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STUDIES ON DEGRADATION OF SYNTHETIC DYES BY USING LACCASE PRODUCING *Aspergillus nidulans* ISOLATED FROM TEXTILE EFFLUENT

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ABSTRACT

Waste water from textile, paper and pulp industry are known to accumulate in soils and cause number of significant environmental problems. Different synthetic dyes present in this effluent of the dye industry are harmful for the living organisms and they cause environmental pollution. Therefore there is a need to find some solution to control this type of environmental pollution. One of the options is use of microorganisms to degrade the harmful dyes from the effluent of the textile industry. In the present study one potent Laccase producing fungus *A. nidulans* isolated from soil samples (near from textile and dye industries) are used. Using the gene specific sequencing primers, the purified PCR amplicons was sequenced.The sequences were analyzed using Sequencing Analysis 5.2 software. Blast result and phylogenetic tree analysis clearly indicate that fungal strain is *A. nidulans*. Dye decolourization efficiency of fungi *A. nidulans* was also determined. Initially the activities of Laccase against 10 different synthetic dyes were determined. On the basis of % Decolourization three dyes namely Congo red, Methyl orange and Alizarin red were selected for further studies. Decolourization efficiency of free fungal mycelium and crude laccase enzyme was determined and it was found that maximum % degradation was obtained against Congo red (88.48%). Fungal spores were immobilized by using calcium alginate gel entrapment method and activity of immobilized laccase was determined by using Congo redsolution of different concentration. Maximum activity was observed when fresh beads of immobilized fungus were used (78.67 %) in 50 ppm dye solution.

KEYWORDS : *A. nidulans*, Synthetic Dye, Fungal Laccase, Textile Effluent

Textile dyes are classified as azo, diazo, cationic, basic, anthraquinone base and metal complex dyes based on the nature of their chemical structure. Synthetic dyes such as azo dyes, xanthenes dyes and anthraquinone dyes are very harmful to all living organisms. Azo dyes constitute a major class of environmental pollutants. Some of the azo dyes or their breakdown products are known to be highly toxic and mutagenic to living organisms.

The general structural characteristics of azo dyes feature substituted aromatic rings that are joined by one or more azo groups (N=N). The annual world production of azo dyes is estimated to be around one million tons and more than 2000 structurally different azo dyes are currently in use. Due to inefficiency in the dying process, it is estimated that about 2% of these dyes are discharged in aqueous effluents during the manufacturing process while 1050% of applied dyes are lost in textile effluents, leading to severe contamination of surface and ground waters in the vicinity of dyeing industries (Ndasi et al., 2011).

However, textile and pharmaceutical effluents are usually recalcitrant to the standard biological treatments, due to the complex aromatic structures, the extreme physico-chemico-parameters and the presence of an autochthonous bacterial micro flora (Spina et al., 2012).

Paper and pulp industries, molasses based-alcohol distilleries, tanneries, dye-making units and textiles are some of the major industries that produce and discharge highly colored effluents. Each of these industrial effluents creates some specific problem besides producing aesthetically unacceptable intense coloring of soil and water bodies. They block the passage of light to the lower depths of the aquatic system resulting in cessation of photosynthesis, leading to anaerobic conditions, which in turn result in the death of aquatic life causing foul smelling toxic waters (Raghu Kumar et al).

There are more than 100,000 different synthetic dyes available in the market. They are used in the textile, paper, cosmetics, food and pharmaceutical industries. Some of them are dangerous to living organisms due to their possible toxicity and carcinogenicity. Among the numerous water- treatment technologies, research interest in the fungal bioremediation, i.e. decolourization and degradation of synthetic dyes, has increased significantly in the last three decades (pavko).

Extensive research in the field of biological azo dye decolourization has shown promising results, but much of this work has been done with single model compound. However, industrial textile wastewater presents the

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additional complexity of dealing with unknown quantities and varieties of many kinds of dyes, as well as low BOD/COD ratios, which may affect the efficiency of the biological decolourization. Dyeing of textile require water and generates a substantial quantity of effluents containing mineral salts and dyes at high concentration. Chronic effects of dyestuffs, especially of azo dyes, have been studied for several decades. Dyes are stable against breakdown by many microorganisms and most dyes do not biodegrade under the aerobic biological treatments in a municipal sewage treatment plants. Many dyes, including the azo dyes, degrade under anaerobic conditions and the aromatic amines thus formed have been found to degrade further aerobically. Out of several methods that are used in the treatment of textile effluents to achieve decolourization, including physiochemical methods like filtration, specific coagulation, use of activated carbon and chemical flocculation some of the methods are effective but quite expensive (Sriram et al., 2013).

Recently, many studies have shown that fungi are able to degrade dyes by extracellular, nonspecific and non-stereo selective enzyme systems [Reddy, 1995]. Until recently various investigations have been focused on either the decolourization of various dyes by a single fungal strain, or consortium fungal strains. Degradation of a dye involves aromatic ring substituent with the presence of phenolic, acetamido, amino or other easily biodegradable functional groups resulting in a greater amount of degradation (Spadaro et al., 1992). Azo dyes are recalcitrant compounds and these dyes are discharged in the water systems. These dyes are mainly degraded by the fungal strains. Their number and species composition in the habitat differs from place to place depending upon the physical, chemical and biological factors of the particular habitat (Gopi et al., 2012).

Laccase is one of the very few enzymes that have been studied since the end of 19th century. Laccases are typically found in plants and fungi. Plant laccases participate in the radical-based mechanisms of lignin polymer formation (Sterjiades et al., 1992) whereas in fungi laccases probably have more roles including morphogenesis, fungal plant-pathogen/host inter- action, stress defense and lignin degradation . Most laccases are extracellular enzymes, making the purification procedures very easy and laccases generally exhibit a considerable level of stability in the extracellular environment. The inducible expression of the enzyme in most fungal species also contributes to the easy applicability in biotechnological processes (Baldrian.).

In the present studyone potent Laccase producing fungus *A. nidulans* was used to study the dye decolourization efficiency. Initially the activities of Laccase against 10 different synthetic dyes was determined. On the basis of maximum decolourization activity three dyes namely Congo red, Methyl orange and Alizarin red were selected for further study. Percent decolourization of each of this dye was determined by using different concentrations of them.

MATERIALS AND METHODS

Sample Collection, Enrichment and Isolation of Fungi

For isolation of laccase producing microorganisms (fungi) various effluent samples were collected comprising textile waste, dye industries and lignocelluloseic wastes. Sampling has been carried out randomly using sterile plastic bags and sterile bottles.

Selective enrichment has been carried out in presence of 0.02% ABTS using nutrient broth and Sabouraud broth under the conditions of optimum temperature of 25^0C. Three cycles of enrichment have been carried out by successive transfer of pre-enriched samples into fresh media. The samples from last enrichment were used to isolate laccase producing fungi.

The isolated organisms were further subjected to screening by plate assay to select efficient laccase producer. Fungal laccase producers were screened by observing brown zone around the colony. The organism which shows good laccase activity was selected for further study.

The selected fungal strain was identified by using morphological analysis of fungal hypal morphology by using microscopic observation. The strain was further identified up to the species level by using 18S rDNA analysis.

Laccase Assay

Guaiacol Assay Method for Laccase Assay

Guaiacol has been reported as efficient substrate for laccase assay. The intense brown color development due to oxidation of guaiacol by laccase can be correlated to its activity often read at 450 nm. Guaiacol (2mM) in sodium acetate buffer (10mM pH 5.0) was used as substrate. The reaction mixture contained 3ml acetate buffer, 1ml guaiacol and 1ml enzyme source and enzyme blank contained 1ml of distilled water instead of enzyme source. The mixture was incubated at 30ºC for 15min and absorbance was read at 450nm blank using UV spectrophotometer. Enzyme activity was expressed as International Units (IU), where 1 IU is defined as amount of enzyme required to oxidize 1micromole of guaiacol per min. The laccase activity in U/ml is calculated using the extinction coefficient of guaiacol (12,100 M-1 cm-1) at 450 nm by the formula: E.A = (A* V) / (t * e * v), where E.A = Enzyme Activity (U/ml), A = Absorbance at 450nm, V = Total volume of reaction mixture (ml), v = enzyme volume (ml), t = Incubation time (min) and e = Extinction Coefficient ($M^{-1}cm^{-1}$).

Dye Decolourization Studies

Dye decolourization efficiency of fungus *A. nidulans* was determined. Initially the activities of fungi producing Laccase against 10 different selected synthetic dyes were determined by using 100ppm concentration of each dye. Following 10 dyes were selected for decolourization experiments. The lambda Max value of each dye was obtained by measuring the absorption of each dye at various wave-lengths.

The actual lambda Max value of each dye was obtained by measuring the absorption of dye solution at various wave-lengths ranges from 350 nm to 660 nm. This nm range was selected on the basis of literature review available.

The obtained lambda max value is used for further analysis.

Calculation

For calculating % decolourization of dye following formula was used.

% DECOLOURIZATION =
(INITIAL ABSORBANCE- FINAL ABSORBANCE)/
(INITIAL ABSORBANCE) X100

The stock solution (10mg/ml) of each dye was prepared by dissolving dye powder in suitable solvent. 0.1 gm of each dye powder is dissolved in 10 ml of suitable solvent which gives final concentration of 1% (10 mg/ml).

This stock solution was used to make different concentrations of dyes like 25ppm, 50ppm, 75ppm and 100ppm. Dilution was performed as shown in the (Table 2).

On the basis of % decolourization three dyes namely Congo red, Methyl orange and Alizarin red were selected for further study. The efficiency of intact fungal

Table 1 : List of Selected Dyes with Molecular Formula and Molecular Weight.

Sr.No	Dye Name	Molecular Formula	Molecular Mass
I	**Congo red**	$C_{32}H_{22}N_6Na_2O_6S_2$	696.665g.mol^{-1}
II	**Fuchsin acid**	$C_{20}H_{17}N_3Na_2O_9S_3$	585.538 g.mol^{-1}
III	**Thymol blue**	$C_{27}H_{30}O_5S$	466.59 g.mol^{-1}
IV	**Methyl orange**	$C_{14}H_{14}N_3NaO_3S$	327.33 g·mol^{-1}
V	**Crystal violet**	$C_{25}N_3H_{10}CI$	407.979 g.mol^{-1}
VI	**Malachite Green**	$C_{23}H_{25}CIN_2$	364.911 g.mol^{-1}
VII	**Phenol red**	$C_{19}H_{14}O_5S$	354.38 g.mol^{-1}
VIII	**Alizarin red**	$C_{14}H_8O_4$	240.21 g·mol^{-1}
XI	**Bromocresol purple**	$C_{21}H_{16}Br_2O_5S$	540.22 g.mol^{-1}
X	**Bromophenol blue**	$C_{19}H_{10}Br_4O_5S$	669.96 g.mol^{-1}

Table 2 : Dilution Scheme.

Concentration	Stock (ml)	D/W (ml)	Final Volume (ml)
25ppm (25mg/L)	0.25	99.75	100
50ppm (50mg/L)	0.5	99.50	100
75ppm (75mg/L)	0.75	99.25	100
100ppm (100mg/L)	1.0	99.00	100

mycelium against three different concentrations (50ppm, 75ppm and 100ppm) of each selected dye was determined.

Dye Decolourization By Using Crude Laccase

The % decolourization activity of crude fungal la ccase was determined against Congo red dye by incubating the enzyme in different dye concentration (25ppm, 50ppm, 75ppm, 100ppm).

The reactions were carried out directly in the spectrophotometer cuvette. The reaction mixture consisted of crude laccase and an aqueous solution of dye in phosphate buffer pH 7.0 in a total final volume of 3 ml. The cuvette was then inverted using paraffin as a cover, in order to achieve the homogeneity of the mixture prior to the measurement. Decolorized activity was determined by monitoring the decrease in absorbance on a spectrophotometer at a maxima absorbance of dye and expressed in terms of percentage. All experiments were done in triplicate. Controls contained the individual dye solution with inactive enzyme.

Immobilization of Fungi

Sodium alginate and calcium chloride solution was preparedseparatelyby dissolving 2.5 gm of Sodium alginate powder and 2 g of calcium chloride in 100 ml distilled water. Sodium alginate and calcium chloride solutions were autoclaved at 121^0 C for 15 minutes, and then were cooled. For immobilization of fungi for the production of laccase use fungal spores dissolved in distilled water. The solution thus obtained was stirred slowly, for 15 minutes at 35^0 C. The solution was allowed to settle for 40 minutes, and then it was stirred again for another 5 minutes. First, the solution of sodium alginate obtained previously was mixed with the fungal spore suspension. To produced uniform size beads the mixture of sodium alginate and fungal spore was added drop wise into calcium chloride solution. The samples were refrigerated for 24 hrs. After 24 hrs. The beads were washed several times with distilled water before use.

Then the beads were used to set the degradation experiment, in which dye solution of selected dye in MSM medium were continuously recycled over the column containing beads with the help of electric pump and % degradation was determined by periodic removal of sample after each 12 hrs. The efficiency of recycled beads was also determined by same method.

RESULTS AND DISCUSSION

Enrichment, Isolation and Screening for Potent Fungal Strain

From the enriched culture broth 22 fungal isolate were obtained which exhibit considerable laccase activity were selected and subjected for screening procedure.

The 22 positive fungal laccase producers were screened by observing brown zone around the colony, out of it 6 presume to be potent, 10 showed medium potency and 6 showed weak laccase activity.

All the isolated and sub cultured fungal colonies were inoculated on petri plates containing PDA, amended with 1% 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). Development of dark green to purple colour indicates the presence of laccase produced by the fungus. The organism which showed rapid growth was selected for further study. Out of 6 potent Laccase producing fungi one namely R.S1was selected for further study depending upon productivity.

Characterization of Screened Fungal Strain

Microscopic study of the isolated fungal strain showed that it is having conidial heads, are short columnar and biseriate, conidiophores are usually short, brownish and

Table 3 : Lambda (λ) Max of Selected Dyes

Dye Name	Given Lambda Max value	Observed Lambda Max Value
Congo red	460	470
Fuchsin acid	544-548	560
Thymol blue	594	600
Methyl orange	430	422
Crystal violet	590	590
Malachite Green	615-619	620
Phenol red	350-640	550
Alizarin red	555	556
Bromocresol purple	590	600
Bromophenol blue	615	600

Table 4 : Dye Degradation Study

Dye Name	Initial Absorbance	Final Absorbance	% Decolourization
Congo red	0.4703	0.0410	81.38
Fuchsin acid	1.335	0.940	29.58
Thymol blue	0.205	0.110	46.34
Methyl orange	0.5821	0.1102	81.06
Crystal violet	1.715	1.425	16.90
Malachite green	1.439	0.726	49.54
Phenol red	0.557	0.272	51.16
Alizarin red	0.4183	0.0575	86.25
Bromocresol purple	1.167	1.001	14.22
Bromophenol blue	2.834	1.442	49.11

smooth walled, conidia are globose and rough walled.

Using the gene specific sequencing primers, the purified PCR amplicons was sequenced. The sequences were .analyzed using Sequencing Analysis 5.2 software. Blast result and phylogenetic tree analysis clearly indicate that screened fungal strain is *A. nidulans*.

Dye Decolourization Studies

The 100ppm concentration of each of 10 selected dyes was used to determine their actual lambda max values and it was found that no significant change observed in the given and calculated value.

The optical density value obtained for each dye at different wavelength (nm) by spectroscopic analysis was used to determine their Lambda max.

The obtained lambda max value is used for further analysis.

The initial and final spectroscopic reading was used to calculate % decolourization which shows that *A. nidulans* show maximum dye degradation activity against Alizarin red (86.25%) followed by Congo red (81.38%) and Methyl orange (81.06) at 100ppm concentration of each dye.

On the basis of % decolourization three dyes namely Alizarin red, Congo redand Methyl orange were selected for further study.

The effect of different concentration of three selecteddyes on degradation process was determined by calculating initial and final absorbance at 50ppm, 75ppm and 100ppm.The dyes shows % degradation as follows.

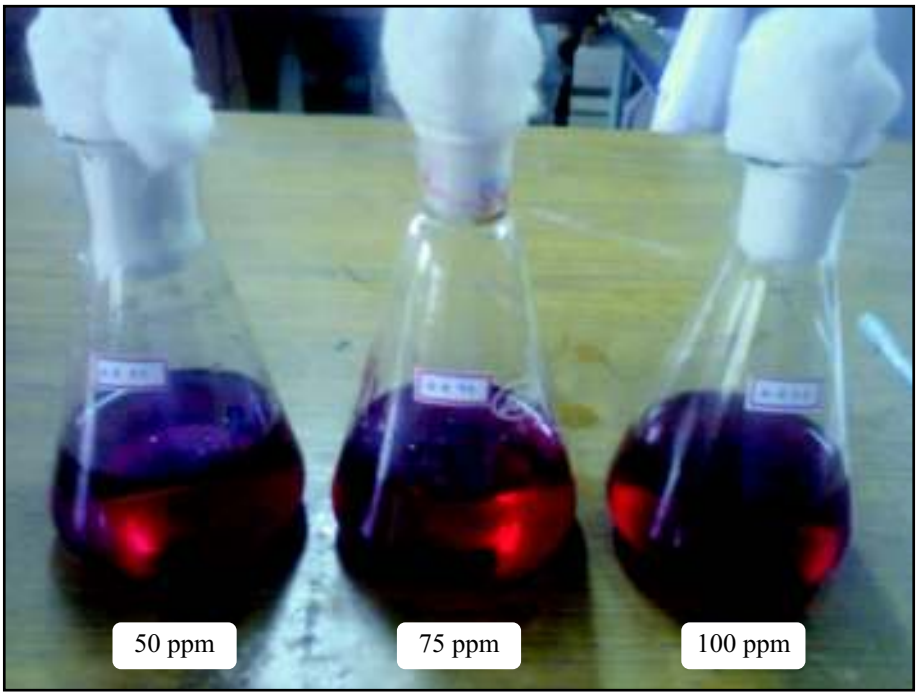

Figure 1 : Solution of Congo Red Before Incubation

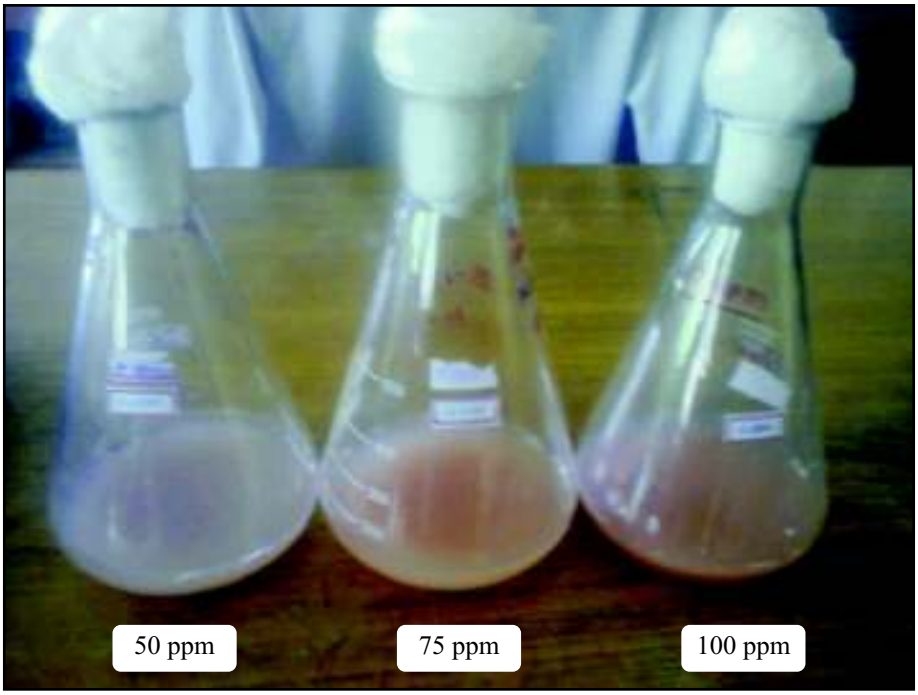

Figure 2 : Solution of Congo Red After Incubation

Table 5 : % Dye Degradation Activity of Free Fungal Mycelium Against Congo Red

Time in hr.	50ppm	75ppm	100ppm
24	12.82	5.00	13.45
48	15.18	16.37	34.27
72	55.15	57.01	46.93
96	88.48	81.38	51.41

Table 6 : % Dye Degradation Activity of Free Fungal Mycelium Against Methyl Orange

Time in hr.	50ppm	75ppm	100ppm
24	14.15	9.52	5.32
48	34.49	23.72	17.51
72	48.61	32.98	31.32
96	65.62	56.04	33.70

Table 7 : % Dye Degradation Activity of Free Fungal Mycelium Against Alizarin Red

Time in hr.	50ppm	75ppm	100ppm
24	25.98	10.07	15.95
48	5001	21.53	29.06
72	74.30	33.71	41.00
96	86.25	69.85	51.89

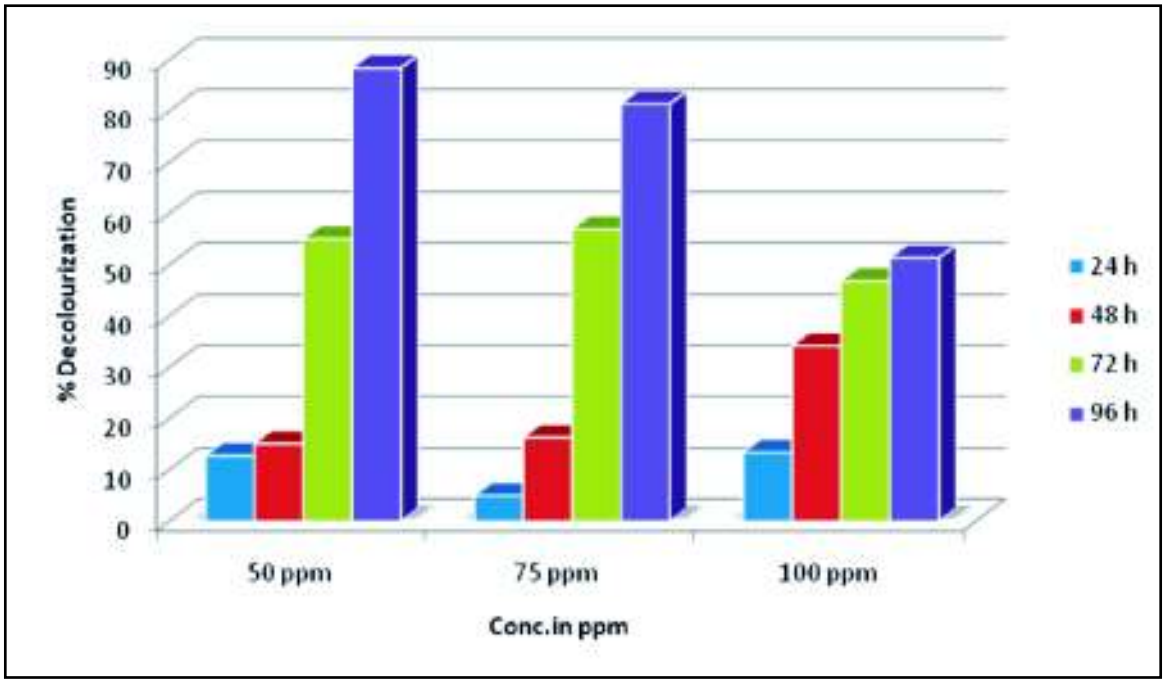

Figure 3 : Activity of Free Fungal Mycelium against Congo Red.

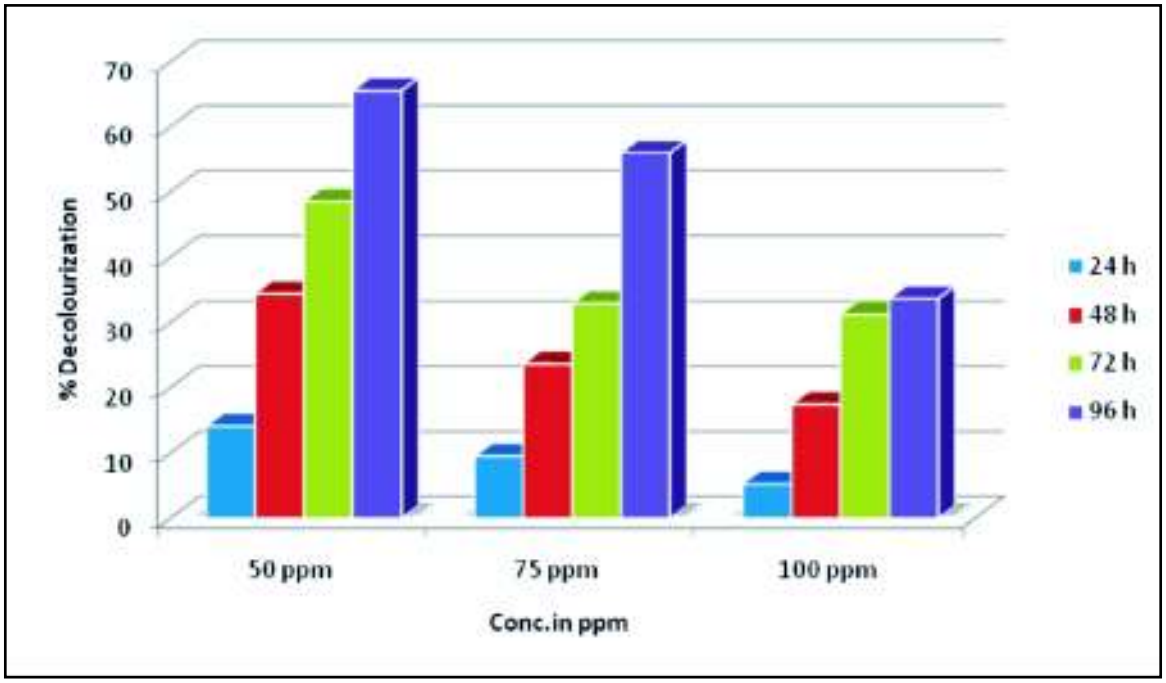

Figure 4 : Activity of Free Fungal Mycelium Against Methyl Orange.

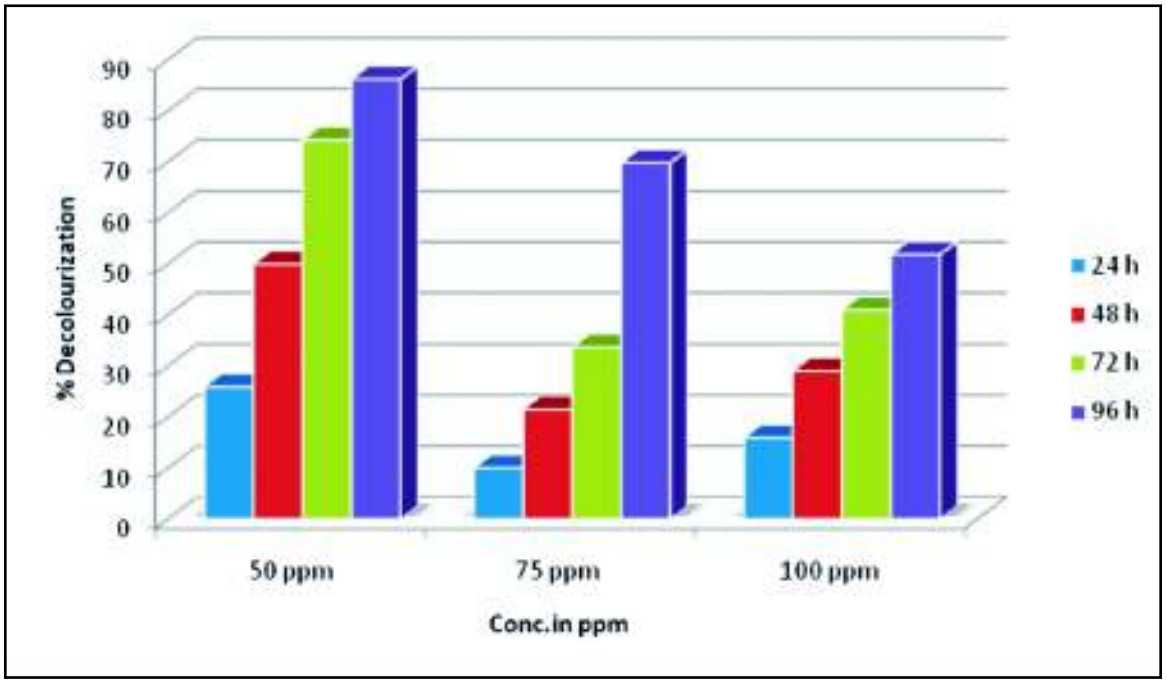

Figure 5 : Activity of Free Fungal Mycelium Against Alizarin Red

Table 8 : Activity of Crude Fungal Laccase Against Congo Red

Dye Conc.	Initial Absorbance	Final Absorbance	% Decolourization
50 ppm	0.4703	0.1074	78
75 ppm	0.9579	0.4231	55.83
100 ppm	1.5079	0.7218	52.13
125 ppm	1.7821	1.0100	43.32

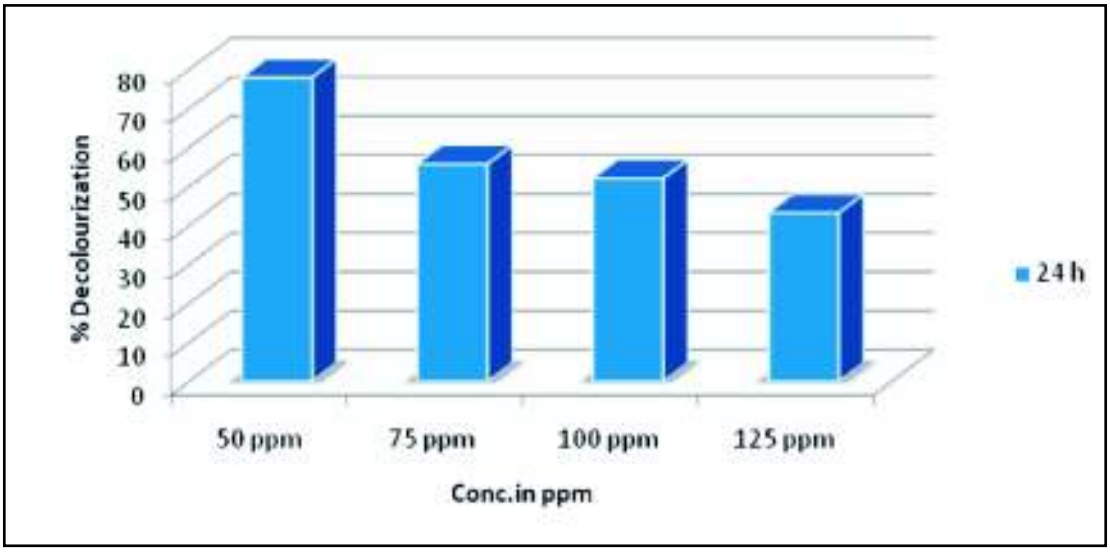

Figure 6 : Activity of Crude Fungal Laccase Against Congo Red.

Initial dye concentration can significantly affect the overall degradation rate. Maximum value of percent dye degradation was obtained when fungus was inoculated in MSM medium containing 50ppm initial concentration of each of the three selected dyes. As initial concentration of dye was increased from 50ppm to 100ppm value of percent degradation start to decrease.

The rate of dye degradation was also found to be significantly affected by time of incubation, this is because fungus produces number of enzymes (Laccases) as well as metabolite in exponential and stationary phase of growth which play crucial role in degradation pathways. So % degradation value increases with time of incubation.

On the basis of result Congo red (88.48 % at 50ppm Conc.) was selected for further study.

Dye Decolourization By Using Crude Laccase

The Laccases are important candidates for degradation of various synthetic dyes. Crude preparation of Laccase shows maximum activity against Congo red solution of 50ppm (78%). It was found that % decolourization decrease (55.83 %, 52.13%, 43.32%) with increasing concentration of Congo red from 75ppm, 100ppm and 125ppm respectively. It can be presumed that the major mechanism of decolorization in the cells is mostly because of the biotransformation by the enzymes i.e. laccase, lignin peroxidase (Raghu Kumar et al.,1996) The relative contributions of laccase, lignin peroxidase to decolorization of dyes may be different for each microorganism.

Activity of Immobilized Fungi Against Congo Red

The *A. nidulans* were successfully immobilized using calcium alginate method as described in methodology. The Fig 9 and 10 show the decolorization ability of freshly prepared beads and recycled beads of immobilized fungi against Congo red solution of 100 ppm.

The experiment was conducted up to 2 rounds and decrease in decolorization rate was observed after 96 hours of incubation. % Decolorization of Congo red remain nearly

(Using Fresh Beads)

Table 9 : % Dye Degradation Activity of Fresh Lmmobilized Fungal Beadsagainst Congo Red.

Time in hr.	50ppm	75ppm	100ppm
24	47.90	50.22	47.76
48	55.09	54.88	47.95
72	59.57	58.67	48.76
96	78.67	67.50	53.98

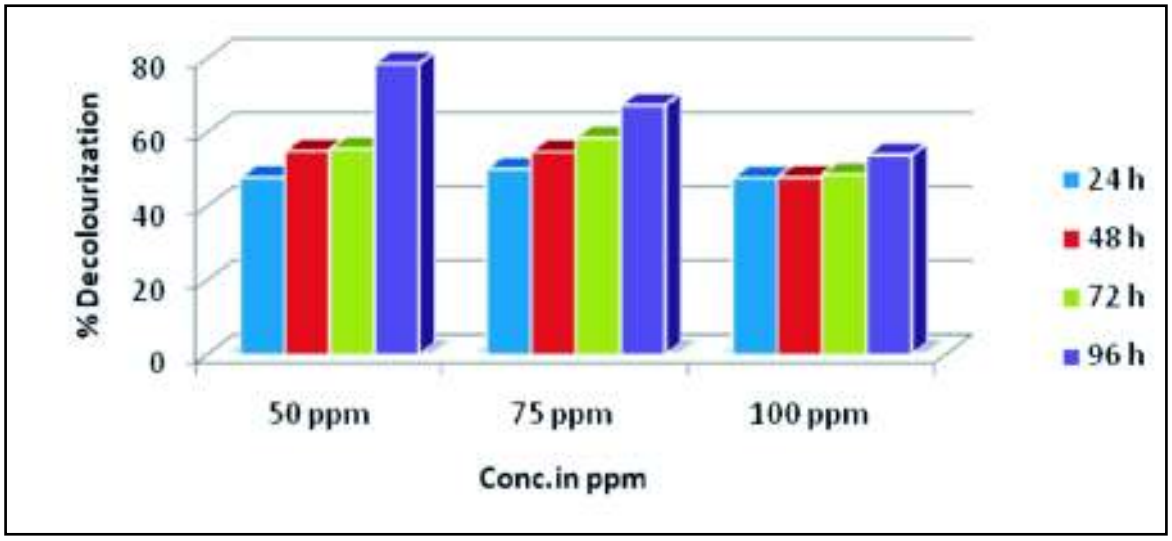

Figure 7 : Activity of Immobilized Fungal Laccase against Congo Red Using Fresh Beads.

stable in second round of incubation in which recycled beads were used.

The Dye degrading efficiency of immobilized fungus *A. nidulans* was determined, and it wasf ound that the freshly prepared beads of immobilized *A. nidulans* effectively decolorized solution containing Congo reddye of different concentration. Percent degradation value increases with time of incubation from 24 hrs. to 96 hrs. Maximum activity was found after incubation for 96 hrs. (78.67%), at 50ppm followed by 67.50% and 53.98% at 75ppm and 100ppm respectively.

The colour removal ability of fungi present in recycled beads was also checked and it was found that the recycled beads (used beads) of immobilized *A. nidulans* effectively decolorized solution containing selected dye of concentration 50ppm, 75ppm and 100ppm. Percent degradation value increases with time of incubation from 24 hrs. to 96 hrs. Maximum degradation value obtained after incubation for 96hrs. (66.17%) at 50ppm followed by 59.17%, 53.69% at 75ppm and 100ppm respectively.

According to Dorthy et al. (2012) fungal isolates were highly effective in decolourization of textile effluent and synthetic dyes in both form (Free and Immobilized) when sodium alginate was used as a matrix. The medium

(Using Recycle Beads)

Table 10 : % Dye Degradation Activity of Recycle Immobilized Fungal Beads against Congo Red.

Time in hr.	50ppm	75ppm	100ppm
24	46.20	46.77	45.70
48	49.59	47.88	46.54
72	54.36	49.73	47.90
96	66.17	59.17	53.69

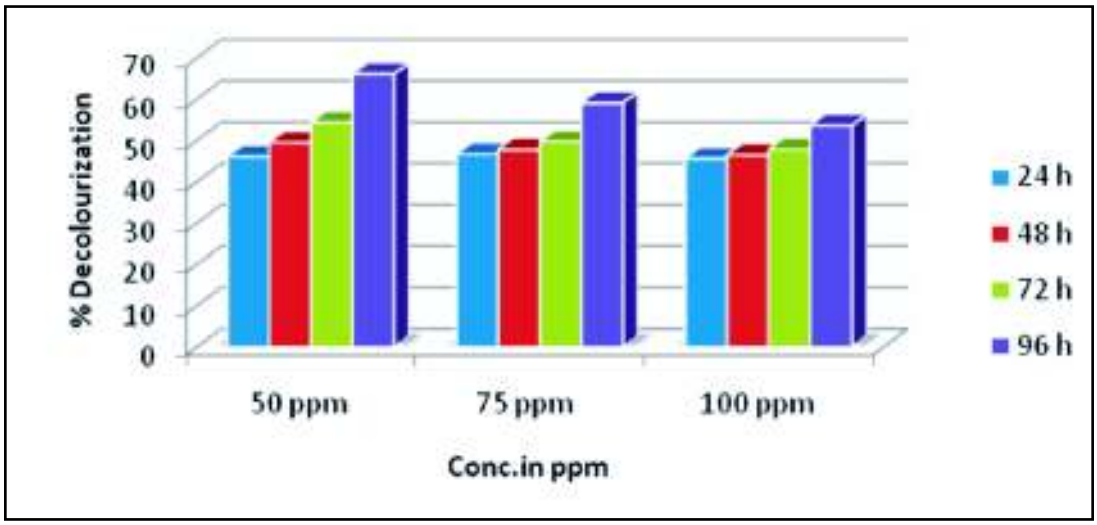

Figure 8 : Activity of Immobilized Fungal Laccase Against Congo Red Using Recycled Beads.

immobilized *A. flavus* was shown 96.32% of decolourization and reduced the pH of effluent.

Senthil Kumar et al., (2013) demonstrates the decolourization capabilities of the fungi *P. chrysosporium* on Amido black B and procion blue- 2G dye solutions. A different decolourization capability was exhibited by the fungus, *P. chrysosporium* based on the nature of dyeas well as whether it existed as free or immobilized cells.

The resultsobtained in this study are in agreement with the result obtained by Dorthy, *A. nidulans* was also proved to be very effective dye degrader when immobilized with sodium alginate. It was found that dye removing ability of fungi remain nearly stable after recycling of beads in second round of reaction.

The isolated strains of *A. nidulans* were found to be very efficient in degradation of synthetic dyes. Initially the activities of *A. nidulans* against 10 different synthetic dyes were determined. Out of which three dyes namely Alizarin red, Congo red and Methyl orange were selected for further study on the basis of % decolourization activity.

The efficiency of crude enzyme laccase in textile processing has been recognized for many years and increasingly gained importance as biocatalysts in textile effluent processing.

The present study result also shows that one can use crude laccase and immobilized form of fungi *A. nidulans* as a coast effective and viable option for treatment of textile industry effluent containing Congo red.

CONCLUSION

Effluent from textile, pulp and paper industries are known to accumulate in soils and water and cause environmental problem. Most of the effluents of the dye industry are harmful for the living organisms and they cause environmental pollution. One possible alternative for treatment of textile waste water is the use of fungi or their enzymes like laccase which can oxidize a wide spectrum of synthetic dyes. Enzymatic treatments have less impact on the environment and ecosystem as they present no risk of contamination.

REFERENCES

Dorthy A. M., Sivaraj C. R. and Venckatesh R., 2012. Decolorization Efficiencies of Dyes and Effluent by Free and Immobilized Fungal Isolates. International J.of Environmental Sciences & research, **1** : 109-113.

Gopi V., Upgade A. and Soundararajan N., 2012. Bioremediation potential of individual and consortium Non-adapted fungal strains on Azo dye containing textile effluent. Advances in Applied Science Research, **3**(1) : 303-311.

Janshekar H. and Fiechter A., 1988. Cultivation of Phanerochaete chrysosporium and production of lignin peroxidase in submerged stirred tank reactors. Journal of Biotechnology, **8** : 97112

Jayasinghe C, Imtiaj A., Lee G. W., I. M. K., Hur H., Lee M., Hee-dun Yang and Tae-Soo Lee, 2008. Degradation of Three Aromatic Dyes by White Rot Fungi and the Production of Ligninolytic Enzymes. Mycobiology, **36**(2) : 114-120.

Khanongnuch C., Wanphrut N., Lumyong S. and Watanabe T., 2004. Thermotolerant wood rotting fungi isolated from northern Thailand and their potential uses in lignin degrading application. Fungal Diversity, **15** : 187-196.

Kim S. J., Ishikawa I. L., Hirai M. and Shoda M., 1995. Characteristics of a newly isolated Geotrichum candidum Decl which decolorizes various dyes. Journal of FermentationBioengineering, **79** : 601-607.

Kumar Praveen G. N. and Bhat S. K., 2012. Fungal Degradation of Azo dye-Red 3BN and Optimization of Physico-Chemical Parameters. ISCA J.of BiologicalSciences, **1** (2) : 17-24.

Meena R. C. and Pachwarya R. B., 2009. Photocatalytic degradation of model textile azo dyes in textile waste water using methylene blue immobilized resindowex-11.J.of Scientific and Industrial Research, **68** : 730-734.

Mohan G., Logambol K. and Ravikumar R., 2012. Investigation on the Removal of Direct Red Dye Using Aspergillus Niger and Aspergillus Flavus under Static and Shaking Condition with Modeling. International J.of science, environmental andtechnology, **1** : 144-153.

Ndasi N. P., Augustin M. and Bosco T. J., 2011. Biodecolourisation of textile dyes by local microbial consortia isolated from dye polluted soils in ngaoundere (Cameroon). International J. Of Environmental Sciences, **1** : No.7.

Raghukumar C., Souza-Ticlo D. D. and Verma A. K., Treatment of coloured effluents with lignin-degrading enzymes: An emerging role of marine-derived fungi. Author version: Critical Reviews in Microbiology, **34** : 189-206.

Senthil Kumar S., Balasubramanian P. and Swaminathan G., 2013. Degradation Potential of Free and Immobilized Cells Of White Rot Fungus Phanerochaete Chrysosporium On Synthetic Dyes. International J. of chem Tech research, **5** : 565-571.

Spina F., Anaslani A., Prigione V., Tigini V. and Gioranna C. Varese, 2012. Biological Treatment of Industrial Waste Waters: a Fungal Approach, : 27.

Sriram N., Reetha D. and Saranraj P., 2013. Biological Degradation of Reactive Dyes by Using Bacteria Isolated from Dye Effluent Contaminated Soil. Middle-East J.of ScientificResearch, **17** (12) : 1695-170.