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ORIGINAL RESEARCH PAPER

A comparative dye decolorization study for using by bacterial and fungal laccases: a broad spectrum green technology

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Abstract

Technology Area

This technology falls under environmental biotechnology, specifically focusing on enzymatic bioremediation of textile dye effluents using microbial enzymes such as laccases.

Technology

The technology utilizes laccase enzymes derived from the fungus Ganoderma lucidum and recombinant Escherichia coli BL21 expressing the laccase (yacK) gene from Yersinia enterocolitica. These laccases belong to the oxidoreductase enzyme family and catalyze oxidative degradation of textile dyes. The fungal laccase demonstrated superior efficiency, achieving 99.62% decolorization of Bromophenol blue dye within 24 hours. The technology enables enzymatic degradation of azo dyes, preventing formation of toxic, carcinogenic, and mutagenic intermediates. The enzyme activity is influenced by pH, indicating the need for optimized process conditions.

Potential Market / Customers

Textile manufacturers (cotton, polyester, blended fabric industries), Environmental engineering firms, Wastewater treatment solution providers, Industrial enzyme manufacturers, Government pollution control bodies and municipal wastewater facilities

Technology Valuation (Commercial Potential & Value Proposition)

Provides an eco-friendly, sustainable alternative to chemical dye treatment., Reduces environmental pollution and regulatory compliance costs, Prevents formation of toxic degradation byproducts, Highly efficient dye removal (>99%), making it commercially viable.

Keywords Dye Decolourization; Laccase; Ganoderma; Yersinia; Enzyme

Introduction

Commercially, 7×10^5 tons of dye stuff are produced annually by dyes available in industries (Pootset *al.*, 1976). Textile industries uses large amount of chemicals and water for wet processing which accounts for two-third of the total dye-stuff market (Upadhyayet *al.*, 2016). Approximately, 84,000 tons of dyes are released into water and 90 million tons of water contributes to production of dye and its applications such as textiles, leather industries. In India, textile industries are the reason for 20% of the water pollution due to industries. Discharge of textile industries contains various types of dyes including azo and recalcitrant dyes. As a result, water gets polluted due to their color and formation of carcinogenic and teratogenic compounds. Chemical compounds used in dyeing are very different in chemical composition, varying from organic compounds and inorganic compounds to polymers (Zollinger, 2002). Dye industries related to dye applications are considered as most polluting industries (Sundaret *al.*, 2011). Environmental problems are produced due to effluents discharged in water (Pereira *et al.*, 2012; Saratalet *al.*, 2011). Additionally, corrosion, blockage, and bioaccumulation in pipes give rise to dangerous problems (Leinget *al.*, 1991). Azo dyes are constant pollutants because they are not deteriorated under aerobic conditions (Chen, 2006). Under anaerobic conditions, azo dyes can be degraded by intestinal bacteria and some microorganisms present in nature to colorless amines and the compounds formed are toxic, carcinogenic, and mutagenic to animals and humans (Chung, 2000). Anthaquinone dyes have stable and complex structure and these are more toxic as compared to azo dyes (Novotny *et al.*, 2006).

In twenty first century serious environmental problem is caused by untreated dyeing effluents. Synthetic dyes present in water can lead to increase in level of BOD (biochemical oxygen demand) and COD (chemical oxygen demand). The chomophore present in synthetic dyes can absorb sunlight easily and strongly, as a result inhibition in photosynthetic activity of organisms takes place (Da Silva *et al.*, 2010; Kagalkaret *al.*, 2010). Generally, individual cultures are tested for dye degradation but when consortia of microorganisms are used and this improve the degradation of dye as compared to the individual culture (Parmaret *al.*, 2015). Enzymes are very

effective in dye decolorization because they are highly specific catalysts which produce byproducts with less toxicity and volume. The enzymes available for dye degradation mainly belong to the family of oxidoreductases including peroxidases, reductases, and laccases (Kaushik *et al.*, 2010; Fu *et al.*, 2001). As compared to whole cells, isolated enzymes are easier to use in harsh environmental conditions and their handling and storage is also easier (Forgacs*et al.*, 2004; Husain *et al.*, 2011; Nicell*et al.*, 2001). Phenol oxidases catalyze the oxidation of phenolic compounds without any cofactors but presence of oxygen is necessary for catalyzing the reaction. Laccases belongs to this group of enzymes. They degrade dye by removal of hydrogen atom from hydroxyl group via electron oxidation and it forms non-toxic products (Telke*et al.*, 2011; Adnan *et al.*, 2015).

Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) are the enzymes which consists of multicopper atoms and belongs to the group of blue oxidases (Thurston 1994). Laccases are extensively distributed in higher plants and fungi. In fungi, the enzyme is present in *Ascomycetes*,*Duteromycetes*,*Basidiomyetes* but it is principally rich in a variety of white rot fungi which functions in lignin degradation (Desai *et al.*, 2011). Other laccase producers among the wood-degrading fungi are: *Trametes*(*Coriolus*) *versicolor*,*Ganodermalucidum*,*Cerrena maxima*,*Coriolopsispolyzona*,*Trameteshirsuta*,*Lentinustigrinus*,*Plreurotuseryngii*, *Trametesochacea* (Arora and Gill, 2000). They are also been characterized in saprophytic ascomycetes found in composts (*Chaetomium thermophile*,*Myceliophthorathermophila*) (Berka*et al.*, 1997; Chefetz*et al.*, 1998; Vasil'chenko*et al.*, 2000). First bacterial laccase was discovered in roots of the plant associated with bacterium *Azospirillumlipoferum*(Givaudan*et al.*, 1993). In *Azospirillumlipoferum*, it was involved in formation of melanin pigment (Faure *et al.*, 1994). Laccases usually occur as isoenzymes in fungal species. They perform different functions in different organisms. In fungi, they have role in formation of pigments, degradation and detoxification of lignins. In plants, they participate in lignin formation (Singh *et al.*, 2014). Laccases are also involved in morphogenesis, pathogenicity, and immunity of organisms (Claus, 2004). In bacteria, they have role in morphogenesis, copper detoxification (Sharma *et al.*, 2007), spore coat resistance (Hullo *et al.*, 2001; Martins *et al.*, 2002), and synthesis of melanin (Singh *et al.*, 2014). The main role of laccase in phenolic and lignin compound deterioration has been classified

in a wide range of biotechnological applications like dye decolorization, bioremediation of hazardous chemical compounds i.e., pesticides, chlorinated aromatic compounds, nitroaromatics, aromatic anhydrocarbons, and biosensor advancements (Rodŕıguez Couto *et al.*, 2006; Śanchez*et al.*, 2011; Gonzalez *et al.*, 2013). The catalytic performance of laccases affect by their stability and activity at different pH and temperature conditions. The pH activity profiles of laccases are often bell-shaped, with optima around 4.6, when measured with phenolic substrates (Viswanath*et al.*, 2014). In contrast to their activity, the stability of laccases is generally highest at pH values around 8-9 (Shaddha*et al.*, 2011). Temperature stability of laccases varies considerably, depending on the source from which they are isolated. In general laccases are stable at 30-50°C and rapidly lose activity at temperatures above 60°C (Monssef e*t al.*, 2016).

Mediators are small compounds with low molecular weight and high redox potential as compared to laccases. Non-phenolic parts of chemical compounds can be oxidized by the use of mediators. In previous years, more efficient synthetic mediators were discovered which increases the laccase catalysis for non-phenolic substrates (Desai *et al.*, 2011). Currently, the mediators are divided into three categories: the natural mediators (Acetosyringone, Syringaldehyde, Vanillin, Acetovanillone, Methyl vanillate, p-coumaric acid), the synthetic mediators (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonate (ABTS), 1-Hydroxybenzotriazole (HBT), Violuric Acid (VIO), and others (Polyoxometalates) (Fabbrini*et al.*, 2002, Cantarella*et al.*, 2004, Galli and Gentili, 2004).

In present study, four different dyes, i.e, Phenol red (PR), Bromophenol blue (BRB), Crystal violet (CV), Malachite green (MG) were used for decolorization using both bacterial (recombinant *E. coli* BL 21 having *yac*K) and fungal (*G. lucidum*) laccase.

Materials and Methods

Chemicals and Reagents

All the chemicals used in this work were of high analytical grade. Imidazole used in the preparation of lysis buffer was purchased from Sigma-Aldrich, India. The chemicals Malt extract, Potassium dihydrogen phosphate (KH_2PO_4), Magnesium sulfateheptahydrate ($MgSO_4.7H_2O$), Calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$, Agar, Sodium chloride (NaCl), Ammonium sulfate $(NH_4)_2SO_4$), Calcium chloride ($CaCl_2$), Dibasic sodium phosphate

(Na_2HPO_4), Citric acid ($C_6H_8O_7$), Guaiacol, Sodium sulfite (Na_2SO_3), Sulfuric acid (H_2SO_4), Methanol (CH_3OH), Ethanol (C_2H_5OH), Isopropyl β-D-1- thiogalactopyranoside (IPTG), Copper chloride ($CuCl_2$), Phenyl methylsulfonyl fluoride (PMSF), Sodium dihydrogen phosphate (NaH_2PO_4), Sodium carbonate (Na_2CO_3), Sodium hydroxide (NaOH), Copper sulphate ($CuSO_4$), Sodium potassium tartrate, Folin, Pottasium hydrogen phosphate (K_2HPO_4), Tris Cl were purchased from Hi-Media and Merck. The dyes Phenol red (PR), Bromophenol blue (BPB), Malachite green (MG), Crystal violet (CV) were also purchased from Hi-Media, India. All the glasswares were purchased from Borosil and Schott Duran, Germany.

Purification of bacterial laccase

The recombinant strain *Escherichia coli* BL 21 having PET 28a vector with laccase gene were inoculated by loop inoculation from the plates of bacterial isolates in 100 ml Erlenmeyer flask having 20 ml media Luria broth containing 100 µg/ ml kanamycin (primary inoculation). The flasks were then incubated at 37° C, 210 rpm for 16 hours. After incubation the flasks were taken out from incubator. Secondary inoculation was done by pipetting 2 ml of culture broth into 1 L Erlenmeyer fask having 200 ml luria broth containing 100 µg/ml kanamycin. The flasks were then incubated at 37 °C, 210 rpm until OD_{600} reached 0.5 to 0.6. When appropriate O.D was attained, 1.0mM IPTG and 0.25 mM $CuCl_2$ was added to the flask. The flasks were then incubated at 16 °C, 100 rpm for 20 hours. After incubation, culture was centrifuged at 9000 rpm for 10 minutes at 4 °C. The obtained pellets were re-suspended in 2 ml NaH_2PO_4 buffer (pH 8) and 0.25 mM $CuCl_2$. The culture was stored at 4 °C and then centrifuged at 9000 rpm for 10 minutes at 4 °C. Obtained pellets were again resuspended in 5-6 ml lysis buffer. Lysis buffer contains 50 mM Na_3PO_4 and 10 mM imidazole (pH 8). After adding lysis buffer, 1 mg/ml lysozyme and 0.25 mM $CuCl_2$ was added. Then incubation was done in ice for 30 minutes. Then, 1 mM PMSF (protease inhibitor) was added and sonication was performed. Centrifugation was done at 9000 rpm for 15 minutes at 4 °C. Supernatant was stored at 4 °C.

Purification of Fungal laccase

Culture of *Ganodermalucidum* MDU-7 was inoculated by disc inoculation on malt extract agar (MEA) plates. The plates were incubated at 30 °C for 4-5 days in static incubator for the growth of fungal mycelia on plates. Substrate was prepared for the production of laccase by adding 30 ml of

mineral salt in 10 g of substrate (wheat bran and mixed grain bran), i.e. 1:3 ratio in 250 ml Erlenmeyer flasks. The flasks were autoclaved at 20 psi for 20 minutes. 4-5 discs of MEA plates containing fungus spores were inoculated in substrate (1×10^8) ml/ 1 g. the flasks were incubated at 30 °C for 5-6 hours in static incubator. After incubation, 50 ml of CP buffer (pH 4) was added in flask. Again the flasks were incubated at 30 °C and 150 rpm for 2 hours until the solid converts into slurry like appearance. Then, the enzyme was filtered through double layered muslin cloth. After filtration, the content was centrifuged at 8000 rpm for 10 minutes at 4 °C. Supernatant was stored at 4 °C.

Ammonium sulfate was added slowly by continuous stirring, according to 20-80 kDa cut.The sample was incubated overnight at 4 °C.Sample was centrifuged at 9000 rpm for 20 minutes.Precipitates were dissolved in 20 mM CP buffer (pH 4).Dialysis was performed against same buffer at 4 °C (Kumar et al., 2017).

Dye decolorization

Four dyes were used for this experiment namely phenol red (PR), Bromophenol blue (BPB), Malachite green (MG) and Crystal violet (CV). All the dyes were dissolved in respective buffers except malachite green because it was partially soluble in water. Therefore, malachite green stock solution was prepared by dissolving it in ethanol. Dye stock (10µg/ml) was prepared for all the dyes by dissolving the dyes in respective buffers. For studying the decolorization properties of bacterial and fungal laccases, the reaction mixture was prepared by dissolving dye solution with 25 U/ml laccase isolated from bacterial as well as fungal laccase according to different pH. Dye decolorization by bacterial laccase was performed on pH ranging from 7 to 9 while dye decolorization by fungal laccase was performed on pH 4 to 6. The reaction mixture was vortexed for 2 minutes. Enzyme control (without dye) and dye control (without laccase) were also taken. For bacterial laccase, the reaction mixtures were incubated at 37 °C for different time intervals. For fungal laccases, the reaction mixtures were incubated at 30 °C for different time intervals. After incubation, the tubes were centrifuged at 10,000 rpm for 5 minutes; thereafter, centrifugation, O.D. was measured at their respective wavelengths. For phenol red, bromophenol blue, malachite green and crystal violet absorbance was taken on 430 nm, 590 nm, 618 nm and 598 nm, respectively. Each experiment was done in triplicates, and % dye decolorization was calculated accordingly.

Calculation of enzyme activity by performing laccase assay

Approx. 250 µl supernatant was taken in eppendorf. Approximately 250µl guaiacol (25 mMguaiacol in 100 mM citrate phosphate buffer (pH 7-7.5) and 2 mM copper chloride was added. Tubes were vortexed for 2 minutes and then incubated at 37 °C for 30 minutes. Tubes were observed for development of red brown colour. Guaiacol(500µl) was used as blank, and OD was measured at 470 nm.

Dye decolorization % was calculated by:

Dye decolorization (%) = $[(A_i - A_t)/A_i] \times 100$ A_i and A_t represents initial absorbance and absorbance after incubation.

RESULTS

Determination of activity of laccase from fungi Ganodermalucidum MDU-7

Enzyme assay was performed as described by Kumar *et al*., 2015 using guaiacol as a substrate to determine the laccase activity. Assay was performed with different dilutions in triplicates and the blank was guaiacol. Absorbance was taken at 470 nm.

Enzyme activity = O.D. × D.F. × Reaction volume/0.01 × 1/Reaction time

Where O.D. is the optical density and D.F. is the dilution factor.

Determination of activity of recombinant bacterial laccase

Recombinant bacterial laccase was obtained after induction (using IPTG) from recombinant strain of *E. coli* BL 21 harboring*yac*K gene from *Yersinia enterocolitica*. To determine its activity, enzyme assay was performed as described by Singh *et al*., 2016 using guaiacol as a substrate. The laccase assay was performed in triplicates with guaiacol as a blank. Absorbance was taken at 470 nm.

Enzyme activity = O.D. × D.F. × Reaction volume/0.01 × 1/Reaction time

Table 1 Laccase activity when wheat bran is used as substrate

Dilution factor	**1**	**2**	**3**	**Av.**	**Enzyme activity (U/ml)**
100 X	0.9	1.3	0.9	1.0	3582.9

	35	52	39	75	75
200 X	0.565	0.531	0.552	0.549	3659.634

Table 2 Activity of laccase when grain bran is used as substrate

Dilution factor	1	2	3	Av.	Enzyme activity (U/ml)
100 X	1.867	1.827	1.898	-	-
200 X	1.172	1.193	1.173	-	-
400 X	0.769	0.615	0.673	0.685	9132.42

Table 3 Activity of laccase after partial purification with wheat bran as a substrate

Dilution factor	1	2	3	Av.	Enzyme activity (U/ml)
1000 X	0.973	1.007	1.004	-	-
1500 X	0.728	0.730	0.757	0.738	36896.31

Table 4 Activity of laccase after partial purification with grain bran as a substrate

Dilution factor	1	2	3	Av.	Enzyme activity (U/ml)
1000 X	1.417	1.461	1.543	-	-
1500 X	1.078	1.099	1.122	-	-
2000 X	1.015	0.978	0.998	0.997	66460.02

Table 5 Estimation of bacterial laccase activity

Dilution factor	1	2	3	Av.	Enzyme activity (U/ml)
50 X	0.382	0.445	0.405	0.410	136.735

Determination of specific activity by Lowry method

Lowry was performed to determine the total protein, which was calculated from the absorbance at OD_{660}.

Table 6 Estimation of protein concentration

Abs	1	2	3	Av.	Protein conc(mg)
OD_6	0.94	0.89	0.88	0.90	15.14

60	7	5	4	8	

Specific activity (U/mg) = Enzyme activity/ Protein conc. 136.73/15.14 = 9.02 U/mg

Dye decolorization using laccases

The decolorization of phenol red (PR) was done using both bacterial and fungal laccases, while other thee dyes used in this study were decolorized using fungal laccases only. This was due to the fact that the decolorization achieved using the fungal laccase was better than using the bacterial laccase. Further, it was more convenient and economic to produce the laccase from the fungus rather than from the recombinant bacteria.

Table 7 Decolorization of phenol red at pH 4.0-9.0 after 4h treatment with bacterial and fungal laccases

Fungal laccase	**Time (4 h)**	**Bacterial laccase**	**Time (4 h)**
pH	**% D**	**pH**	**% D**
4.0	30.101	7.0	19.158
5.0	28.854	8.0	27.255
6.0	31.467	9.0	6.444

In addition, the fungal laccases were shown to have more activity than the recombinant bacterial laccases. Conclusively, keeping in mind the above points, the fungal laccase was selected over the bacterial recombinant laccase to decolorize the dyes (Table 7).

To decolorize the phenol red (PR) using bacterial recombinant laccase (rLac), buffers with pH 7.0, 8.0, and 9.0 were used, as rLac has pH optima in alkaline range (~8.0). While for decolorizing the PR using fungal laccase obtained from *G. lucidum*, buffers with pH 4.0, 5.0, and 6.0 were used, as fungal laccase has pH optima in acidic range (~5.0).After 4h of treatment with laccases, maximum decolorization (31.46 %) of PR was achieved at pH 6.0 using fungal laccase. Therefore, further decolorization of PR at different time intervals (2 h, 4 h, 16 h, and 24 h) was done with fungal laccase. This was done to check the effect of incubation time on the decolorization property of the laccase enzyme.

Phenol red decolorization using fungal laccase

Phenol red dye (1 µg/ ml) was mixed in respective buffers and incubated with bacterial or fungal laccase at 30 °C for different time intervals (2 h, 4 h, 16 h, and 24 h). Percent decolorization was calculated by taking absorbance at 430 nm.

Table 8 Decolorization of phenol red (PR) using fungal laccase at different time intervals

pH	% D (2 h)	% D (4 h)	%D (16 h)	%D (24 h)
4.0	29.794	35.226	38.271	40.987
5.0	30.104	33.333	40.312	42.395
6.0	32.326	38.365	43.635	46.536

Maximum decolorization (46.53 %) of phenol red (PR) was observed at pH 6.0 after 24 h treatment with the fungal laccase, as shown in figure1.

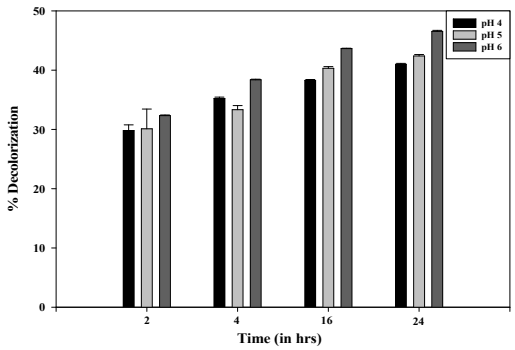

Fig 1. Decolorization of phenol red by fungal laccase at pH 4.0, 5.0, and 6.0 at different time intervals (2 h, 4 h, 16 h, and 24 h).

Bromophenol blue decolorization

Bromophenol blue dye (1 µg/ ml) was mixed with respective buffers and incubated with fungal laccase at 30 °C for different time intervals (2 h, 4 h, 16 h, and 24 h). Further, percent decolorization was calculated by taking absorbance at 590 nm.

Table 9 Decolorization of bromophenol blue using fungal laccase at different time intervals

pH	% D (2 h)	% D (4 h)	% D (16 h)	% D (24 h)
4.0	90.904	92.830	99.250	99.625
5.0	60.373	67.288	90.447	95.199
6.0	11.304	11.520	14.664	16.795

Maximum decolorization (99.62 %) of bromophenol blue (BRB) was observed at pH 4.0, as shown in figure 2. Percentdecolorization of BRB was almost similar after treatment of 16 h and 24 h with fungal laccase at pH 4.0. Surprisingly, the dye was decolorized completely after 16 h treatment with the fungal laccase. This highlighted the efficiency of the laccase, especially the fungal laccase obtained from *G. lucidum* in decolorizing the commercially used, water-polluting recalcitrant, toxic dyes including BRB.

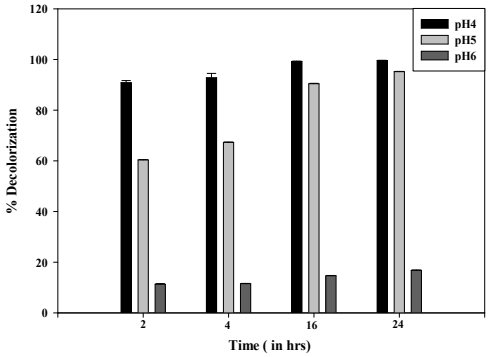

Fig 2. Decolorization of bromophenol blue (BRB) using fungal laccase at pH 4.0, 5.0, and 6.0 at different time intervals (2 h, 4 h, 16 h, and 24 h).

Crystal violet decolorization by fungal laccase

Crystal violet (CV) dye (1 µg/ ml) was mixed with respective buffers and incubated with fungal laccase at 30 °C for different time intervals (2 h, 4 h, 16 h, and 24 h). % decolorization was calculated by taking absorbance at 598 nm.

Table 10 Decolorization of crystal violet using fungal laccase at different time intervals

pH	% D (2 h)	% D (4 h)	% D (16 h)	% D (24 h)
4.0	43.71	40.50	59.39	71.29
5.0	6.09	8.61	26.70	43.56
6.0	0	0	6.03	17.49

Maximum decolorization (71.29 %) of crystal violet using fungal laccase was observed at pH 4.0 after 24 h of treatment, as shown in figure 3.

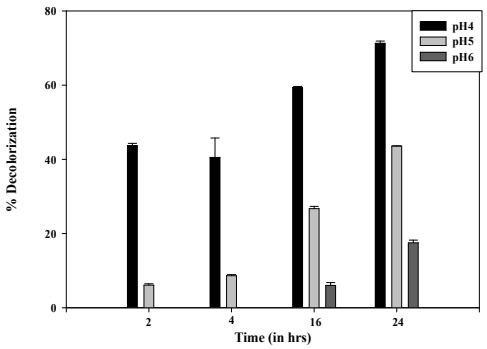

Fig 3. Decolorization of crystal violet using fungal laccase at pH 4.0, 5.0, and 6.0 at different time intervals.

Malachite green decolorization by fungal laccase

Malachite green dye (1 µg/ ml) was mixed with respective buffers and incubated with fungal laccase at 30 °C for different time intervals (2 h, 4 h, 16 h, and 24 h). Percentdecolorization was calculated by taking absorbance at 618 nm.

Table 11 Decolorization of malachite green (MG) using fungal laccaseat different time intervals

pH	% D (2 h)	% D (4 h)	% D (16 h)	% D (24 h)
4.0	30.330	28.886	47.996	68.415
5.0	24.379	29.873	66.864	77.655
6.0	85.795	87.755	95.729	98.736

Maximum decolorization (98.73 %) of malachite green (MG) using fungal laccase was observed at pH 6.0 after 24 h of incubation, as shown in figure 4. Malachite green (MG) is a teratogenic and carcinogenic recalcitrant dye and it's up to ~98 % decolorization using laccase from *G. lucidum* again reflects the high decolorizing ability of the laccase towards the commercially used dyes.

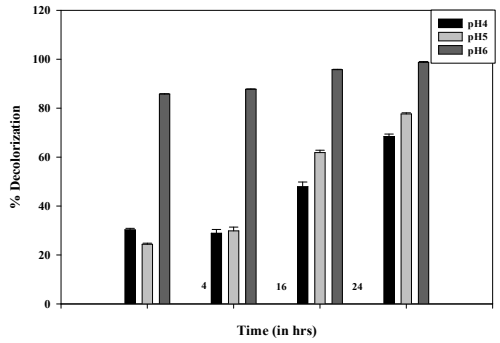

Fig 4. Decolorization of malachite green using fungal laccase at pH 4.0, 5.0, and 6.0 at different time intervals (2 h, 4 h, 16 h, and 24 h).

DISCUSSION

Synthetic dyes are used in a large number of industrial sectors like biomedical, chemical, food, leather, plastic, printing, pharmaceuticals, and textiles (Othman *et al.*, 2018). Discharge of textile industries contains various types of dyes including azo and recalcitrant dyes. As a result, water gets polluted due to their color and formation of carcinogenic and teratogenic compounds. Chemical compounds used in dyeing are very different in chemical composition, varying from organic compounds and inorganic compounds to polymers (Zollinger, 2002). Environmental problems are produced due to effluents discharged in water (Pereira *et al.*, 2012; Saratale*et al.*, 2011). Additionally, corrosion, blockage, and bioaccumulation in pipes give rise to dangerous problems. Textile industries are under pressure to remove it by environmental friendly strategies (Ahlawat et. al. 2019).

The enzymes have potential for degrading dyes by either forming the precipitants or transforming the dye chemically into less toxic form (Borujeni*et al.*, 2011). As compared to whole cells, isolated enzymes are easier to use in harsh environmental conditions and their handling and storage is also easier (Sharma et al. 2015). It is necessary to degrade dyes because these are very harmful for environment and cause serious health problems for living beings. Rate of decolorization of synthetic dyes widely depends on sources of laccases, types of dyes, chemical nature of applied dyes, and other physical factors such as pH and temperature. Due to complex structure, azo dyes are recognized as recalcitrant for laccases; thus, results in slow oxidation whereas anthaquinone dyes are easily oxidized by laccases (Fang *et al*., 2015). We can further improve the rate of decolorization by adding laccase mediators which oxidizes the dyes and make the degradation more feasible.

Keeping in mind the above point, in this study, the dyes were degraded by laccase enzymes obtained from fungus *G. lucidum*and recombinant *E. coli* BL 21 having *yac*K (laccase) gene from *Y. enterocolitica.* As known earlier, laccases can be used in decolorization of dyes

either as extracellular culture fluid (Lorenza *et al*., 2002) or as fungal pellets (Yesiladа*et al*., 2003).In present work, the culture fluid was due for decolorizing dyes and the ability of dye degradation was studied by calculating the % decolorization. By performing the experiment, it was observed that the % decolorization of dyes varied at different pH values. This might be due to the fact that the structure of laccase might got affected at different pH values, as we know that the structure of protein changes at different pH values. Further, due to variation in enzyme structure (especially the active site); the enzyme activity also gets affected.

Using fungal laccase, maximum decolorization (46.53 %) of phenol red (PR) was observed at pH 6.0 and 30 °C after 24 h of incubation. Phenol red (PR) is said to exhibit a slow oxidation rate by laccase (D' Acunzo and Gali, 2003). Earlier, laccase isolated from *Trametes versicolor* decolorized phenol red up to 23 % in 24 h, at pH 4.5 and temperature 30 °C). Further, maximum decolorization (99.62 %) of bromophenol blue (BPB) was observed at pH 4.0 and 30 °C in 24 h. Similarly, maximum decolorization (98.73 %) of malachite green (MG) was observed at pH 6.0 and 30 °C after 24 h, while it was 71.290 % for crystal violet (CV) pH 4.0 and 30 °C after 24 h. However, previously, alkali and thermostable laccase isolated from novel *Alcaligenesfaecalis* showed 48 % decolorization of malachite green and 36 % decolorization of crystal violet at pH 7.0 in 3 h (Mehandia*et al*., 2020). Further, the culture supernatant of the extracellular laccase of *Pseudomonas putida* showed 36-94 % decolorization of dyes (brilliant green, bromophenol blue, crystal violet, and congo red) within 12-24 h at room temperature.

Earlier, Alvarez *et al* used *G. lucidum* supernatant for the degradation of crystal violet and malachite green dyes such that 23 % decolorization of CV and 52 % of MG was observed in 24 h. Similarly, Zhuo*et al* also evaluated the decolorization of several synthetic dyes like crystal violet and malachite green. They employed culture extracts obtained from *Ganoderma*sp. and observed 66 % decolorization of crystal violet and 95.4 % decolorization of malachite green (Zhuo*et al*., 2011). Moreover, % decolorizaion of malachite green (MG) was observed to be 77.65 % and 70.45 % by laccases isolated from *Bacillus pumilis* DSKK1 and *Yersinia enterocolitica* strain 7 at pH 8.0 and temperature 37 °C in 16 h (Ahlawat*et al*., 2019). In present study, we observed maximum decolorizaton (95.72 %) of malachite green (MG) after 16 hs of

incubation with laccase from *G. lucidum* MDU-7 at pH 6.0 and temperature 30 °C. It was higher than bacterial laccase-decolorized MG, obtained from previous work by our lab, thereby suggesting the better potential of fungal laccase over bacterial in decolorizing synthetic dyes. Furthermore, in an recent report, a novel textile dye degrading extracellular laccase extracted from symbiotic bacterium *Bacillus sp.* CF96 isolated from gut termite (*Anacanthotermes*) showed just 10 % decolorization of malachite green (Javadzadehand Asoodeh, 2019). Another report with two laccase isozymes (Lac 1 & Lac 2) isolated from *Trameteshirsuta* strain showed less than 15 % decolorization of crystal violet at pH 5.0 and room temperature in 3 h when incubated in dark (Huang *et al*., 2019). However, laccase from *Ganoderma* sp. showed 70 % decolorization in presence of 1 mM HBT (laccase mediator) at pH 5.0, 30 °C, and 150 rpm under solid-state fermentation in 36 h and showed no decolorization in absence of HBT (Sharma *et al*., 2015).

Conclusion

Bacterial recombinant laccase (rLac) and fungal (*Ganodermalucidum* MDU-7) laccase were used for decolorization of dyes (PR, BPB, CV, and MG). It was observed that decolorization of dyes varied at different pH values. Using fungal laccase, maximum decolorization (46.53 %) of phenol red was observed at pH 6.0 and 30 °C after 24 h of incubation. Further, maximum decolorization (99.62 %) of bromophenol blue (BPB) was observed at pH 4.0 and 30 °C in 24 h. Similarly, maximum decolorization (98.73 %) of malachite green was observed at pH 6.0 and 30 °C after 24 h, while it was 71.29 % for crystal violet at pH 4.0 and 30 °C after 24 h. Reason for the variations at different pH is the fact that the laccase protein structure might got affected at different pH values, as we know that the structure of protein changes at different pH values (Kumar et. al. 2021).

Further, due to variation in enzyme structure, especially the active site, the enzyme activity also gets affected. It was also concluded that % decolorization achieved using the fungal laccase was better than using the bacterial laccase. Also, it was more convenient and economic to produce the laccase from the fungus rather than from the recombinant bacteria. In addition, the fungal laccases were shown to have more activity than the recombinant bacterial laccases. Therefore, fungal laccases can be applied in removing the toxic azo and anthaquinone dyes from textile effluent to decrease the rate of environmental pollution. Further, we can

improve the rate of decolorization by adding laccase mediators that oxidizes the dyes and make the degradation more feasible.

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