International Journal of Research Studies in Biosciences (IJRSB)
Volume 2, Issue 11, December 2014, PP 49-61
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

Biodegradation of Diazodye, Trypan Blue by Aspergillus Species from Dye Contaminated Sites

Ravuri Jaya Madhuri

Assistant Professor,
Department of Applied Microbiology
Sri Padmavati Mahila Visvavidyalayam,
Tirupati, Andhra Pradesh, India
drjayaravuri@gmail.com,

Gangavarapu Vijayalakshmi

Research Scholar,
Dept of Applied Microbiology
Sri Padmavathi Mahila Visvavidyalayam
Tirupati, Andra Pradesh, India
Vijaya.gangavarapu@gmail.com

Abstract: *In the present study, the decolourisation potential of three Aspergillus species, Aspergillusniger, Aspergillusflavus, Aspergillusfumigatus, isolated from dye contaminated soils, on a di azo dye, Trypan Blue, under different physicochemical conditions was investigated. The maximum decolourisation (92%) was recorded with Aspergilliusniger under static conditions at optimal parameters such as pH 4,40^{0}C temperature, 0 - 0.5 % Nacl concentration, 200mgL^{-1} initial dye concentration and 2% glucose as carbon source. Apparently, maximum extent of decolourisation was found to be associated with increased fungal biomass (Biosorption) and high level of production of extra cellular enzymes. A part from the traditional methods of decolourisation, an alternative attempt was made by using immobilized growing cells and obtained 90% of decolourisation with in 24 hrsof incubation. By the above results it is evident that, Aspergillus species are the potent dye degrading fungi*

Keywords: *Trypan Blue, Aspergillus, enzymes, decolourization, immobilization.*

1. INTRODUCTION

It is reported that there are over 100 000 commercially available dyes with a production of over 7×10^5 metric tons per year [1] [2]. Azo dyes are the most widely used and constitute 60% of the total dyes synthesized. Among the industrial waste waters, dye waste water from textile and dyestuff industries is one of the most difficult to treat. This is because dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to be biodegraded. The colour in the waste water is highly visible and affects esthetics, water transparency and gas solubility in water bodies. Thus decolourisation is a challenge for textile industry as well as for waste water treatment systems. Usually, the elimination of these colored effluents in waste water treatment systems is mainly based on physical or chemical procedures such as adsorption, concentration, chemical transformation and incineration [3,4]. The above ways for cleanup of textile effluents are either ineffective, expensive, complicated or having disposable problems which limits their application [3]. Despite the existence of a variety of physical and chemical processes, bioremediation of textile effluent has received increasing attention in recent years due to its reputation as a low cost, environmentally friendly and publicly acceptable approach [5]. Development of efficient dye degradation requires a suitable strain and its use under favourable conditions to realize the degradation potential. A large number of microorganisms belonging to different taxonomic groups of Bacteria, algae, fungi and Yeast and actinomycetes have been reported for their ability to decolouriseazo dyes [6]. However a variety of fungi are capable of decolourizing a wide range of dyes with high efficiency and the fungal biomass can be produced cheaply, using relatively

simple fermentation techniques and inexpensive growth media. The best studied white rot fungi is *Phanerochaetechrysosporium* which degrades the dye by producing lignin peroxidase, manganese peroxidase, laccase. Among the non white rot fungi, newly screened *Aspergillus* species from dye contaminated sites were examined for its ability to decolourise the dye industry effluent [7] [8] [9].The current study has evaluated the potential of three *Aspergillus* species namely *Aspergillusniger*, *Aspergillusflavus* and *Aspergillusfumigatus* isolated from dye contaminated sites for their decolourisation efficiency of a diazo dye, Trypan Blue under in vitro conditions in both free and immobilized states and optimization of the factors influencing the process.

2. MATERIALS AND METHOD

2.1. Collection of Soil Samples and Dyes

The soil samples were collected from nearby places where the dye effluents were discharged from the small and medium scale textile dyeing units as described earlier[10] [11].Structural information of the selected dye Trypan Blue is depicted in Fig.1and Table 1. Detailed dye information is represented earlier [10]. Concentration of dye used is 0.02g/L as followed by [12] [13].

Table 1. *Details of trypan blue*

Character	Trypan Blue
Chemical Nature	Diazo dye
Molecular formula	3, 3^1 – [3, 3^1 – dimethyl - 4, 4^1-biphenylene) bis(azo)] bis (5-amino-4-hydroxy 2,7-naphthalene di sulfonic acid)
Molecular Mass	$C_{34}H_{28}N_6O_{14}S_4$
Supplier of dyes	872.88 g mol^{-1}
	National Scientific Products,Guntur

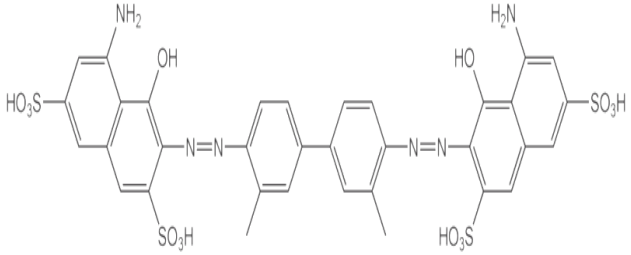

Fig1. *Structure of trypan blue*

2.2. Isolation and Screening of Soil Fungi for Dye Decolourization

Soil fungi were isolated on Potato Dextrose Agar (PDA) medium following the standard method of [14].Morphologically different fungi were screened on PDA medium incorporated with the selected azo dye, by dye agar plate assay technique [15] [16].

2.3. Characterization of Selected Fungi

High potential dye decolorizing fungi were selected by clear zone formation and characterized according to [17]. These fungal cultures were streaked on PDA slants and stock cultures were sub cultured monthly and stored at 4^0C for further use.

2.4. Dye Decolourisation Assay

The assay was performed following the method of [18]. The percentage of decolourisation was expressed in terms of degree of decrease in the absorbance at 597 nm for Trypan Blue against the initial absorbance at the same wavelength. Decolourisation yield was calculated by using the formula given by [19].

2.5. Optimization Studies of Fungal Decolourisation of AZO Dyes

As different physico-chemical and biological parameters influence the ability to decolorize dyes, the optimization studies were also performed with different parameters such as temperature, pH, carbon source, Nacl concentration, and microbial biomass. The experimental procedures were described earlier with reference to Methyl Red azo dye [11].

2.6. Enzymes in Dye Decolorisation

Assay of different enzymes (amylase, protease, catalase, glucose oxidase and laccases) involved in biotransformation of the selected dye was performed in the present research work following the standard procedures respectively [20-24]. Experimental details were mentioned earlier with reference to Congo Redazo dye [10].

2.7. Decolourisation Studies with Immobilized Cells

Immobilized cells can be used as alternative to whole cells and more attractive because of the reuse of biomass and more resilient to environmental perturbations [25]. Hence in the present work, the effect of immobilization of three *Aspergillus* cultures using sodium alginate beads on the rate of decolourisation of selected azo dye was determined. Preparation of alginate immobilized cells was given in earlier reference of Congo red [10].

3. RESULTS AND DISCUSSION

3.1. Screening and Identification of Potent Fungal Isolates Involved in Dye Decolorization

InTable2, it was observed that out of 19 species, 17 species had shown positive reaction for dye decolourisation test. Among the 17 positive organisms, five species of *Aspergillus,* and *Rhizopusoryzae we*re found as efficient (3+) dye degraders. They were identified as *A.flavus, A. foetidus, A. fumigatus, A. niger*and *A.terreus*based on lactophenol cotton blue staining. Seven fungal strains namely Alternaria alternate, *Aspergillusochraceus, Drechslerarostrata, Fusarumoxysporium, Mucormucedo, Pencilliumnotatum* and *Trichodermaviridae* showed moderate (2+) level of dye decolourisation and four fungi (*Alternariasolani, Aspergilluscandidus, Aspergillusoryzae* and *Curvularialunanta* exhibited meagre (1+) activity of dye degradation.

Among the 17 positive species, the most frequently encountered potential dye degrading fungi selected for different decolourisation studies were *Aspergillusniger, Aspergillusflavus* and *Aspergillusfumigatus* (Table 2).

Similar to present observations, many researchers,[26-29]. Have investigated on various diversified fungal species and identified the species of *Aspergillus* capable of removing dyes from aqueous solution. Based on our results and going through the literature, it may be concluded that the contaminated sites are the potential centers for the isolation of completely or partially mutated or adopted fungi capable of degradation and decolourisation of dyes.

Table 2. *Screening of isolated soil fungi for dye decolourisation*

S.No	Name of The Species	Trypan Blue
1	*Alternariaalternata*	2+
2	*Alternariasolani*	-
3	*Aspergilluscandidus*	1+
4	*Aspergillusflavus*	3+
5	*Aspergillusfoetidus*	2+
6	*Aspergillusfumigatus*	3+
7	*Apergillusniger*	3+
8	*Aspergillusoryzae*	1+
9	*Aspergillusterreus*	2+
10	*Aspergillusochraceus*	2+
11	*Cephalosporiumacremonium*	-
12	*Curvularialunanta*	2+
13	*Drechslerarostrata*	2+
14	*Fusariumoxysporum*	-
15	*Fusariummoniliformis*	-
16	*Mucormucedo*	2+
17	*Pencilliumnotatum*	2+
18	*Rhizopusoryzae*	2+
19	*Trichodermaviridae*	2+

3.2. Decolourisation Activity of Isolated Soil Fungi for Trypan Blue

From Table 3, it was evident that, decolourisation of Trypan Blue was to the maximum extent (92%) in Aspergillusniger followed by 90% in Aspergillusflavus at the end of 21 days of incubation. Minimum amount of dye decolourisation (74%) was noticed in Aspergillusfumigatus. (Fig.2-4).

Similar results were observed by [7] on decolourisation of azo dyes like Amaranth, Sudan 111 and Congo red by *Aspergillussojae* B10. Mineralization of the same polymeric azo dye, Tryphan blue by a novel *Penicillium* isolate was recorded by [30].

References [8] and [28] also reported removal of a Basic blue dye from an aqueous solution by the fungus *Aspergillusniger*. Fungal decolourisation of azo dyes by *A.foetidus* was indicated by [9]. According to their data, the extent of colour removal of textile dye was greater than 95% within 72 hours of growth. Reference [13] also made a similar study on decolourisation of textile dyes and showed that *Aspergillusflavus* was more efficient in decolourisation (99%) of T. blue within 8 days.

Table 3. *Decolourisation of trypan blue by isolated soil fungi*

Fungi	Days of incubation	Percentage of Decolourisation
Aspergillusniger	7	70
	14	85
	21	92
Aspergillusflavus	7	72
	14	84
	21	90
Aspergillusfumigatus	7	44
	14	63
	21	74

Fig2. *Decolourisation of trypan blue by Aspergillusniger*

Likewise, reference [31] stated that *Aspergillusfoetidus* was efficient in decolourising Reactive black 5 dye (100 mg/lt) with more than 99% efficiency at acidic P^H .Extensive decolourisation of orange II and reactive diazo dye by *Aspergillusniger* SA1 isolated from textile waste water sludge was noted. [16, 32].

Fig3. *Decolourisation of trypan blue by aspergillusflavus*

Fig4. *Decolourisation of trypan blue by aspergillusfumigatus*

3.3. Effect of Temperature on the Rate of Decolourisation of Trypan Blue

The optimum temperature for Trypan blue decolourisation was noticed at 30-40^{0}C (Table 4). There was a decrease in the rate of decolourisation at temperature above 40^{0}C. A significant rate of decolourisation was observed for the sulfanylated di azo dye at all the temperatures studied, except at 60^{0}C where the activity was minimum. The temperature above 40^{0}C decreased the rate of decolourisation indicating a thermal denaturation of enzymes. At the optimum temperature of decolourisation, *Aspergillusniger* and *Aspergillusflavus* showed 74-76% of decolourisation at the end of 7 days. A minimum of 50% decolourisation was found with respect to *Aspergillusfumigatus*. The present finding of maximum decolourisation at 40^{0}C was coinciding with the recent report of [33]. As per their experimental data decolourisation of Trypan blue, Remasol Brilliant blue, Amido black.etc by *Pleurotussajorcaju* was also noticed at temperature range of 30^{0}C-35^{0}C.

In a similar manner, Zilly [3] made an experimental study on decolourisation of industrial dyes Congo red, Trypan blue. Etc by using *Pleurotuspulmonarius*. He described all of them were decolourised to the maximum extent at 40^{0}C. More over Ali [16] mentioned that the mesophilic temperature of 25-30^{0}C suited best for the maximum removal of AR – 151 dye by *Aspergillusniger*.

Table 4. *Effect of temperature on trypan blue decolourisation at 7days incubation*

Fungi	Temperature (^{o}C)	Percentage of Decolourisation
Aspergillusniger	20	57
	30	63
	40	74
	50	59
	60	36
Aspergillusflavus	20	54
	30	68
	40	76
	50	58
	60	31
Aspergillusfumigatus	20	31
	30	43
	40	50
	50	38
	60	26

3.4. Effect of P^H on the Rate of Decolourisation of Trypan Blue

The dye was decolourised maximally (70-72%) at P^H -4. High rate of decolourisation at P^H 4 indicates that an acidic environment is required for enhancing rate of reaction as well as decolourisation (Table 5).

Trypan blue decolourisation was minimal (18%) with *A.fumigatus* at alkaline P^H of 8. Decolourisation was more than 50% upto P^H 6 except in *A. fumigatus*. However decolourisation was sustained even at P^H -8 with the dye tested. Recent observations made by Singh [33]had

shown that the decolourisation of Trypan blue and other dyes by white rot fungi was to the greatest extent at P^H 4. In the same manner Baldrian [24] said that the white rot fungus *Daedaleaquercina* could decolourise synthetic dye, Trypan blue at very low P^H of 2.5. Similar evidence of acidic P^H 3.4 favourable for significant rate of decolourisation was given by [34] in case of Reactive Blue 4 and Reactive orange 86.

Table 5. *Effect of pH on trypan blue decolourisation at 7 days of incubation period*

Fungi	pH	Percentage of decolourisation
Aspergillusniger	2	40
	4	72
	6	63
	8	32
	10	24
Aspergillusflavus	2	38
	4	70
	6	59
	8	31
	10	23
Aspergillusfumigatus	2	32
	4	50
	6	39
	8	22
	10	18

Contradictory to the above statement, Zilly[3] proved the occurance of maximum decolourisation at P^H 6.5 regarding Trypan blue by the activity of *Pleurotuspulmonarius*. Similar observation of high alkaline P^H of 6 leading to a maximum decolouration was made by Swamy and Ramsay [35] in the evaluation of white rot fungi towards decolourisation of textile dye.

3.5. Effect of Carbon Source on the Rate of Decolorisation of Trypan Blue

Of the different carbon sources tested, glucose was found to be the most suitable substrate for dye decolourisation (95%) in the defined media followed by starch (8.3%). Mannitol supported little (or) poor decolourisation (43%).Similar trend of decolourisation was noticed with respect to three selected fungal species *A.niger*, *A.flavus*, and *A.fumigatus* (Table 6).

Experimental work carried on by Ryu and Weon[7] focussed on decolourisation of azo dyes by *Aspergillussojae* B_{10} which showed greatest decolourisation ability when it was cultivated in medium containing Congo red, Sudan III and Amaranath with 2% glucose for 5 days. Decolourisation yield of Congo red was 91% with glucose and 47% with mannitol.

Research findings of [36] revealed that, mycelial pellets of *Trametesversicolor* grown at 10 gm /lt glucose concentration decolourised successive additions of same the dye and of different dye mixtures. Declourisation commenced by day three and all dyes Amarnath, Reactive blue etc. were decolourised at high rates during the next 12 days when glucose was consumed at the steady rate.

Table 6. *Effect of carbon source on trypan blue decolourisation at 7 days of incubation*

Carbon Source	Percentage of decolourisation		
	Aspergillusniger	***Aspergillusflavus***	***Aspergillusfumigatus***
Glucose	95	92	78
Starch	83	80	64
Mannitol	62	65	43

Experimental study on Dimarene dyes showed that, isolated *Aspergillusfoetidus* was effective in decolourising media containing azo reactive dyes [9]. The extent of colour removal was greater than 95% within 48 hours of fungal growth and has requirement for a biodegradable substrate such as glucose.

Further Oranusi and Ogugbue[37] experimented on the effect of co-substrates on biodegradation of triphenyl methane dyes by *Pseudomonas*. Biodegradation was enhanced in cultures with

glucose substrate supplementation. Glucose and starch were the best among the substrates tested at a concentration of 0.2 mg /lt. and 0.3 mg/lt per starch.

According to [32], optimisation of the culture medium by different co-substrates has been found critical in initiating fungal growth and related decolourisation of Acid red, especially when fungal strain *A.niger* SA_1 was used as in oculum source. Among the different carbon sources glucose, gave the best results in colour removal efficiency. Increase in glucose concentration from 1-10 gm/lt triggered a considerable increase in biomass production in *A.niger*SA_1 which in turn lead to the increased rate of decolourisation

3.6. Effect of Nacl Concentration on Fungal Growth and Decolorisation of Trypan Blue

The data from Table 7 indicates that the process of decolourisation is significantly inhibited by the presence of high concentration of NaCl in *A.fumigatus* i.e., only 17% of decolourisation was observed.

The mycelial growth which occurs under the condition of 0.5 and 1% NaCl, may be related to the level of decolourisation at the end of incubation period.

This was in close correlation with the experimental result of [9]. Corresponding to the luxurious growth of *A. niger*and *A. flavus* and *A.fumigatus* at 0.5%, there was a concomitant increase in decolourisation activity from 36-63%.

Comparatively remarkable growth was noticed at 1.5% NaCl of *A. fumigatus*. Similar to this, *Halomonas* showed tolerance towards 2 % of sodium chloride and exhibited significant growth in the medium [38]. Contradictory to the growth pattern, there was a significant reduction in percentage of decolourisation for *A.fumigatus.* Probably, the two (N=N) azo bonds and four sulfonated groups present in the ring structure of Trypan blue has less tendency for microbial attack. Hence the degree of decolourisation is low in the fungal culture studied, when compared to control. The extent of decolourisation is gradually decreased with the increment of salt concentration

Table 7. *Effect of Nacl concentration on fungal growth and trypan blue decolourisation at 7 days of incubation*

Fungi	NaclConc (%)	Mycelial growth	Decolourisation (%)
Aspergillusniger	0	+++	70
	0.5	+++	63
	1.0	++	55
	1.5	+	46
Aspergillusflavus	0	+++	72
	0.5	+++	62
	1.0	++	54
	1.5	+	42
Aspergillusfumigatus	0	+++	44
	0.5	+++	36
	1.0	++	25
	1.5	++	17

3.7. Effect of Microbial Biomass on the Rate of Decolorisation of Trypan Blue

The experimental results were given in Table -8. Mycelial biomass in terms of dry weight has a positive correlation with the degree of decolourisation of the azo dye. The microbial biomass was more prominent with reference to *A.niger* and *A.flavus* as incubation commences from 7-21 days and to a limited extent in case of *A.fumigatus*. Decolourisation was maximum (93%) with 3 gms biomass of A.niger and *A.flavus* respectively. Regarding *A.fumigatus* 77% of decolourisation of Trypan blue corresponding to 2.8 gms of mycelial biomass was noticed. Culture medium amended with Trypan blue and inoculated with 3 *Aspergillus* species mediated a pronounced rate of decolourisation when compared to control. The improvement in decolouration of dyes was associated with increasing amount of fungal biomass and culture conditions.

In coincidence with the present experimental result few studies on dye removal by fungal biomass have been carried out. Therefore there is very limited information available on the interactions between fungal biomass and decolourisation of a variety of dyes with complicated chemical structure.

Fu and Viraraghavan[8] investigated on the effect of microbial biomass on the rate of decolourisation by the fungus *A.niger*. Their investigation data revealed that increased biosorption by approximately 6 times with live biomass ranging from 1.17 to 18.39 mg/gm.

Table 8. *Effect of Microbial biomass on Trypan Blue decolourisation at 7 days of incubation*

Fungi	Days of incubation	Dry wt of mycelial bio mass (gm)		Percentage of decolourisation
		control	test	
Aspergillusniger	7	2.1	2.3	73
	14	2.5	2.8	89
	21	2.9	3.0	93
Aspergillusflavus	7	2.1	2.3	72
	14	2.3	2.7	87
	21	2.8	3.0	91
Aspergillusfumigatus	7	1.9	2.2	46
	14	2.2	2.5	65
	21	2.5	2.8	77

They also proved that pretreatment of *Aspergillusniger* with sodium carbonate further increased biosorption of Basic blue 9 dye to a significant extent. According to [16] and [33], decolourisation was maximum in Orange II i.e., 78.08% with 1.73 mg/lt of biomass production where as it was 67.26 with 1.40 mg/lt biomass and 13.74% with 1.87 mg/lt of biomass in case of Acid red 151 with *Aspergillusniger*SA_1. Kinetic studies made by Patel and Suresh [31] on biosorption of Reactive black 5 (100 mg/lt) by *Aspergillusfoetidus* clearly shows that pre-treatment of fungal biomass by autoclaving or on exposure to 0.1 M NaoH facilitate more efficient uptake of dye. Contrarily, it was reported in another study made by Fu and Viraraghavan [28] that *A.niger* is responsible for 98% reduction in biosorption of the four dyes by chemical modification within a short period.

3.8. Enzymatic Activity of Isolated Soil Fungi During the Decolourisation of Trypan Blue

From the tabulated data (Table 9) it is evident that *Aspergillusniger* is the efficient producer of all the enzymes studied than the remaining isolated fungi.The production of amylase, an extra cellular hydrolytic enzyme playing an indirect role in azo dye decolourisation, was high at 14 day incubation in *A.niger* (3.25 U/ml.). Initially at 7 day incubation, the amylase enzymatic activity is low ranging from (2.68 – 2.88 U/ml). *A.flavus* is the second efficient amylase producer during the course of decolourisation of Trypan blue next to the A niger. *A.fumigatus* reported the least enzyme activity (2.52 U/ml) at 21 day incubation period. The enzyme production is found to be decreased from 14^{th} day to 21^{st} day with reference to all the three fungi tested.

Our experimental results are in correlation with data recorded by [39]. In their study amylase activity was determined to play an active role during the decolourisation of Congo red by *Polyporusostreiformis*.

Table 9. *Enzyme activity of isolated soil fungi during decolourisation of Trypan Blue*

Fungi	*Days of incubation*	*Amylase*	*Protease*	*Catalase*	*Glucose Oxidase*	*Laccase*
Aspergillusniger	7	2.88	0.99	1.90	2.20	1.83
	14	3.25	1.25	2.21	2.50	2.06
	21	2.79	0.86	1.70	2.40	2.41
Aspergillusflavus	7	2.80	0.98	1.85	2.28	1.81
	14	3.20	1.23	2.18	2.48	2.09
	21	2.75	0.88	1.78	2.10	2.30
Aspergillusfumigatus	7	2.68	0.82	1.79	2.02	1.52
	14	2.90	1.02	2.12	2.20	1.71
	21	2.52	0.78	1.68	1.92	2.01

In general, 14 days of incubation showed maximum protease production by all the fungi and ranged from (1.02 to 1.25 U/ml). The three fungi showed decreased trend of protease production in 21 days period. As the enzyme activity is increased, the rate of decolourisation of Trypan blue was also enhanced in all the 3

Our experimental data is coinciding with the data provided by [39] and [40]. They also estimated and analyzed the functional role of proteases in the azo dye degradation produced by the selected white rot fungi, *Polyporusostreiformis* and *Pleurotusostreatus.* Beside fungal members, [38] represented the role of bacteria in the degradation of azo dyes by protease production.

The catalase production was high during 14 days and reduced in the subsequent incubations. The highest range of enzyme production (2.21 U/ml) was reported with *A. niger*. The lower range of enzyme production (2.12U/ml) was found in *A. fumigatus*. Regarding *A. flavus*, the level of secretion of this enzyme was far nearer to that of *A. niger*(2.18U/ml) .85% of decolourisation was recorded at 14 days of incubation against 2.21 U/ml secretion of catalase by *A.*where as 84% of decolourisation was observed in case of *A.flavus* against 2.18 U/ml production of catalase. These observations confirm the role of catalase in mediating azo dye decolourisation.

The glucose oxidase enzyme secreted by two fungi is slightly varied in the range of 1.92 -2.50 U/ml at the 3 incubation periods. At 7 day incubation period, the production of glucose oxidase is 2.20 U/ml in *A.niger*, 2.28 U/ml in *A.flavus* and 2.02 U/ml in *A. fumigatus.* The efficient enzyme activity is seen with *A.niger* (2.50 U/ml) at 14 day interval. *A.flavus* recorded its maximum production of enzyme i.e. 2.48 U/ml at 14 day incubation and lowest production (2.10 U/ml) at 21 day interval. Of the three fungi studied, *A.fumigatus* reported the least enzyme activity, 1.92 U/ml at 21 day incubation period. From the data presented in Table 3 and 9, the relation between the rate of decolourisation of Trypan blue and level of enzyme production is well understood. Similar experimental results were obtained by the data of [41].They proved the participation of glucose oxidase in the decolourisation of azo dye by two white rot fungi *Phanerocheatechrysosporium* and *Pleurotussajor-caju* in the treatment of textile industrial effluent.

Laccase, the most studied enzyme, playing a prominent role in azo dye degradation was assayed at different incubation periods. As per the data obtained, laccase enzyme production is found to be highest at 21 day incubation, where, as the other enzymes show their maximum level at 14 day interval. The reduced level of laccase production was observed at 7 day and 14 day periods. The range of enzyme activity is in between 1.52 U/ml to 2.41 U/ml with reference to all the three fungi at the three incubation periods. *Aspergillusniger* produced 1.83 U/ml at 7 day and 2.06 U/ml at 14 day and 2.41 U/ml laccase at 21 day period of incubation. The maximum quantity of laccase in *A. flavus*is 2.30 U/ml and in *A. fumigatus* is 2.01 U/ml. Of all the there fungi under study, the least level of enzyme activity is recorded with *A. fumigatus* 1.52 U/ml at 7 days of incubation.

The highest rate of decolourisation of Trypan blue in *A.niger* is 92% which is recorded against the maximum laccase activity (2.41 U/ml) at 21 day incubation. Similar findings are reported regarding rate of decolourisation and laccase production with *A.flavus* and *A.fumigatus* too. These observations confirm the major role of laccase in dye degradation than the other four enzyme assayed.

In coincidence with the above reports, Tychanowicz*et al.*,[42] out lined the proportionality between the extent of decolourisation and laccase production during the decolourisation of Trypan blue and Congo red by *Pleurotuspulmonarius.* It was further confirmed by [3]. They revealed laccase as the sole phenol oxidizing enzyme involved during the decolourisation of Congo red and Trypan blue by *Pleurotuspulmonarius* in case of Methyl violet. Similarly Abadulla*et al.*,[43] made a study and established the role of laccase in detoxification of textile azo dyes with *Trametesversicolor*.

On the whole, the present experimental data and various references proved that decolourisation of azo dyes by fungi occur mainly due to ring cleavage by laccase enzymes.

3.9. Decolourisation of Trypan Blue by Immobilized Soil Fungi

At the end of 24 hours, almost complete degradation of Trypan blue, a diazo dye (93 and 95%) is *A.flavus* and *A.niger* respectively was observed and the data was outlined in Table 10. Despite the

effective role in decolourisation, there was no significant difference between the normal and immobilized culture of *A. fumigatus* in particular (74% in normal and 72% in immobilization Technique). On the whole, as the incubation time is enhanced decolourisation activity by means of immobilized culture was more pronounced.

Table 10. *Decolourisation of Trypan Blue by Immobilised soil*

Fungi	Time required for decolourisation					
	4h	**8h**	**12h**	**16h**	**20h**	**24h**
Aspergillusniger	20	32	47	59	74	95
Aspergillusflavus	13	23	38	48	68	93
Aspergillusfumigatus	6	18	30	45	57	72

Interestingly in agreement with our experimental data, Reference [44] clearly demonstrated the role of immobilized white rot fungi (*Phanerochaete*and *Trametesversicolor*) unidentified basidiomycetes member using alginate beads could mediate complete degradation of Congo red and significant amount of Trypan blue after 6 days of cultivation.

Refernce [47] found that the white rot fungus *Dichomitussqualence* immobilized on poly urethane foam and pine wood cubes in a fixed bed reactor was able to decolourize the synthetic dyes in only 1 day.

A maximum decolourisation of 89.7% was attained at a concentration of 142.6 mg/lt of Congo red after 4 days was reported by [48] after 4 days of incubation ascribed to optimizing the process parameter i.e. adaptation of immobilization technique by several earlier works [48-51].

At the outset, except [52] and very few scattered isolated studies, no information was available on Trypan blue by immobilized *Aspergillus* species to the best of our knowledge.

4. CONCLUSION

Based on the above investigation, it can be concluded that Aspergillus species showed great ability in decolourising the selected azo dyeindicating their potential in anti pollution treatment. Immobilization studies made by using alginate beads on decolourisation activity of fungal isolates in the present experiment revealed enhancement in the rate of decolourisation from 4h to 24h. About 72-95% of decolourisation was found within 24 hrs in immobilised fungal cultures when compared to decolourisation activity of free fungal cells. Thus, the usage of immobilization technique in dye remediation technology is highly recommended.

REFERENCES

[1] Meyer U. Biodegradation of synthetic organic colorants. In: Leisinger,T, Cook, A.M, Hutter R, Nuesch, J. (Eds), Microbial degradation of Xenobiotics and Recalcitrant Compounds. Academic Press, London, pp.387-399,(1981).

[2] Zollinger H. Colur chemistry-syntheses, properties and applications of organic dyes and pigments, p. 92-102. VCH Publishers, New York,(1987).

[3] Zilly A., Souza CGM.,Tessmann IPB. and Peralta RM. Decolourization of industrial dyes by a Brazilian strain of *Pleurotuspulmonarius* producing laccase as the sole phenol oxidizing enzyme. Folia Microbiol. 47 (3): 273-277, (2002).

[4] Zhao X., Hardin IR. and Hwang HM. Biodegradation of a model azo disperse dye by the white rot fungus *Pleurotusostreatus*. Int. Biodeterioration and Biodegradation. 57: 1-6, (2006).

[5] McMullan G., Meehan C., Connely A., Kirby N., Robinson J., Nigam P., Banat 1M., Marchant R. and Smyth WF. Microbial decolourisation and degradation of textile dyes. Appl. Microbiol. Biotechnol. 56: 81-87, (2001).

[6] Seyis I. and Subasioglu T. Comparison of live and dead biomass of fungi on decolourisation of Methyl orange. Afr. J. Biotechnol. 7(13): 2212-2216, (2008)

[7] Ryu BH. andWeon YD. Decolourisation of azo dyes by *Aspergillussojae*B10. J. Microbiol. Biotechnol. 2(3): 215-219, (1992).

[8] Fu. andViraraghavan T. Removal of a dye from an aqueous solution by the fungus *Aspegillusniger*. Water Qual. Res. J. 35(1): 95-111, (2000).

[9] Sumathi S. and Manju BS. Uptake of reactive textile dyes by *Aspergillusfoetidus*. Enz. Microbial. Technol. 27: 347-355, (2000).

[10] Ravurijayamadhuri. And Gangavarapuvijayalakshmi. Biodegradation of Congo Red Azo dye by Aspergillus spp. Isolated from dye contaminated sites. J. Agr.Sci. Tech.$A_{4.}$ pp. 422-434(2014a).

[11] R. Jaya Madhuri. And G. Vijayalakshmi. Biodegradation of the Azo dye Methyl red by Aspergillus species, from dye contaminated sites. Int. J. Trends in Microbiol. Biotechnol. Vol.3 issue 9. (2014b).

[12] Kirby N., Marchant R. and McMullan G. Decolourisation of synthetic textile dyes by *Phlebiatremellosa*. FEMS Microbiol. Lett. 188: 93-96, (2000).

[13] Raju NS., Venktaramana GV., Girish ST., Raghavendra VB. and Shivashankar P. Isolation and evaluation of indigenous soil fungi for decolourisation of textile dyes. J. Appl. Sci. 7(2): 298-301, (2007).

[14] Aneja KR. *Experiments in Microbiology , Plant Pathology and Biotechnology*. New Age International Pvt Ltd Publishers. 4th Edition 69-71,(2008).

[15] Arora S., Harvinder Singh S. and Kamaljit Singh. Decolourisation of a mono azo disperse dye with *Candida tropicalis*. Colouration Technol. 121: 298-303, (2005).

[16] Ali N., Hameed A., Ahmed S. and Khan AG. Decolourisation of structurally different textile dyes by *Aspergillusniger* SA1World. J. Microbiol. Biotechnol. 24: 1067-1072, (2008a).

[17] Gilman CJ. A manual of soil fungi. Oxford and IBH Publications, India. Revised 2ndEdition: 219-229, (1957).

[18] Dias AA., Bezerra RM., Lemos PM. and Pereira AN. Invivo and laccasecatalyseddecolourisation of xenobiotic azo dyes by a basidiomycetous fungus: Characterization of its ligninolytic system. World. J. Microbiol. Biotechnol. 19: 969-975, (2003).

[19] Yatome C., Yamada S., Ogawa T. and Matsui M. Degradation of Crystal violet by *Nocardia coralline*. Appl. Microbiol. Biotechnol. 38: 565-569, (1993).

[20] Ross DJ. A seasonal study of oxygen uptake of some pasture soils and activities of enzymes hydrolyzing sucrose and starch. J. Soil. Sci. 16: 74-85, (1965)

[21] Nannipieri P.., Grego S. and Ceccanti B. Ecological significance of the biological activity in soil. Soil. Biochem. 6: 293-355, (1990).

[22] Sadasivam. andManickam A. Biochemical methods.. New Age International Publishsers,New Delhi, India. Third edition. 99-100, (2009).

[23] Kelley RL. and Reddy A. Glucose oxidase of *Phanerochaetechrysosporium*. Meth. Enzymol. 161: 307-316, (1988).

[24] Baldrian P. Purification and characterization of laccase from the white rot fungus *Daedaleaquercina* and decolourisation of synthetic dyes by the enzyme. Appl. Microbiol. Biotechnol. 63: 560-563, (2004).

[25] Steffan S., Bardi L. and Marzona M. Azodye biodegradation by microbial cultures immobilized in alginate beads. Environ. Int. 31: 201-205, (2005).

[26] Kim JH., Rhee CH., Woo CJ., Park Joo GJ. andSeoSk . Isolation and characterization of terephthalic acid degrading bacteria. Korean J. Appl. Microbiol. Biotechnol. 27: 118-123, (1999).

[27] Fu Y. and Viraraghavan T. Review on fungal decolourisation of dye waste waters :Bioresource. Technol. 79: 251-262, (2001).

[28] Fu Y. and Viraraghavan T. Dye biosorption sites in*Aspergillusniger*. Bioresource. Technol. 82: 139-145, (2002).

[29] Nagai M., Saito T., Watanabe H., Saito K., Kawata M. and Enei H. Purification and characterization of an extracellular laccase from the edible mushroom *Lentinulaedodes* and decolourisation of chemically different dyes. Appl. Microb. Biotechnol. 60: 327-335, (2002).

[30] Zhang F., Knapp JS. andTapley KN. Decolourisation of cotton bleaching effluent with wood

rotting fungus. Water Res. 33(4): 919-928, (1999).

[31] Patel R. and Suresh S. Kinetic and equilibrium studies on the biosorption of reactive black 5 dye by Aspergillusfoetidus. Bioresource. Technol. 99(1): 51-58, (2008).

[32] Ali N., Ikramullah, Ghosia L., Hameed A. and Ahmed S. Decolourisation of Acid red 151 by *Aspergillusniger* SA1 under different physicochemical conditions. World. J. Microbiol. Biotechnol. 24: 1099-1105, (2008b).

[33] Singh AD., Sabaratnam V., Abdullah N., Annuar MSM. andRamachandran KB. Decolourisation of chemically different dyes by enzymes from spent compost of *Pleurotussajorcaju* and their kinetics. Afr. J. Biotechnol. 9(1): 041-054, (2010).

[34] Khan AA. and Husain Q. Decolourisation and removal of textile and non-textile dyes from polluted waste water and dyeing effluent by using potato (*Solanumtuberosum*) soluble and immobilized polyphenol oxidase Doi:10.1016/j.biortech. 2006.04.008., (2006).

[35] Swamy. and Ramsay JA. The evaluation of white rot fungi in the decolourisation of textile dyes. Enz. Microbial. Technol. 24: 130-137, (1999a).

[36] Swamy J. and Ramsay JA. Effects of glucose and $NH4^+$ concentrations on sequential dye decolourisation by *Trametesversicolor*. Enz. Microb. Technol. 25: 278-284, (1999b).

[37] Oranusi NA. andOgugbue CJ. Effect of co substrates on primary biodegradation of triphenyl methane dyes by *Pseudomonas.* Afr. J. Appl. Zool. Environ. Biol. 7: 38-44, (2005).

[38] Yang C., Zhe W., Yang L., Yu N., Miaofen D., Xia FH., Cuiqing M., Hongzhi J. and Ping Y. Metabolic versatility of halotolerant and alkaliphilic strains of*Halomonas* isolated from alkaline black liquor. Bioresource. Technol. 101: 6778-6784, (2010).

[39] Dey S., Maiti TK. and Bhattacharya BC. Production of some extra cellular enzymes by a lignin peroxidase producing brown rot fungus *Polyporusostreiformis* and its comparative abilities for lignin degradation and dye decolourisation. Appl. Environ. Microbiol. 60: 4216-4218, (1994).

[40] Gianna P., Carmen B., Cennamo G., Giardina P., Marino G., Maria M. and Sannia G. Purification, characterrization and functional role of a novel extra cellular protease from *Pleurotusostreatus*. Appl. Environ. Microbiol. 67 (6): 2754-2759, (2001).

[41] Chagas EP. andDurrant LR. Decolourisation of azo dyes by *Phanerochaetechrysosporium* and *Pleurotussajorcaju.* Enz. Microbial. Technol. 29: 473-477, (2001).

[42] Tychanowicz GK., Zilly A., Giatti C., de Souza M. and Peralta RM. Decolourisation of industrial dyes by solid state cultures of *Pleurotuspulmonarius*. Process. Biochem. 39: 855 - 859, (2004).

[43] Abadulla E., Tzanov T., Costa S., Karl-Heinz R., Artur CP. and Gubitz GM. Decolourisation and detoxification of textile dyes with a laccase from *Trameteshirsuta*. Appl. Env. Microbiol. 66: 3357-3362, (2000).

[44] Couto SR. Dye removal by immobilised fungi. Biotechnol. Adv. 27: 227-235, (2009).

[45] Couto RS., Sanroman MA., Hofer D. and Gubitz GM. Stainless steel sponge: a novel carrier for the immobilization of the white rot fungus *Trameteshirsuta* for decolourisation of textile dyes. Bioresource Technol. 95: 67-72, (2004).

[46] Dominguez A., Couto RS. andSanroman MA. Dye decolourisation by *Trameteshirsuta* immobilized into alginate beads. World. J. Microbiol. Biotechnol. 21: 405-409, (2005).

[47] Susla M., Novotny C. and Svobodova K. The implication of *Dichomitussqualens*laccaseisoenzymes in dye decolourisation by immobilized fungal cultures. Bioresource. Technol. 98: 2109 – 2115, (2007).

[48] Li X. and Jia R. Decolourisation and biosorption for Congo red by system rice hull – *Schizophyllum* sp. F_{17} under solid state condition in a continuous flow packed bed bioreactor, Bioresource Technol. 99: 6885-92, (2008).

[49] Diorio L., Mercuri AA., Nahabedian DE. andForchiassin F. Development of a bioreactor system for the decolourisation of dyes by *Coriolusversicolor f. antarcticus*. Chemosphere. 72:150-156, (2008).

[50] Enayatzamir K., Alikhani HA., and Couto RS. Simultaneous production of laccase and

decolourisation of the diazo dye Reactive Black 5 in a fixed bed bioreactor. J. Hazard. Mater. Doi:10.1016/j.jhazmat. 2008.08.032, (2008).

[51] Gao D., Zeng Y., Wen X. and Qian Y. Competition strategies for the incubation of white rot fungi under non sterile conditions. Process. Biochem. 43: 937-944, (2008).

[52] Fu Y. and Viraraghavan T. Column studies for biosorption of dyes from aqueous solutions on immobilized *Aspergillusniger* fungal biomass. Water SA. 29 (4): 465-472, (2003).

AUTHORS' BIOGRAPHY

Dr. R. Jaya Madhuri is working as Assistant Professor in Department of Applied Microbiology, Sri Padmavati Women's University, Tirupati – Andhra Pradesh. She is an active researcher in the field of biodegradation and published 25 national and international papers. More than 40 research articles are presented by her in various scientific events and is a life member of five scientific organizations. To her credit, three research degrees were awarded.herher.guidance

G.Vijaya Lakshmi, M.Sc, M.Phil (Ph.D), is actively engaged in Research and Teaching. She started her carrier as a lecturer in the field of microbiology at both UG and PG level later extended her administrative services in motivating students in academic excellence and practical & research expertise. she is energetic, ambitious and self- motivated person. She has published 2 papers in National & International journals, and attended several seminars and workshops. As a Research Scholar under the supervision of **Dr. R.JayaMadhuri**, Assistant Professor, carrying her research work in the area of Bioremediation.