Biodegradation of azo dyes using *Pleurotus platypus* and its phytotoxicity study

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Abstract : In the present study, the potentiality of *Pleurotus platypus* in degrading four different azo dyes, namely methylene blue, methyl red, congo red, and orange –G, under optimum conditions were studied and the degraded sample was analyzed for its efficiency in plant growth. Kirk's media was used for the degradation study and it was found out that the organism degraded the dye best in Kirk's media on the fifth day of incubation under neutral pH at 40ºC. The decolourization of the dye was estimated by using UV-VIS spectrophotometer. TLC was performed to find out the Rƒ value of the degraded dyes. Azoreductase enzyme activity was also analyzed. Phytotoxic analysis of the treated dye was compared with the control. Germination count of green gram seeds with the treated dye sample is compared with the untreated dye and tap water. The phytotoxicity analysis of the green gram plants showed better results with the treated dye sample .Enzyme activity of the treated dye soil sample was compared with the enzyme activity of the soil with untreated dye. The results are very promising and confirms the potentiality of the white rot fungi, *Pleurotus platypus* in degrading azo dyes.

IndexTerms: **degradation, Kirk's media, Rf value, Azo dyes, Dehydrogenase, Phosphatase and cellulase**

I. INTRODUCTION

The wide use of synthetic dyes in several industries such as textile, paper, printing, cosmetics, pharmaceuticals, colour photography and petroleum has drastically increased the large scale use of dyes.Based on the structure dyes are classified into acidic, basic, disperse, azo, diazo, anthraquinone and metal complex. Based on the dyeing process, textile dyes are classified as reactive, direct, disperse, acid, basic and vat dyes.Textile industries generates large volume of polluted water during processing. The increased production of textile industry effluents in recent years has proportionally increased the rate of water pollution. Approximately 75% of the dyes discharged by textile processing industries belong to the classes of reactive (36%), acid (25%) and direct (15%) dyes. Approximately 50% of the applied dye is lost in the effluent during the textile dyeing process.(GuoW.et.al;2015).One of the most obvious indicators of water pollution are the coloured water generated by the textile industry. Coloured dye wastewater causes severe effects on aquatic environment and also contains other pollutants like degradable organics, nutrients, pH altering agent, salts, sulphur, toxicants and refractory organics (Somasiri et al. 2008; Haroun and Idris 2009). A number of physicochemical methods employed for the efficient removal of residual azo dyes from industrial effluents have shown limitations.The effectiveness of these methods is limited due to the low efficiency, incomplete (20–30 %) colour removal, limited versatility, high cost, production of large amounts of sludge and handling of the effluents generated (Saroj et al. 2014). Hence, biotechnologists have focused on biological treatment as the best alternative which is more cost efficient when compared with conventional technologies. Many microorganisms, including bacteria, fungi and actinomycetes, have been reported for their ability to decolourize dyes. Efficiency of biodegradation of azo dyes by the bacteria *Bacillus megaterium* has been reported. (Lekha et.al.2017; P.A Joshi et al 2015).The efficiency of the biodegradation of azo dyes by a consortium of fungi at low concentration of the dye has been reported.(Samta Saroj,et.al 2015)

Among these microorganisms, white rot fungi are the most intensively studied dye decolourizing microbes. These fungi produce large quantities of extracellular enzymes that help to remove dyes from industrial effluent and also have the ability to resist unfavourable environmental conditions. In this study, a white rot fungal strain, *Pleurotus Platypus* , was examined for its ability to decolourize four different textile dyes namely methylene blue,methyl red, congo red,and orange –G under optimized condition and the % of degradation were carried out by UV –Visible Spectroscopy and TLC analysis. The dye treated sample was analysed for its efficiency in germinating green gram seeds and phytotoxic analysis of the plants were done. Soil enzymes namely phosphatase, cellulose and dehydrogenase activity are also performed.

II. MATERIALS AND METHODS

2.1Microorganism and Culture media

The *Pleurotus platypus* were obtained from TNAU, Coimbatore, India. The sample was inoculated in malt agar medium and incubated at 37° C for 24 hrs and then stored at 4° C and periodically sub-cultured for further study. Biomass assay, to find the optimum day of growth was done by measuring the mycelia weight every day. The decolorization study was carried out on Kirk' s

medium (D-glucose 10g, KH2PO4 2 gm, MgSO4.7H2SO4 0.5gm, CaCl2.2H2O 0.1gm, L- Asparagine monohydrate 93 mg, NH4NO3 50mg, 6M KOH, Nitrogen supplements, Trace element solution 1ml, thiamine 100 µg, dialyzed poly acrylic acid 0.72gm/L of distilled water). All microbiological media and medium ingredients were purchased from HiMedia Laboratories (Mumbai, MH,India).

2.2Azo Dyes

Methylene blue, Methyl red, Orange G and Congo red dyes also obtained from Hi Media, Mumbai were used for the study. All chemicals and dyes were of highest purity and were of analytical grade.

2.3Optimum Day

The fungal culture was inoculated into Kirk's Medium (pH 6.0) in the 100ml Erlenmeyer flask. The flasks were then incubated at room temperature in orbital incubator- 150 rpm for 8 days. The biomass was calculated to find the optimum day of growth of the organism.

2.4Optimisation of Carbon and Nitrogen source

Decolourisation effect of the *Pleurotus Platypus* was tested using three different carbon sources and nitrogen sources. The Kirk's media (Selvam and Swaminathan 2003) is optimized with carbon sources (Glucose, Sucrose and maltose) and nitrogen sources (K2HPO4, KH2PO4, and Peptone) for attaining high decolorisation effect.

2.5Degradation Analysis

The fungal culture was inoculated into Kirk's Medium (pH 6.0) in the 100ml Erlenmeyer flask. The flasks were then incubated at room temperature in orbital incubator- 150 rpm for 8 days. 5ml of the dyes of concentration 500mg/L and 1000 mg /L were added to the culture on the second day. Simultaneously, controls were also maintained in the same conditions without the addition of inoculums at neutral pH. The biomass, decoloration and pH were observed every 6 h.

2.6U-V visible Nano Drop Spectrophotometer

The biomass and decoloration were calculated by measuring the OD at specific nm : Methyl Red : (560nm) ; Malachite green : (617nm) ; Methyl orange : (487nm) ; Congo red (Merck) (580nm) and Methylene blue (660nm). The degradation study was monitored by measuring the absorbance as a UV-Vis spectrum of reaction medium by drawing 1cm3 medium on UV visible ELICO SL 159 nanodrop spectrophotometer. The percentage decolourization was calculated from the following equation,

$$\% \text{ Decolourization} = (\text{Initial OD} - \text{Final OD}) / (\text{Initial OD}) \times 100 \quad (1)$$

2.7TLC Analysis of biodegradable product

The degraded product was extracted from the strain. Cells were centrifuged and supernatant was extracted with the equal volume of ethyl acetate and then dried over anhydrous sodium sulphate. The residue was dissolved in a small amount of methanol and this was utilized for a TLC test. The developing solvent systems used were ethylacetate: hexane (2:3v/v) for biotransformed intermediates/products and ethyl acetate: methanol (7:3, v/v) for residual dye. The bands of aromatic compounds were observed under UV light (365 nm).

2.8 Enzyme assay

2.8.1Purification and characterization of azoreductase enzyme

Cells from the mid log phase culture were harvested by centrifugation at 10,000 rpm for 10 minutes at 4° C. Pellets were disrupted by sonication for 6 minutes. The cell lysate was subjected to fractionated ammonium sulfate precipitation at 40% saturation to remove impurities, followed by 70% saturation in a second step to precipitate the azoreductase. After 24 hrs, the precipitated protein is centrifuged for 10 minutes at 10000 rpm at 4° C and the pellet was dissolved in equal volume of 50mM potassium phosphate buffer (pH 7.2). Ammonium sulfate precipitated sample was then desalted by dialysis against phosphate buffer (50 mM, pH 7) overnight under room temperature. 2mL of the resulting solution was fractionated by anion exchange chromatography using DEAE sephadex column. Elution buffer (sodium phosphate buffer containing 1M NaCl was set to a gradient of 100% for 150 minutes. Proteins were eluted at a flow rate of 1 mL/minute. The fractionated sample was concentrated using protein purification column. Active fractions were collected and stored as the purified enzyme preparation.

2.8.2Assay of Azoreductase activity (Zimmermann et al. (1982)

Assays were carried out in cuvettes with a total volume of 1mL using ELICO SL159 UV- VIS Nanodrop Spectrophotometer. The reaction mixture consists of 400 µl of potassium phosphate buffer (50mM) with 200 µl of sample and 200 µl of reactive dyes (500 mg/l). The reaction was started by addition of 200 µl of 2mM NADH (7mg/mL) and was monitored photometrically at 532 nm. The linear decrease of absorption was used to calculate the azoreductase activity. One unit of azoreductase can be defined as the amount of enzyme required to decolorize 1 µ mol of acid red per minute. The assay procedure is based on the principle that with the addition of NADH to the reaction mixture containing substrate, buffer and enzyme solution, the substrate azodyes azo bond is

degraded and there is a decrease in the absorbance of the dye after an initial lag phase. One unit will reduce 1.0 µ mole of azo dye per minute in the presence of NADH using Millimolar extinction coefficient of azo dye at pH 7.0 and 30° C temperature

$$\text{Units / ml enzyme} = \frac{\text{A 532 nm / min Test} - \text{A532 nm / min blank}}{\text{B X (0.1)}} \quad 2$$

A = Total volume (in milliliters) of assay; B = Millimolar extinction coefficient of azo dye, Orange G (1.5x105); 0.1 = Volume (in milliliter) of enzyme used.

2.9 Phytotoxicity study

2.9.1Germination count

The seeds of green gram (Vignaradiata) were obtained from TamilNadu Agricultural University, Coimbatore and were treated with the tape water, dye alone and treated effluent with dye. Then the seeds were spread on the sterilized petridishes lined with filter paper. Germination was observed after every 24 hours.

$$\text{Germination percentage} = \frac{\text{No.of seeds germinated x 100}}{\text{No.of seeds sown}} \quad 3$$

The plants were treated with tap water, dye contaminated water and *Pleurotus Platypus* treated dye water.Shoot and root lengths are equal in (control)tap water and treated dye water samples, whereas in the dye treated samples the shoot and root lengths are less when compared with the control.

Phytotoxicity study were done to identify the problems and nutrient level changes of the plants. In this study, green gram plant were selected and three types of treatment were given to the plants ie; with tap water as control; dye treatment (orange G) and with *Pleurotus platypus* treated dye waste water as a sample. The above mentioned were used as water sources for the plants and studied for two weeks. The changes were noticeable and recorded. The root and shoot length, height of the plants were studied. Nutrient analysis was performed to access protein and carbohydrate , chlorophyllcontent of the plant extract.The toxicity studies on the sample dyes (untreated) and the extracted metabolites (treated) obtained after the biodegradation of the selected azo dyes by *Pleurotus platypus* on the plants was carried out in two concentrations 500mg/l and 1000mg/l.Enzyme activity of the soil enzymes namely dehyrogenase, phosphatase and cellulase were also found.

2.9.2Estimation of Carbohydrate (Anthrone method) and Protein (Lowry's Method)

The plants grown in tap water were taken as control. They were compared with the dye contaminated water grown plants and dye treated water grown plants. Estimation of carbohydrate and proteins were done by anthrone methods and Lowry's method respectively, Spectrophotometric methods were utilised and the OD were taken at 620nm and 660nm respectively for the Anthrone and Lowry's et al (1951) respectively. The amount of carbohydrates and proteins of the plant extracts were tested for all the plants. It was noted that the plants grown using treated water contained almost the same concentration of carbohydrates and proteins as the control. Hence, it can be inferred that the decolorisation has been proved effective since they did not affect the carbohydrate and protein concentration of the plant. Glucose was used as a standard for the anthrone method and were prepared by mixing 10 mg /100 ml concentration

2.9.3Estimation of Chlorophyll (Tanaka, A et.al)

Five hundred mg of fresh leaf material was taken and ground with help of pestle and mortar with 10 ml of 80% acetone. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved. The residue were re-extracted with 80% acetone. The supernatant was saved and utilized for chlorophyll estimation. Absorbance was read at 645, 663 and 480 nm in the UV-spectrophotometer.

Acetone grinded plant leaves were used to check chlorophyll using UV – VIS spectrophotometer and OD was measured .The amount of Chlorophyl a and b was calculated using the following formula

$$Ca = 11.75\ A662 - 2.350\ A645$$
$$Cb = 18.61\ A645 - 3.960\ A662 \quad 4$$

In all these parameters treated dye and tap water poured plants (Green gram) showed better results compared to untreated dyes.

2.9.4 Soil Enzyme analysis

Dehydrogenase activity in the soil sample was determined by following the procedure as described by Casida et al., (1964). Ten gram of soil from each treatment was thoroughly mixed with 0.2 gram of CaCO3 and dispensed in test tubes. Each tube was added with one ml of 1.5 per cent aqueous solutions of 2, 3, 5 triphenyl tetrazolium chloride (TTC), one ml of one per cent glucose solution

and eight ml of distilled water i.e sufficient to leave a thin film of water above the soil layer. The test tubes were stopper with rubber bands and incubated at 300C for 24 hours. After incubation, the contents of the tubes were rinsed down into a small beaker and converted into slurry by adding 10 ml methanol. The slurry was filtered through Whatman No. 42 filter paper. Repeated rinsing of soil with methanol was continued till filtrate ran free of red colour. The intensity of red colour was measured at 485 nm, against a methanol blank using spectrophotometer. The concentration of formazon formed in the soil sample was determined. The results were expressed in microgram of triphenylformazon (TPF) formed per gram of soil per day by referring to the standard curve of TPF.

Phosphatase activity of soil sample was determined by following the procedure of Eivazi and Tabatabai (1979). One gram of soil sample was placed in 50 ml Erlenmeyer flask to which 0.2 ml toluene followed by four ml of modified universal buffer (pH 6.5) were added. One ml of paranitrophenyl phosphate solution made in modified universal buffer was added to the flasks and contents of the flasks were mixed by swirling for two minutes. The flasks were stoppered and incubated at 370 C for one hour. After incubation, one ml of 0.5M CaCl2 and four ml of 0.5M NaOH were added to the flask, swirled and filtered through whatman No. 42 filter paper. The intensity of yellow color developed was measured at 420 nm using spectrophotometer. Control sample was analyzed for each soil sample following the same procedure described above, except that the paranitrophenyl phosphate solution was added after the addition of 0.5M CaCl2 and 0.5M NaOH and just before filtration. The phosphatase activity in the soil samples was expressed as µg paranitrophenol (PNP) per gram soil per hour with reference to the standard curve prepared by using graded concentrations of paranitrophenol solution.

The estimation of soil cellulase activity was done by modified method as described by Miller (1959). The method uses Paper as the source of cellulose; the reducing sugar was released as a result of cellulase reaction on cellulose. Cellulose present in the paper was be estimated by dinitro-salicylic acid (DNSA) method, using DGlucose as sugar standard. Finely cut-pieces of Whatman No. 1 paper, 0.01 g was incubated with the soil sample dissolved in 0.2 M Na-acetate buffer, pH 4.5 at 300C for 1 h. One mL of this sample (without the paper) + 1 mL of DNSA reagent were incubated in a boiling water bath for 10 min. It was then cooled to RT, the total volume was brought to 10 mL with DW and the absorbance was spectrophotometrically recorded at 546 nm. The blank was enzyme free. The cellulase activity in the soil samples was expressed as µg reducing sugar produced per gram soil per hour with reference to the standard curve prepared by using graded concentrations of D-Glucose solution.

III. RESULTS AND DISCUSSION

Fascination for coloured clothes is always seen in human beings .Dyes are used to colour the textiles during the dyeing and printing process. Textile dying and printing is thus the most successful industrial sector. The dyes used cause pollution of water and are harmful to all living organisms. Though there are various physiochemical methods available for the removal of dyes from waste water, these methods has many draw backs which reduce the efficiency. Thus there is a requirement for a method which is ecofriendly and efficient. The removal of azo dyes using microorganisms is gaining momentum as it is the most cost efficient and environmental friendly method. Therefore an attempt has been made in the present study is to verify the biodecolorization and biodegradation of four different textile azo dyes using *Pleurotus platypus*.

Kirks medium (Selvam and Swaminathan (2003) were used for the degradation study, after preparation of the medium 5 ml of congo red, methyl red, methylene blue and orange G were added in different concentration of 500 and 1000mg separately. And this were incubated for 24 hrs and added the culture Pleurotus platypus and again incubated and the growth of organism and decolorisation were studied using different parameters.

3.1Estimation of biomass

The primary focus is to find the optimum day of growth for *Pleurotus platypus* .The white rot fungi showed best growth on 7th day of inoculation. The growth chart of *Pleurotus platypus* is shown in "**Fig.1**"

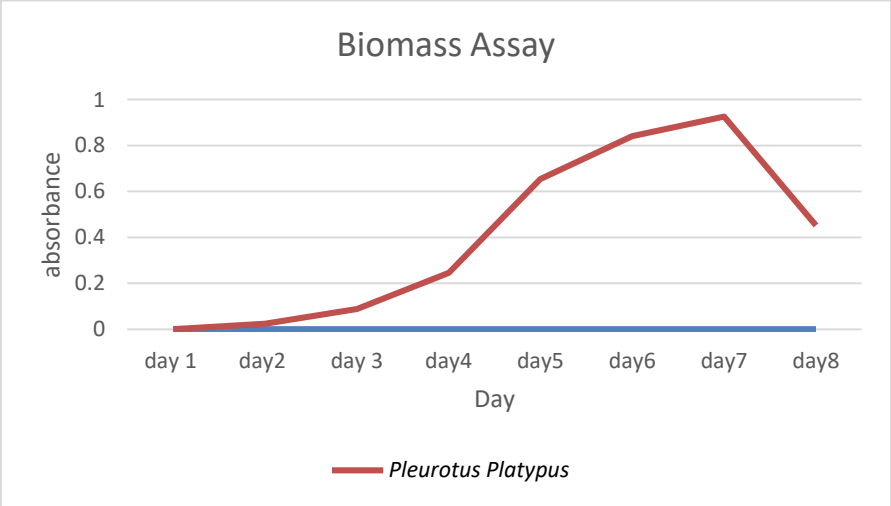

Figure 1 Growth of Pleurotus *platypus* at pH 6.0

3.2 Degradation study

Dye decolourisation assay was measured in the terms of percentage decolourization using UV – Spectrophotometer. In this study orange G shows best decolourisation in both 500 and 1000 mg/ L concentration, methylene blue and congo red showed equal percentage of decolorisation with 500 mg concentration and decolourisation of methelyene blue dropped with 1000mg. Methyl red also shows remarkable decolourisation as shown in "**Fig.2**".Biodegradation and detoxification of dyes, Malachite green, Nigrosin and Basic fuchsin have been carried out using two fungal isolates Aspergillus niger, and Phanerochaete chrysosporium, isolated from dye effluent soil. Aspergillus niger recorded maximum decolorization of the dye Basic fuchsin (81.85%) followed by Nigrosin (77.47%), Malachite green (72.77%) and dye mixture (33.08%) under shaking condition. Whereas, P. chrysosporium recorded decolorization to the maximum with the Nigrosin (90.15%) followed by Basic fuchsin (89.8%), Malachite green (83.25%) and mixture (78.4%)(Babita Rani,et.al,2014).

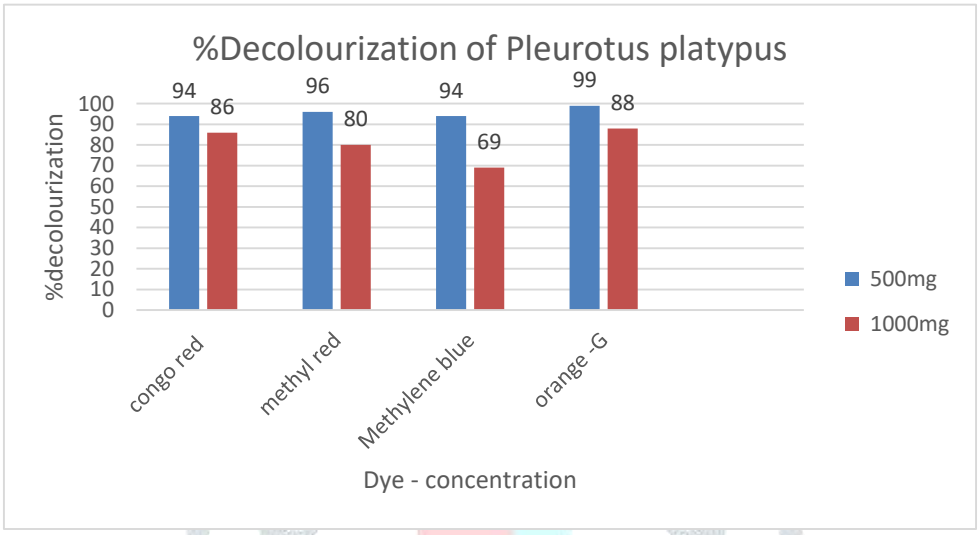

Figure 2 Percentage of decolonization in different dyes

3.3Optimization of carbon and nitrogen source

To provide the best environment for decolourisation by the organism, the fungi *Pleurotus Platypus* was grown in different carbon and nitrogen sources and the source which supported the best growth and decolourisation was found. Glucose, sucrose and maltose were used as carbon source. Each carbon sources were added separately in equal concentration and were observed. The fungal strain showed high decolourization capacity with glucose as carbon source for all dyes. Three different type of nitrogen sources K2HPO4, KH2PO4, and peptone were used as nitrogen source, among which K2HPO4 showed best results when compared to the other sources as shown in "**Fig. 3 – 8**".

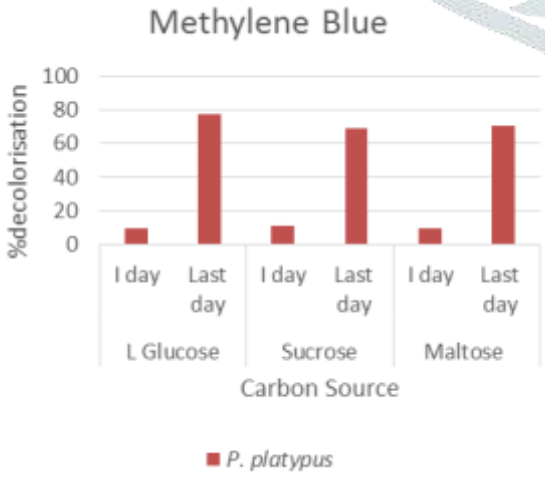

Figure 3 % decolorisation of methylene blue with various carbon source

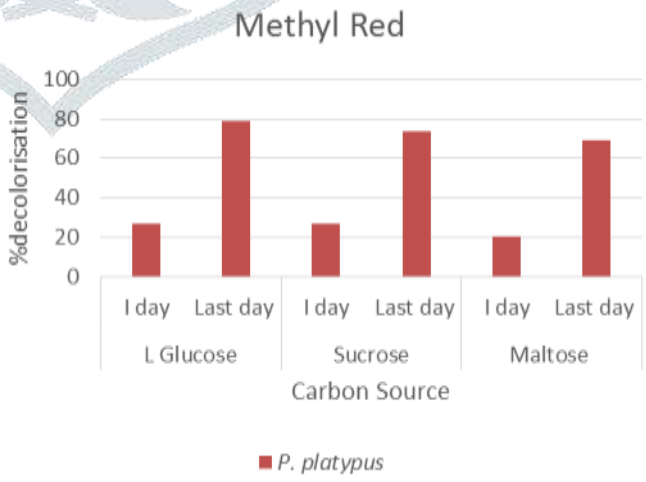

Figure 4 % decolorisation of methyl red with various carbon source

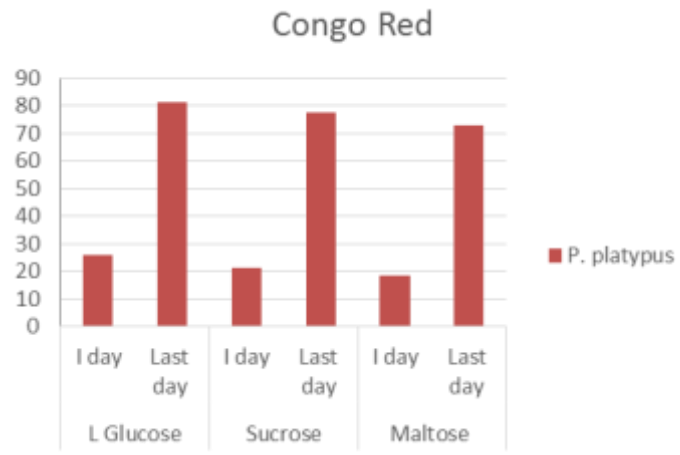

Figure5 % decolorisation of congo red with various source

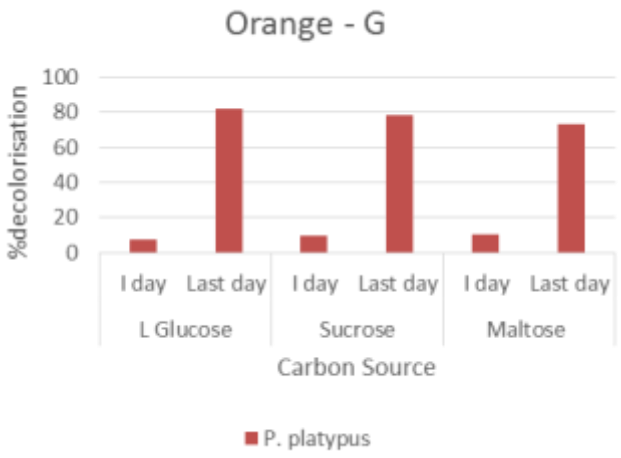

Figure 6 % decolorisation of orange G with various carbon carbon source

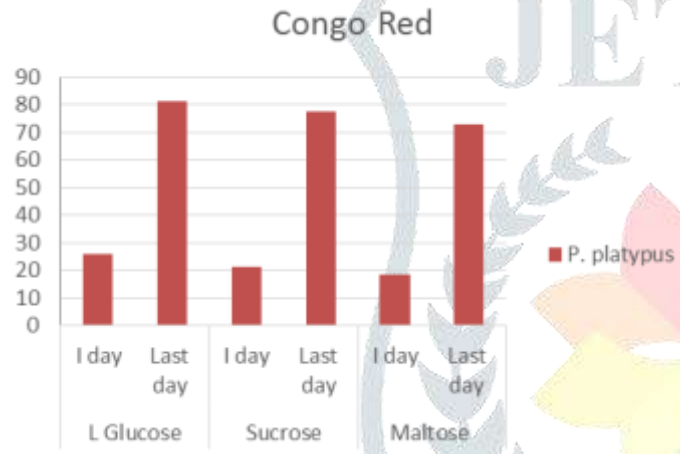

Figure 7 % decolorisation of congo red with various nitrogen source

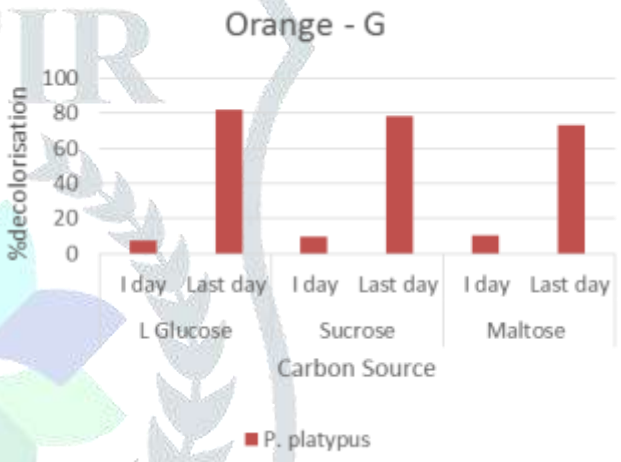

Figure 8 % decolorisation of methylene Blue with various nitrogen source

Since the dyes are deficient in carbon source, it seems necessary to supplement additional carbon or nitrogen source to assist biodegradation of dyes by fungi. Negligible decolorization in the presence of sucrose (25%) whereas moderate activity was shown in presence of glucose (55%), lactose (80%) and maximum decolorization was reported in presence of starch (95%). In addition, supplying urea as a nitrogen source exhibited less decolorizing ability. In contrast, addition of carbon sources seemed to be less effective to promote the decolorization probably due to the preference of the cells in assimilating the added carbon sources over using the dye compound as the carbon source.

3.4TLC study

The TLC analysis of dye degraded products has shown remarkable difference in Rf values which ensures that the dyes have been degraded as shown in table 1. The Rf value of the dye methylene blue is 0.90.The Rf value after treatment with *P.Platypus*, 500mg concentration is 0.64 and for 1000mg concentration is 0.65. Rf value of methyl red before treatment is 0.83 after treatment for 500mg 0.51 and for 1000mg 0.67. Rf value of Congo red before treatment is 0.88 and after treatment it is decreased to 0.45 and 0.54 for500 mg and 1000mg concentration respectively. Orange G has also shown positive results.Rf value without treatment is 0.78 and after treatment for 500mg is 0.42 and for 1000mg it is 0.54 which depicts that the dye has been degraded "Fig 9".The TLC analysis of dye has shown Rf value of the dye sample 0.42, and the Rf value of degraded products of Alcaligenes sp. treated dye was found to be 0.02. Therefore, the TLC analysis has confirmed that there was no starting material, present in the degraded sample and two new degraded products were formed (Ajay Kumar Pandey et.al,2012).

Table 1 Rf valueof dye and degraded compounds

Dye	Control	Concentration mg/L Rf Value 500	1000
Methylene Blue	0.9	0.64	0.65
Methyl Red	0.83	0.51	0.67
Congo Red	0.88	0.45	0.51
Orange - G	0.78	0.42	0.54

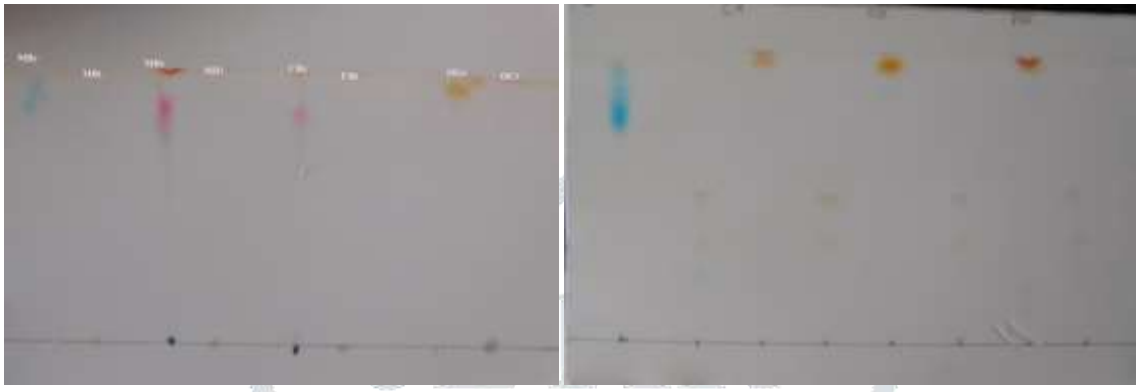

Figure 9 TLC plate for 500mg and 1000 mg.

3.5Azo reducatse Enzyme assay

The azo reductase activity of the purified sample of *Pleurotus platypus* was assessed and the protein content was estimated. The sample showed azo reductase activity of 12.65 U/mL after 8 days of degradation. Azoreductase is the key enzyme responsible for the reductive azo dye degradation. As this enzyme activity is remarkably good, it suggests that the organism can degrade the azo dyes efficiently.

Table 2 Azo reductase activity and protein content

Fungi	OD	Protein (mg/L)	OD	Enzyme activity (U/mL)
P. platypus	0.526	76.0	1.265	12.65

3.6Phyotoxicity

The green gram seeds were treated with tap water, dye contaminated water and *Pleurotus platypus* treated dye water. The germination count was observed. The treated samples showed better germination of seeds when compared to the dye sample.

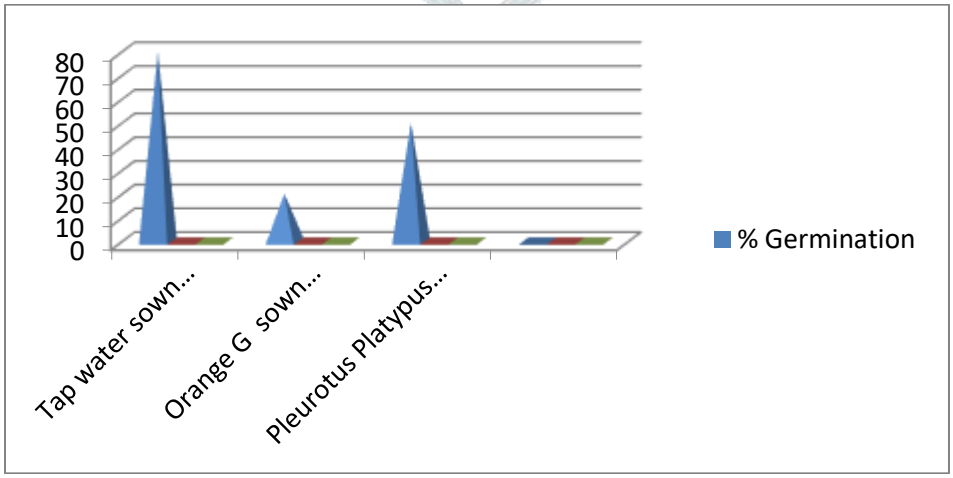

Figure 10 Percent germination

Phytoxicity tests were done to check for the efficiency of the *Pleurotus platypus* treated dye in the growth of plants. In all these parameters treated dye and tap water poured plants (Green gram) has shown better results compared to untreated dyes. Apart from this nutrient analysis is also done, which also has shown better results for treated dyes.
Shoot and root lengths are equal in control (tap water and treated dye water samples), whereas in the dye treated samples the shoot and root lengths are less when compared with the control as shown in table 3.Similar results are shown by tthe phytotoxicity analysis of the treated textile dye effluent on *S. vulgare and P. mungo* by a bacterial consortium (Harshad Lade,et.al: 2015)It was also observed that seeds exposed to treated dye wastewaters showed similar germination as well as shoot and root lengths as like that of distilled water. These results clearly indicate that the treated dye and textile effluent samples were almost as non-toxic as distilled water. Moreover, the plants grown with treated samples were healthy in terms of shoot and root lengths, suggesting the conversion of complex dyes into simple oxidizable forms of a non-toxic nature.

Table 3 Shoot and root length

Dye	Length (in cm)					
	Control(Tap Water)		Dye		Treated	
	Shoot	Root	Shoot	Root	Shoot	Root
MB	5.13	1.23	4.10	1.2	4.56	1.62
MR	4.2	3.1	2.4	0.8	4.21	3.4
CR	3.7	2.7	1.2	2.0	4.02	3.2
OG	4.4	1.9	1.6	0.7	3.98	2.8

Estimation of carbohydrate (Anthrone method) and Protein (Lowrys Method)

Nutrient analysis of plants treated with P. platypus treated dye effluent is compared with tap water and dye treated plants. Carbohydrate, protein and cholorophyll content of the plant treated with *P.platypus* and tap water treated plants showed better results when compared to the dye treated plants.The results are depicted as a graph in the "**Fig. 11 - 12**".

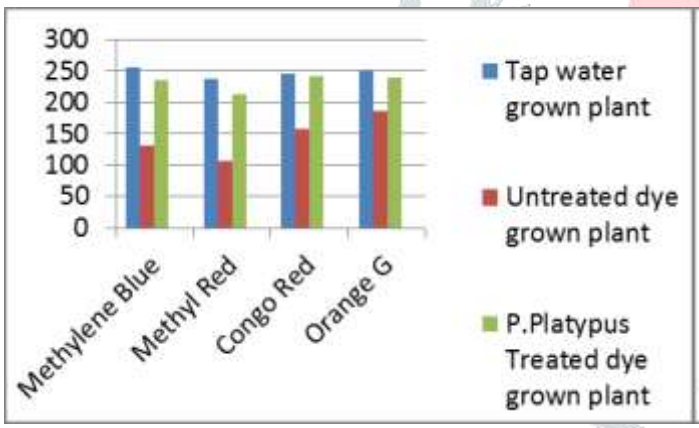

Figure 11 Carbohydrate content

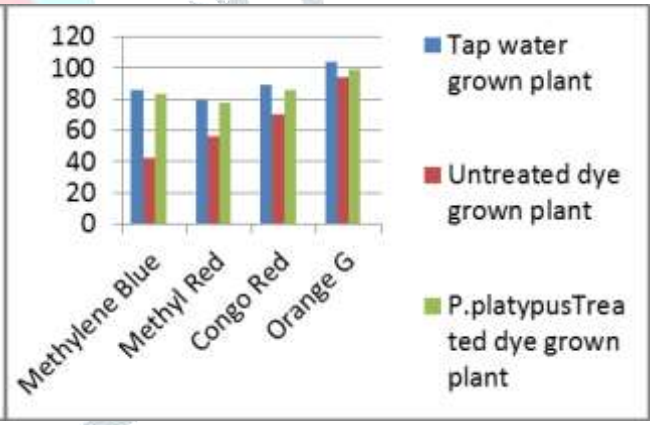

Figure 12 Protein content

Estimation of chlorophyll content
Chlorophyll content of the leaves of the plants treated with dye water, *Pleurotus platypus* treated water and control was compared and found that chlorophyll content of the plants irrigated with P.platypus treated dye water showed better results when compared to the dye treated plants .Both chlorophyll a and b content of the treated dye grown plants are almost at par with the control.

Table 4 Estimation of chlorophyll content

Sample	Chlorophyl ((µ g/gfw))	
	Cla	Clb
Control	52.36	26.65
Dye	16.24	18.12
Dye treated	42.16	20.86

Soil Analysis

To check the effect of *P.platypus* treated dye on the soil enzyme, selected enzyme activities were compared with the dye treated soil and tap water as control .Dehyrogenase, phosphatase and cellulase activity of the soil was analysed are shown in "**Fig. 13**". The dehydrogenase, cellulase and phosphatase activity of the three soil samples when compared shows that the *Pleurotus platypus* treated soil enzyme the activity is almost the same as the tap water treated soil.

Table 5 Soil enzyme activity

Sample	Dehydrogenase activity µg TPF/g soil/day	Phosphatase activity µg TPF/g soil/day	Cellulase activity µg TPF/g soil/day
Tape water	**21.06**	**16.21**	**28.34**
Dye	**5.63**	**1.02**	**9.56**
Fungi (T)	**16.21**	**10.56**	**24.63**

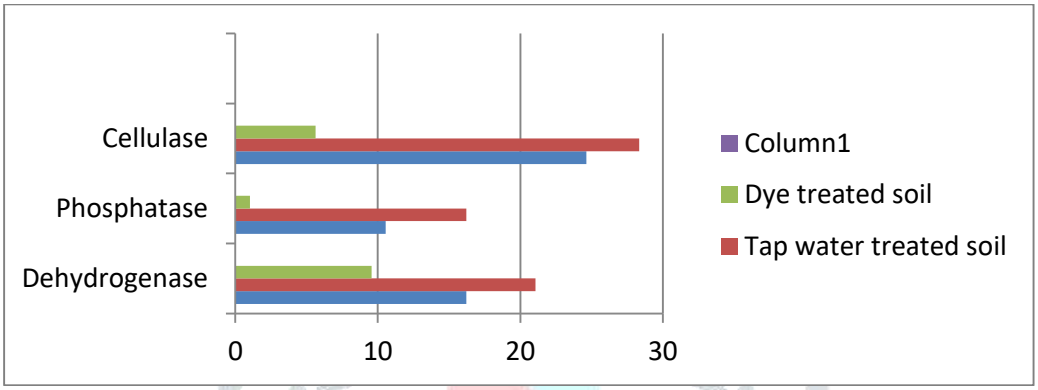

Figure 13 Soil enzyme activity of *Pleurotus Platypus*

IV. Conclusion

Biological degradation is one of the best techniques to detoxify and decolour the azo dyes. The present study reports the potency of *Pleurotus platypus* to decolorize and degrade azo dyes. The sample dye contaminated water showed degradation of around 95% for all the dyes and further degradation of the azo compounds is confirmed through TLC analysis. The azoreductase activity exhibited by the fungi is remarkable. Phytotxicity analysis and soil enzyme analysis of the treated dye sample shows a very encouraging result and suggests that the treated dye effluent can be used in irrigation purpose. Hence, through this study, we can confirm that *Pleurotus platypus*(so far only less reports are available) can be used for the treatment of dye contaminated soils and water. Though decolorization is obtained through efficient studies, there is still the need for further studies to understand the degradation mechanism of azo compounds under natural conditions by *Pleurotus platypus.*

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