* 21.98%, *Fusarium semetectum* 14.40%, *Alternaria triticola* 12.84%, followed by minimum decolourization activity was observed in *Cladosporium uredinicolas* 10.45%, *Aspergilllus niger* 8.93% *Curuvalaria tritici* 7.14.

Zone formation in industrial effluent soil

Maximum zone formation was observed in *Aspergillus fumiculosis* 29mm, followed by *Fusarium semetectum* 25mm. *Drechslera tripogonis* 28mm. *Alterneria dennsii* 28mm, *Aspergillus fumigatus* 27mm, *Aspergillus oryzae* 25mm. curuvalaria tritici 25mm. *bipolaris sorokiniana* 25mm, *Cladosporium uredinicola* 23mm, *Aspergikllus sulphureus* 122mm, Cladosporium *nigriellum* 21mm, *Parapericonia angusii* 19mm. minimum zone formation was observed in *Penicillum citrinum* 10mm.

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