World Applied Sciences Journal 18 (2): 181-185, 2012
ISSN 1818-4952
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DOI: 10.5829/idosi.wasj.2012.18.02.63245

A Study on the Biodegradation of Some Reactive Textile Dyes by White Rot Fungus (*Pleurotus ostreatus)*

[1]Maryyam Siddique, [2]Ansar Mahmood, [1]Sheikh Munir, [2]Abdul Ghafoor, [3]Sohail Khaliq, [3]Mohsin Bukhai, [1]Kamran Yousaf, [2]Khali-Ur-Rehman, [1]Shahla Andleeb and [1]Mian Muhammad Naeem

[1]Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan
[2]Department of Wildlife and Fisheries, GCU, Faisalabad, Pakistan
[3]Department of Chemistry, GC University, Gujrat, Pakistan

Abstract: A study was performed to assess the decolorization (%) of textile reactive dyes and their biodegradation by *Pleurotus ostreatus*. Four reactive dyes; Remazol RG, Livafix Red CA, Prucion Navy PXG and Prucian Blue PX5R were studied. After 10 days incubation the maximum decolorization (%) of Livafix Red CA (81.01±2.10%) was observed at temperature 30°C and pH 4.5. After the incubation of five days maximum biodegradation was exhibited in the presence of glucose (91.58±2.56%) followed by starch (58.8±3.71%) and minimum with fructose (7.17±3.22%). Similarly maximum decolorization (%) was observed in the presence of ammonium-pyro-phosphate (64.78±2.10%) followed by peptone (29.9±1.12%) & ammonium nitrate (29.0±2.33%) and minimum by ammonium sulphate (4.99±1.27%). Further studies were necessary to screen out the cheapest source of carbon and nitrogen as compared to glucose and ammonium-pyro-phosphate.

Key words: Dyes · Decolorization · Biodegradation · Carbon · Nitrogen

INTRODUCTION

The population of the world is increasing in a geometric progression. With the growing population, the need for food, clothes and shelter also increased. To fulfill these requirements more industries are being established, the most prominent being the textile industry. Synthetic dyes can be released into the environment from two major sources: i) as effluents from synthetics plants and ii) from dye using industries, such as textile factories. It is estimated that between 10 and 15% of the total dye used in the dying process may be found in wastewater. Reactive azo dyes are widely used in textile industries because they are cheap, easy to apply on fabric and give good color and light fastness [1]. Dye house effluents contain large amounts of dyes. Unbound reactive dyes undergo hydrolysis due to elevated temperature and pH values during the dyeing processes. The strong color of discharged dyes even at very small concentrations has a huge impact on the aquatic environment caused by its turbidity and high pollution strength. Additional toxic degradation products can be formed. Azo dyes, which constitute the largest group of colorants used in industry, leave municipal wastewater plants highly diluted but nearly unchanged because they resist aerobic and short-term anaerobic treatment [2].

The traditional textile finishing industry consumes about 100 liters of water to process about 1 kg of textile materials. New closed-loop technologies such as the reuse of microbial or enzymatically treated dyeing effluent could help to reduce this enormous water consumption [3]. Vat textile dyes are commonly employed on cellulose fibers. About 10,000 dyes have an assured future. Cellulose consumption of dyes in world wide is about 60.000 tons year^{-1} [4].

Fungi can remove organic pollutants, excessive nutrients and dyes and lead to a decrease in their toxicities. It depends upon media and culture condition, in addition to the degradation of organic pollutant, fungi produce added-value products such as (Lip, Mnp, Lacc, amylase) and single cell protein. An additional C and N sources are therefore, required for primary metabolism by white rot fungi [5]. Several studies have showed that the degradation of azo, anthraquinone, heterocyclic triphenylmethance and polymorphic dyes is endorsed by *Phanerochaete chrysosporium.* Many studies revealed

Corresponding Author: Ansar Mahmood, Department of Wildlife and Fisheries, GCU, Faisalabad, Pakistan.

the use of white rot fungus for the decolorization as well as partial mineralization of various reactive textile dyes [1, 4, 6-8]. With global attention and research now focused on looking for the abatement of pollution, white rot fungi is one of the hopes of the future. The lignin degrading ability of the fungi have been the focus of attention for many years and have been exploited for a wide array of human benefits [1, 5]. In statically grown cultures a decrease in dye adsorption was accompanied by visible adsorption of the dyes to fungal mat. The superior and increased performance of the agitated cultures may be due to the physiological state of the fungi as pellets and increased mass and oxygen transfer between the pellets and medium due to mixing [6].

Biological decolorization is employed under either aerobic or anaerobic environment. A number of reports discourage the azo dye decolorization by microorganisms under anaerobic conditions as it leads to the formation of corresponding aromatic amines. Even though their reductive cleavage is responsible for color removal the formation of aromatic amines is highly undesired as they are reported to be carcinogenic. Waste of textile industry show low biological oxygen demand (BOD) and high chemical oxygen demand (COD) and is highly colored. Chemical treatment is necessary to produce biodegradable compounds. Wastewater containing water soluble dyes are not effectively decolorized by biological treatment [2, 5]. Keeping in view the significance of water pollution and importance of biodegradation, the present project has been undertaken to degrade different dyes by *P. ostreatus* and efforts have been made to degrade reactive dyes using this microorganism to avoid accumulation of notorious toxic intermediates.

MATERIALS AND METHODS

Four reactive dyes from Dyestar Pakistan Faisalabad Pakistan were collected in order to study their biodegradation by *Pleurotus ostreatus* in shake flasks experiment. Dyes were Remazole Red RB, Livafix Red CA, Prucion Blue PX5R and Prucion Navy PXG. A locally isolated *P. ostreatus* fungus strain was used for depolarization studies. The slants were incubated at pH 4.5 at temperature 32ºC for sporulation. The growth medium [9] was prepared and the spores of *P. ostreatus* were transferred aseptically by streaking with inoculation loop on to the slants in laminar air flow. For inoculums preparation the inoculums medium was inoculated in Laminar air flow with *P. ostreatus* from slant culture and placed on a shaking incubator at (120 rpm) at 30ºC for three days to get a homogeneous spore inoculum having 1×10^8spores ml^{-1}. The number of spores in inoculums were counted using haemocytometer [10]. Fresh inoculum was prepared for each parameter investigation.

Duplicate flasks were prepared for all of the four reactive dyes. Each flask contained 100 ml sterilized basal nutrient medium [9] with 0.05% concentration of the respective dyes. The entire decolorization medium was maintained at pH 4.5. All of the flasks were autoclaved, cooled and inoculated with 5ml homogeneous inoculum of *P. ostreatus* in laminar air flow aseptically. All the flasks were incubated on orbital shaker for 10 days at 120 rpm and 30ºC for decolorization of dyes by the microorganism. One ml sample from each flask was removed after every 24 hours by micropipette and diluted to 10 ml. The sample was centrifuged at 10,000 rpm for 30 minutes and each of the samples was run in spectrophotometer. The % decolorization of all four dyes was determined after 10 days decolorization. The λ max values for four dyes viz., Remazol Red RB, Livafix Red CA, Prucion Blue PX5R and Prucion Navy PXG were determined at 513, 515, 568 and 600nm [9, 10]. The *P. ostreatus* (white rot fungus) was cultured in decolorization medium of the selected dye Livafix Red CA by using nutrient medium [9] for decolorization. The selected medium on the basis of best performance under specific culture condition of pH and temperature was used in subsequent process optimization. For optimization of pH the cultures were adjusted to varying initial pH values viz., 3, 4, 4.5, 5, 5.5 and 6 and different temperature 30, 35, 40, 45 and 50ºC. The medium was maintained to initial optimum pH 4.5. Maximum decolorization (%) of the dye Livafix Red CA was observed at temperature 30ºC.

Addition of Carbon and Nitrogen Sources: The fungi were grown on readily available carbon sources at initial stages of growth to produce secondary metabolites and extracellular enzymes for biodegradation of dyes. To find an-inexpensive and most suitable carbon source, different sources such as fructose, sucrose, glucose, starch, molasses and maltose (1gm $100ml^{-1}$) were used for the dye Livafix Red CA in shake flask experiment. The experiment was conducted at optimum pH 4.5 and temperature 30±2°C for five days. Samples were removed daily from each flask by micropipette and diluted to 10 ml with distilled water. Glucose proved the best carbon source for Livafix Red CA decolorization supported by *P. ostreatus*. Urea, peptone, ammonium sulphate $[(NH_4)_2SO_4]$, ammonium nitrate $[(NH_4)_2NO_3]$ and ammonium di-hydrogen phosphate $[(NH_4)_3\ H_2PO_4]$ were used as nitrogen sources for the current study.

Enzyme System: At the end of the study the decolorized, the culture filtrate of the optimum process was assayed for Lignin per-oxidase (Lip), Manganese per oxidase (Mnp) and Laccase (Lac). Laccase activity was determined by 2, 2 azinobis (ABTS) oxidation in a reaction containing 1Mm ABTS in 0.1 molar sodium acetate buffer (pH 4.5) with a 50 µl enzyme sample [11].The oxidation was followed at 436 nm (25ºC). Lignin per-oxidase activity was measured by the method of Kirk and Farrell [11] following the hydrogen-per-oxide (H_2O_2) dependant oxidation of veratryl alcohol to veratrl-aldehyde at 25ºC. The reaction mixture contained 4mM veratryl alcohol at 25°C in 40 mM succinate buffer (pH 3) and the reaction was initiated by the addition of H_2O_2 to a final concentration of 0.4mM. Absorbance was monitored at 310 nm. The activity of Mn peroxidase was measured at 25ºC by the oxidation of manganese malonate complex at 270 nm [9, 10]. The reaction mixture had 1 mM mangonous sulphate in 50 mM manganate buffer (pH 4.5) and 5-50 mL of enzyme sample and was initiated by the addition of H_2O_2 to a final concentration of 0.1 mM. Recorded data were analysed statistically and their Significant and non-significant differences were checked at 0.05 and 0.01% levels.

RESULTS AND DISCUSSION

White rot fungus have broad substrate specificity, ability of transformation and mineralization of organo pollutants for its structural similarities with lignin [1, 5, 12]. Waste waters as effluents of industries with various dyes are the major environmental cancers. Multidimensional approaches for the bio-remediation approached are needed for the treatment of wastewaters bearing health hazardous dyes. Many citations are available who screened out different fungi for the degradation of syringol datives of azo dyes possessing carboxyl or sulphonic groups [13, 14].

Fermentation Time: Erlenmeyer flask experiment was performed for 10 days for biodegradation of different reactive textile dyes by *P. ostreatus*. The results revealed that the decolorization of dyes Remazol Red RB, Livafix Red CA, Prucion Blue PX5R and Prucion Navy PXG after 5 day of incubation were 65.3±1.20%, 81.0±2.10%, 68.1±4.22% and 61.8±2.40% respectively (Table 1). The results presented indicated that maximum decolorization (81.01±2.10) of the dye Livafix Red CA was achieved after 5 days of incubation and decreased thereafter gradually up to 10th days. Balan and Monteiro [15] gave the conclusive remarks that indigo dye extensively used in industries was of a great environmental concerns and found phellinus gilvus started decolorization of Vat textile indigo dye in a few hours and after 4th day it was completely (%) decolorized. Sani *et al*. [6] concluded from his finding that static conditions depicts less decolorization rates than shake culture and also depends upon biomass concentrations. In the current findings Remazol RB was decolorized 65.3±1.20% by *P. ostreatus* after 10 days (Table 1). Similarly Asghar *et al*. [16] demonstrated that all applied dyes gave better responses than remazol brilliant even after eight days. Pourbabaee *et al*. [2] isolated many fungi for the decolorization purpose and found that aerobic species of fungi decolorize some dyes from textile mill effluent. Terasil black dye was decolorized more under current situation in the presence of exogenous source glucose or starch after five days and further that process was enhanced by the presence of 0.5% yeast extract.

PH: Significant ($p<0.01$) variations were recorded among pH values (Table 2). It was observed that fermentation media adjusted at pH 4.5 resulted in a maximum biodegradation of the dye Livafix Red CA (79.28±3.65%) followed by 60.1±2.91, 27.33±1.01, 23.0±2.66, 19.76±2.74 and 18.96±1.21% at 6.0, 5.5, 5.0, 4 and 3.0 pH respectively after 5 days (Table 1).

Table 1: Mean (Mean±SD) decolorization (%) of the four reactive dyes and biodegradation of dyes at varying temperature (°C), pH, nitrogen and carbon sources.

			Decolorization (%) of Livafix Red CA							
No. of variables	Decolorization (%) of four Reactive dyes		Temperature (°C)		Carbon source		Nitrogen source		pH	
1	Remazol RB	65.3±1.20^{c}	30	23.10±1.11^{b}	Fructose	7.17±3.22^{e}	Peptone	29.9±1.12^{b}	3.0	18.96±1.21^{de}
2	Livafix Red CA	81.0±2.10^{a}	35	23.78±1.77^{b}	Sucrose	26.6±1.25^{d}	Urea	28.92±2.24^{bc}	4.0	19.76±2.74^{cd}
3	Prucion navy PXG	68.1±4.22^{b}	45	26.84±1.00^{c}	Maltose	34.0±1.98^{c}	(NH4)2NO3	29.0±2.33^{b}	4.5	20.28±3.65^{bc}
4	Prucian Blue PX5R	68.8±3.40^{b}	50	58.46±4.88^{a}	Molasses	53.7±2.78^{b}	$(NH4)_2SO_4$	4.99±1.27^{d}	5.0	23.0±2.66c^{b}
5	-		-	-	Starch	58.8±3.71^{b}	$(NH_4)_3H_2PO_4$	64.78±2.10^{a}	5.5	27.33±1.01b
6	-		-	-	glucose	91.58±2.56^{a}	-	-	6.0	60.1±2.91^{a}

Similar alphabets in the rows and columns did not differ significantly ($p>0.01$)

Table 2: Analysis of variance (ANOVA) for decolorization (%) of the four dyes at varying pH, temperature (°C), nitrgen and carbon sources.

		Mean sum of squares		
SOV	df	Carbon source	Nitrogen source	Four reactive dyes
Reactive dyes (RD)	3	3175.2**	3068.17**	22912.85**
Carbon (C)	5	364.58**	547.658^{NS}	365858.65**
RD X C	2	6.040^{NS}	363.450^{NS}	36.850**
Nitrogen (N)	4	458.254**	1589.472**	2556.547^{NS}
RD X N	2	358.478^{NS}	369.478^{NS}	35.187^{NS}
C X N	3	298.547^{NS}	365.478^{NS}	3651.478**
RDXC X N	3	547.698**	125.149**	1245.658^{NS}
Error	7	254.25	589.875	54785.258
Total	29	2921.254	24235.658	352599.658
LSD	3.577	10.22	9.20	21.12

**, significant at 1% level of probability; NS, non-significant (p>0.01)

It was noted that dye degradation increased with the increase in pH from 3 to 4.5. A further increase in pH could not produce greater decolorization. The fungus *P. ostreatus* favored the decolorization at pH 4.5 as reported in literature [16, 17] and further reports displayed that acidic pH favored the growth of *P. ostreatus* which favored the decolorization of environmental concerned industrial dyes.

Temperature: Significant (p<0.01) differences were observed among different temperature regimes (Table 2). First of all four respective dyes were assorted out for the better decolorization (%) of dyes with different temperatures and pH. Among all four dyes livafix Red CA was found well resulted at temperature 30°C and pH 4.5. Further process was repeated with *P. ostreatus* for its decolorization (%) efficiency at the best observed optimized temperature and pH. The livafix red CA exhibited maximum decolorization (58.1±1.11%) while least (23.78±1.77%) was found at 30°C in the presence of *P. ostreatus* at pH 4.5 (Table 1). Decolorization and laccase activity were equally effected by the both pH and temperature parameters. Laccase was considered the major enzyme involved in enhancing the ability of *P. pulmonarius* for the discoloring of industrial dyes [7].

Carbon Source: Significant (p<0.01) variations were observed among carbon sources and reactive dyes while non significant (p>0.01) differences were observed among different nitrogen sources (Table 2). Duplicate growth media maintained at pH 4.5 and temperature 35ºC containing different carbon sources (fructose, sucrose, maltose, molasses, starch and glucose) were placed in the shaking incubator at 120 rpm for 5 days (Table 1). It was observed that all additional carbon sources supported fungal growth and increased decolorization of Livafix Red CA by *P. ostreatus.* Decolorization medium of Livafix Red CA however, supplemented by *p. ostreatus* showed maximum (81.0±2.10%) decolorization followed by prucion vavy PXG (68.1±4.22%) after 10days. In the current findings glucose supported the maximum decolorization of livafix red CA (91.5±2.56%) with *P. ostreatus* in 10 days (Table 1). The current results are not in the line of Martins *et al*. [14] who revealed that biodegradation of dyes mainly depends upon sucrose concentration, dye structure and medium used. Even the glucose gave better results than others but major issue was to screened out easily available and less precious carbon source for bio-remediation and decolorization of reactive dyes. Similar findings were found by Sumathi and Manju [18] who revealed that *Aspergillus foetidus* removed prucion dye 90% within 48 hours of fungal growth in the presence of glucose.

Nitrogen Source: Significant (p<0.01) differences were observed among nitrogen sources while non-significant (p>0.01) variations were recorded with reactive dyes (Table 2). Ammonium-pyro-phosphate enhanced 64.78±2.10% decolorization than other sources of nitrogen. Peptone (29.9±1.12%) proved moderate source for current purpose while least decolorization was recorded by the ammonium sulphate (4.99±1.27%) (Table 1). On the contrary Mikiashvili *et al.* [19] stated that peptone followed by casein hydrolysate has proved best nitrogen source for laccase accumulation by p. ostreatus used in the current experiments.

It was observed that all additional nitrogen sources supported fungal growth and increased the decolorization of Livafix Red CA with the inoculation of *P. ostreatus*. After 10 days maximum decolorization (%) was observed in livafix red CA. When different sources were applied the maximum decolorization was observed with glucose (91.58±2.56%) followed by starch (58.8±3.71%) and molasses (53.7±2.78) while minimum with fructose (7.17±3.22%) (Table 1). Results of sucrose (26.6±1.25%) were in accordance with Spadaro *et al*. [4] who observed that under nitrogen limiting white rot fungus with 14C ring labeled azo dye decolorized 23.1-48.1% after 12 days of incubation. Aromatic ring substituting with hydroxyl, amino-acetamido or nitro were mineralized to greater extent than un-substituting rings. It was proved from the current results that *P. ostreatus* fungus gave good results and can be used for the future studies for the biodegradation and decolorization of health hazardous industrial reactive dyes.

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