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Decolourization of synthetic dyes by laccase enzyme produced by *Kluyveromyces dobzhanskii* DW1 and *Pichia manshurica* DW2

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Industrialization has come with environmental challenges. Industries like paper, printing, textile, leather and so on widely use chemical dyes whose waste treatment or degradability is difficult. Among various methods employed, the use of microbial enzymes is the most effective. The study aimed at producing laccase from identified yeast strains for potential industrial use in dye decolourization. Laccase produced by *Kluyveromyces dobzhanskii* DW1 and *Pichia manshurica* DW2 were purified and immobilized up to 65.2 and 73.1%, respectively. The crude, purified and immobilized forms of the enzymes were used to decolourize malachite green and methyl red dyes each at concentrations of 0.05 and 0.1 g/L. The highest percentage decolourization by *K. dobzhanskii* DW1 was 81.50% (immobilized) and 87.50% (purified), respectively for malachite green and methyl red dyes while *P. manshurica* DW2b (crude) had 84.40 and 76.89%, respectively. The Fourier transform infrared (FTIR) spectrum of the dyes was collected within a scanning range of 4000 to 400 cm^{-1}. The spectrum of methyl red dye by the purified *K. dobzhanskii* DW1 laccase showed disappearance of some chemical groups (peak), while the crude *P. manshurica* DW2 laccase removed the main azo-group, alcohol/phenol and higher alkane (1487.17) groups. The spectrum of untreated malachite green also showed 25 peaks with one disulphide, 2 aliphatic halogens, 2 thio ethers, 3 sulphones, 4 imino groups among other chemical groups. The decolourization of the dye with the immobilized *K. dobzhanskii* DW1 laccase showed a spectrum of 17 peaks with the removal of the disulphide (420.5), one aliphatic halogen (C-I), thio ether, 2 sulphone, 4 imino and 2 higher alkanes, while the crude *P. manshurica* DW2 laccase removed the 2 aliphatic halogen, 4 imino, 1 amine, 2 alkane and 2 of the 3 alcohol/phenol chemical groups. The removal of the main components (azo chemical group) of the dyes proved their effectiveness in decolourisation and bioremediation of the textile wastes.

Key words: Decolourisation, azo-dye, laccase, immobilized, FT-IR spectra.

INTRODUCTION

Large amounts of chemically different dyes generated by textile industries are discharged into the environment and have become a major concern in wastewater treatment (Grassi et al., 2011). The suspended dyes in water bodies influence the aquatic ecosystem (Gupta et al., 2007), pose public health risks due to bioaccumulation

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and cause soil contamination (Sriram et al., 2013). Furthermore, the reduced intermediates of azo dyes which are aromatic amines are more toxic than the dyes themselves (Gomi et al., 2011). Discharge of wastewater containing synthetic dyes have carcinogenic health effects and have biodegradable difficulty due to their complex aromatic structure therefore posing an environmentally important problem and this has persuaded environmental engineers to develop new techniques for treatment of such harmful compounds. Many methods are being used for dye removal; they include physical/chemical adsorption, oxidation, biological treatments (Akar et al., 2013), microbial biomass and enzyme treatments (Anjaneyulu et al., 2005). Among these methods, the use of microbial enzymes is most efficient for dye degradation/decolorization (Baldev et al., 2013; Pramanik and Chaudhuri, 2018). Many of the aforementioned methods are not successful in dye removals due to the following reasons: (i) the chemicals are only partially degraded; (ii) the azo-dyes are converted into the toxic metabolites, and (iii) the toxic chemicals are converted to secondary solid wastes with complex binding structure; which has to be either treated again or dumped (Behnajady et al., 2006).

Chemical dyes are widely used in many industries such as paper printing, color photography, pharmaceutical, textile and leather industries (Korbahti and Rauf, 2008; Vidya et al., 2017). Azo/Synthetic dyes contain aromatic and phenolic compounds and microbial enzymes are capable of removing phenolics and aromatic amines present in the azo dyes (Claus, 2003).

Fungal laccase are more advantageous over other sources due to their stability, their substrate non-specific nature and in oxidizing various phenolic compounds and have been widely applied in biotechnology and for various purposes (Shervedani and Amini, 2012). Due to the high sensitivity of laccase to denaturing agents, the use of immobilized laccase has proved effective in the industrial application of laccase (Gochev and Krastanov, 2007). Entrapment of enzymes on agar gel is one of the methods of immobilizing enzymes which has been very effective. Agar (agar-agar) is an agarose polysaccharide which is acid stable and has a strong gelling ability with no great significance in protein reactivity (Om and Nivedita, 2011). Moreover, the cost of this material is low when compared with other materials commonly used for immobilization.

The focus of this research is to investigate the removal of dyes by the crude, purified and immobilized laccase enzyme produced by *Kluyveromyces dobzhanskii* DW1 and *Pichia manshurica* DW2.

MATERIALS AND METHODS

Microorganisms and culture conditions

Yeast strains *K. dobzhanskii* DW1 and *P. manshurica* DW2 obtained from the previous experiments (Wakil et al., 2017) were used for this research. Stock cultures were stored in glucose yeast extract agar slants in sterile McCartney bottles and were kept in the refrigerator at 4°C.

Chemicals and reagents

2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) [ABTS] was purchased from Sigma-Aldrich, USA. Hydroquinone and tannic acid were obtained from Plus Chemical, India. Acetaminophen and catechol were obtained from May and Baker, England. Malachite green and methyl red were obtained from Himedia Chemicals, India and all other chemicals were of analytical grade.

Laccase production, purification and assay

The liquid medium containing rice bran (10 g/l) (*K. dobzhanskii* DW1), cane bagasse (10 g/l) (*P. manshurica* DW2), $NaNO_3$ (3 g/l), $CuSO_4$ (0.2 g/l), $FeSO_4$ (0.05 g/l), $MgSO_4.7H_2O$ (0.5 g/l), $ZnSO_4$ (0.001 g/l), Na_2HPO_4 (0.2 g/l), $MnSO_4$ (0.05 g/l), $CaCl_2$ (0.01 g/l), and KCl (0.1 g/l) at pH (6.0). The liquid medium was sterilized by autoclaving at 121°C for 15 min. After cooling of the sterilized medium, then, 1 ml of yeast extract broth with actively growing *K. dobzhanskii* DW1 and *P. manshurica* DW2 isolates containing 3.1×10^9 and 9.8×10^9 CFU, respectively were inoculated separately and aseptically into a 25 0ml Erlenmeyer flask containing 100 ml of the liquid medium. Cultures were incubated at 30 and 35°C for *K. dobzhanskii* DW1 and *P. manshurica* DW2 isolates, respectively in a shaker incubator at 120 rpm to ensure aerobic conditions. After 14 days of incubation, the cells were removed by filtration through filter paper (Whatman No. 1). The clear supernatant was stored at 4°C and used for purification. The laccase purification was performed according to the method of Ding et al. (2012).

Laccase activity was determined spectrophotometrically by measuring the oxidation of 0.02 M ABTS at 30°C according to the method of Mongkolthanaruk et al., (2012) for the crude and purified enzyme while the method of Faramarzi and Forootanfar (2011) was used for the immobilized laccase. The ABTS was the substrate, and absorbance increase in assay mixture was monitored at 420 nm (ε_{420}=36.0 $mM^{-1}cM^{-1}$), and the enzyme activity was expressed in an international units (U) defined as the amount of enzyme needed to produce 1 µmol product min^{-1} at 30°C. Protein concentration was determined by the method of Lowry et al., (1951) with bovine serum albumin as a standard.

Immobilization of laccase

Entrapment in agar gel

The enzyme was immobilized according to the method of Om and Nivedita (2011). A 4.0% agar solution was prepared in 25 mM sodium acetate buffer (pH 6.0) by warming at 50°C. After cooling down to room temperature, a 1.0 ml enzyme (containing 0.017 mg (DW1) and 0.020 mg (DW2) protein/ml) was mixed with 9.0 ml agar solution (the total volume of matrix and enzyme mixture being 10 ml) and immediately casted on preassembled Petri dishes. After solidification at room temperature, the gel was cut into a small size of 5 × 5 mm to make beads of immobilized enzyme. The beads were stored in 25 mM sodium acetate buffer (pH 6.0) and at 4°C for further use.

Percentage immobilization of laccase enzyme

This was done according to the method of Om and Nivedita (2011).

The percentage immobilization was calculated as the:

$$\frac{\textit{Total activity in immobilized gel}}{\text{Total activity in the soluble enzyme loaded}} \times 100$$

Dye decolourization experiments

The decolourization experiment was done using the forms of laccase enzyme (crude, purified and immobilized). Two dye concentrations (0.05 and 0.1 g of dye in a litre of distilled water) were prepared. Decolourization was determined by measuring the absorbance of decolourization medium at different wavelengths depending on the dye (malachite green at 615 nm and methyl red at 502 nm).

Decolourization experiment of the crude and purified enzyme was according to the method of Mirzadeh et al., (2014) while that of the immobilized enzyme was according to the modified method of Poonkuzhali and Palvannan (2013) where 5 beads were incubated with the decolourization experiment.

Ten millilitres of the different dye concentrations were dispensed into tests tubes and plugged with cotton wool. The dyes in different test tubes were then sterilized at 121°C for 15 min using autoclave before aseptically adding 1 ml of the crude enzyme, 1 ml of the purified laccase enzyme and 0.5 g (5 beads/tablets) of the immobilized enzyme. The control was prepared by adding 10 ml of the dye without any of the laccase enzymes. Each test tube for both test (dye with laccase enzymes) and control (dye without any laccase enzyme) was evenly mixed using a vortex mixer at a low speed (2,000 rpm).

Aliquot (2ml) from the experimental set-up was withdrawn immediately and absorbance was taken at different wavelengths specific for each dye. This was taken as the initial absorbance before incubation at room temperature for 4 days. Subsequent absorbance readings were done every 24 h. These were taken as final absorbance at each time interval.

Percentage decolourization was then calculated according to Olukanni et al., (2010) as follows:

$$\frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy was used to examine the surface functional groups that were involved in decolourization of malachite green dye and methyl red dye treated with crude, purified and immobilized laccase enzymes. This was done according to the methodology described by Poonkuzhali and Palvannan (2013). FTIR analysis was carried out using Spectrophotometer (FTIR-8400S Shimadzu, Japan) and changes in percentage transmission at different wavelengths were observed for 4 days incubated samples. The spectra were collected within a scanning range of 4000 to 400 cm^{-1} for both malachite green and methyl red. The samples were mixed with spectroscopically pure Potassium Bromide in the ratio of 5:95 prior to analyses.

Statistical analysis

The statistical analyses of the aforementioned experiments were carried out using Statistical Package for the Social Sciences (SPSS) software (version 16.0). All the above experiments were carried out in duplicates and their significance level was analysed using the SPSS software.

RESULTS AND DISCUSSION

The laccase enzyme was immobilized on agar gel and percentage immobilization of enzyme of both *K. dobzhanskii* (65.2%) and *P. manshurica* (73.1%) showed that immobilization reduced the enzyme activity in comparison to free enzyme (100%). Many researcher has reported 65% immobilization of laccase in other substrates (Palmieri et al., 2005; Brandi et al., 2006; Chhabra et al., 2015) while the reduced activity of the immobilized enzyme may be as a result of low enzyme loading capacity or largely due to loss in laccase activity due to leaching or inactivation (Palmieri et al., 2005; Brandi et al., 2006).

Dye decolourization of malachite green and methyl red

Table 1 shows the decolourization of two dyes (triarylmethane) malachite green and (azo) methyl red by laccase enzymes of *K. dobzhanskii* DW1. The two dyes (malachite green and methyl red) were decolourized at two different concentrations (0.05 and 0.1 g/l) by the crude, purified and immobilized laccase enzymes of *K. dobzhanskii* DW1. Percentage decolourization of malachite green increases with incubation time in all the laccase enzyme forms (crude, purified and immobilized) and better percentage decolourization recorded at the lower concentration of the dye (0.05 g/l) except with crude laccase. Similar observation was reported by Pramanik and Chaudhuri (2018) where the least dye concentration of 0.5% gave maximum decolourisation. The highest percentage decolourization (81.50%) of malachite green was recorded at 96 h for immobilized laccase enzyme at concentration 0.05 g/l and the least percentage decolourization (2.20%) recorded at 24 h for crude laccase enzyme at concentration 0.1 g/l of the dye. The observed efficient decolorization by immobilised laccase in this study is similar to that reported on the purified and immobilized laccase of *Paraconiothyrium variabile* which efficiently decolourised the removal of two synthetic dyes of acid blue 25 and acid orange 7 compared to the free laccase (Mirzadeh et al., 2014).

Similarly for methyl red, percentage decolourization increases with incubation time in all the laccase enzyme forms. The highest (87.50%) and lowest (13.48%) percentage decolourization of methyl red was recorded at 96 h for purified laccase enzyme at 0.05 g/l concentration and at 0.1 g/l of immobilized laccase concentration after 24 h. Statistically, at all sampling times percentage decolourization of each dye significantly differs ($P \leq 0.05$) with their concentrations. Increase in concentration affected the process of dye decolorization and their efficiency depends upon their chemical structures, enzymes, and system conditions (Sun et al., 2017).

Table 2 shows the decolourization of malachite green and methyl red dyes by laccase enzymes of *P.*

Table 1. Dye decolourization with laccase enzyme of *Kluyveromyces dobzhanskii* DW1 at different incubation times.

Dye	Enzyme	Conc. (g/l)	Decolourization (%)			
			24 h	48 h	72 h	96 h
Malachite Green	Crude	0.05	13.40±0.20^{c}	19.48±0.20^{e}	19.86±0.04^{d}	20.28±0.14^{d}
		0.1	2.20±0.36^{e}	19.42±0.03^{d}	19.77±0.42^{d}	20.47±0.00^{d}
	Purified	0.05	62.38±0.00^{a}	63.36±0.14^{b}	63.86±0.14^{b}	65.84±0.04^{b}
		0.1	38.18±0.00^{b}	38.92±0.04^{c}	39.53±0.20^{c}	40.15±0.00^{c}
	Immobilized	0.05	38.72±0.00^{b}	75.73±0.00^{a}	81.40±0.14^{a}	81.50±0.02^{a}
		0.1	4.49±0.42^{d}	10.26±0.24^{f}	10.79±0.04^{e}	12.39±0.00^{e}
Methyl red	Crude	0.05	35.68±0.04^{c}	45.08±0.42^{c}	60.37±0.20^{d}	65.86±0.03^{d}
		0.1	44.04±0.20^{b}	76.20±0.35^{a}	80.63±0.14^{b}	81.70±0.04^{b}
	Purified	0.05	49.91±0.03^{a}	75.79±0.00^{b}	87.25±0.04^{a}	87.50±0.00^{a}
		0.1	13.95±0.42^{e}	45.54±0.36^{c}	70.27±0.46^{c}	73.91±0.03^{c}
	Immobilized	0.05	32.19±0.12^{d}	36.93±0.20^{d}	47.55±0.04^{e}	52.12±0.03^{e}
		0.1	13.48±0.03^{e}	16.54±0.00^{e}	22.67±0.14^{f}	27.33±0.20^{f}

Values are average of duplicate readings ± standard deviation. Means of values on the same column with the same superscript are not significantly different (P≥0.05) from each other within each dye.

manshurica DW2. The two dyes were decolourized at two different concentrations (0.05 and 0.1 g/l) by the crude, purified and immobilized laccase enzymes of the yeast isolate. With malachite green, at all incubation time and at different forms of laccase enzyme except the crude laccase enzyme, the lower concentration of the dyes (0.05 g/l) had the better percentage decolouration. Percentage decolourization of malachite green also increases with incubation time in all the laccase enzyme forms. The highest percentage decolourization of malachite green (84.40%) was recorded at 96 h and at a concentration of 0.1 g/l for crude laccase enzyme and the lowest percentage decolourization (2.56%) was recorded at 24 h for purified laccase enzyme also at 0.1 g/l concentration of the dye. Similarly for methyl red, percentage decolourization increases with incubation time in all the laccase enzyme forms. The highest (76.89%) and lowest (2.70%) percentage decolourization of methyl red was recorded at 96 h for crude (0.1 g/l) and at 24 h for purified (0.1 g/l) laccases. Generally, for both dyes and at both concentrations used, the purified form of the laccase enzyme gave the least decolourization at most times. Statistically, at all sampling times, percentage decolourization of each dye significantly differs (P≤0.05) with their concentrations.

From Table 1, the best percentage decolourization by laccase of *K. dobzhanskii* Dw1 for methyl red was recorded by the purified laccase at 0.05 g/l concentration of the dye. Meanwhile, the best percentage decolourization for malachite green was recorded by the immobilized enzyme at 0.05 g/l concentration of the dye. Therefore, laccase of *K. dobzhanskii* was observed to work best (highest percentage decolourization) at 0.05 g/l concentration for both dyes. The difference in the extent of decolorization of structurally different dyes by laccase at the wavelength of each dye may be due to the difference of the redox potentials and the suitability of their steric structure with the active site of the enzyme (Afreen et al., 2016).

From Table 2, the best percentage decolourization by laccase enzymes of *P. manshurica* Dw2 for methyl red was recorded by the crude laccase at 0.1 g/l concentration of the dye. Similarly, the best percentage decolourization for malachite green was also recorded by the crude laccase enzyme at 0.1 g/l concentration of the dye. However, for laccase enzymes of *P. manshurica*, it was observed that the highest percentage decolourization was recorded at 0.1 g/l concentration of both dyes and at the same crude laccase treatments.

The observed variation in the decolorization potential of laccases even on the same dye depends on the biological sources of producing microorganism. For example, 60.5% of malachite green was removed after 15 min incubation of the dye in the presence of laccase from *P. variabile* (Forootanfar et al., 2011) while Zhuo et al. (2011) reported 98% of malachite green decolorization using laccase of *Ganoderma* sp.En3 after 72 h incubation. In addition, Vaidyanathan et al. (2011)

Table 2. Dye Decolourization with laccase enzyme of *Pichia manshurica* DW2 at different incubation times.

Dye	Enzyme	Conc. (g/l)	Decolourization (%)			
			24 h	48 h	72 h	96 h
Malachite Green	Crude	0.05	59.73±0.20^{c}	62.18±0.00^{c}	62.72±0.23^{c}	63.13±0.14^{c}
		0.1	77.92±0.14^{a}	79.67±0.00^{a}	81.20±0.03^{a}	84.40±0.26^{a}
	Purified	0.05	30.35±0.35^{d}	34.19±0.14^{d}	38.34±0.23^{d}	39.94±0.03^{d}
		0.1	2.56±0.14^{f}	6.28±0.00^{e}	7.84±0.41^{e}	7.97±0.20^{e}
	Immobilized	0.05	68.81±0.00^{b}	69.28±0.03^{b}	69.40±0.24^{b}	69.49±0.41^{b}
		0.1	6.31±0.36^{e}	6.94±0.03^{e}	7.47±0.04^{e}	7.83±0.20^{e}
Methyl red	Crude	0.05	34.65±0.00^{b}	44.78±0.41^{b}	46.91±0.03^{c}	49.25±0.04^{c}
		0.1	54.43±0.04^{a}	75.74±0.04^{a}	76.69±0.20^{a}	76.89±0.14^{a}
	Purified	0.05	11.68±0.24^{d}	31.37±0.02^{d}	43.32±0.04^{d}	47.06±0.20^{d}
		0.1	2.70±0.42^{e}	14.00±0.12^{e}	15.84±0.12^{f}	22.01±0.04^{f}
	Immobilized	0.05	28.46±0.20^{c}	41.58±0.41^{c}	58.87±0.35^{b}	63.93±0.23^{b}
		0.1	11.22±0.03^{d}	12.85±0.04^{f}	16.44±0.12^{e}	25.71±0.20^{e}

Values are average of duplicate readings ± standard deviation. Means of values on the same column with the same superscript are not significantly different (P≥0.05) from each other within each dye.

reported 72.2% removal of bromophenol blue by laccase of *P. variabile.*

Based on the aforementioned observations, these concentrations of the dyes were chosen for FTIR spectroscopy analysis.

FTIR characterization

The FTIR spectrum of methyl red: untreated by laccase, treated by the purified enzyme of *K. dobzhanskii*, DW1 and treated by the crude laccase of *P. manshurica* DW2 are as shown in Figure 1a to c, respectively.

Figure 1a shows the spectrum of untreated methyl red (control dye). The spectrum shows the changes in percentage transmission at different wavelengths and the spectrum was collected within a scanning range of 4000 to 400 cm^{-1}, while peaks were observed within a scanning range of 500 to 4000 cm^{-1} precisely within 543.94 to 3410.26 cm^{-1}. From the spectrum, sharp peaks were observed between wavenumbers 500 and 200 cm^{-1} while moderate peaks were observed between wavenumbers 2000 to 4000 cm^{-1}. From the figure, 25 peaks were recorded for the undecolourized dye with the presence of chemical groups like aliphatic halogen, alkane, alkene, ether, imine, azo (azide), alkyne, amine and alcohol/phenol.

Figure 1b shows the spectrum of methyl red decolourized by the purified laccase enzyme of *K. dobzhanskii* DW1. The spectrum shows the changes in percentage transmission at different wavelengths and the spectrum was collected within a scanning range of 4000 to 400 cm^{-1} while peaks were observed within a scanning range of 600 to 4000 cm^{-1} precisely within 603.74 to 3416.05 cm^{-1}. The FTIR spectrum of methyl red dye decolourized by the purified laccase enzyme of *K. dobzhanskii* DW1 exhibited ten intense peaks at wavenumbers 2902.96, 2360.95, 1604.83, 1427.37, 1371.43, 1151.54, 931.65, 893.07, 657.75 and 603.74 cm^{-1}. Also, two broad and intense bands were observed at wavenumber 3416.05 and 1074.39 cm^{-1}. The FTIR spectrum showed there were a total of 17 peaks for the decolourized dye with the presence of chemical groups like alkane, alkene, ether, imine, carbonyl, alkyne and amine with total disappearance of azo and halogen groups. The observed reduction in the number of peaks in the FTIR spectra of the degraded/decolourised dye indicates that the number of reactive functional groups has reduced. This is similar to the report of Chatterjee et al., (2017) on the mycoremediation of textile dyes by *Talaromyces funiculosum* JAMS1, a reaction indicating change in the chemical reaction during the decolourisation process.

Figure 1c shows the spectrum of methyl red decolourized by the crude laccase enzyme of *P. manshurica* DW2. The spectrum shows the changes in percentage transmission at different wavelengths and peaks were observed from a scanning range of 500 to 4000 cm^{-1} precisely from 543.94 to 3431.48 cm^{-1}. The FTIR spectrum of methyl red decolourized by the crude

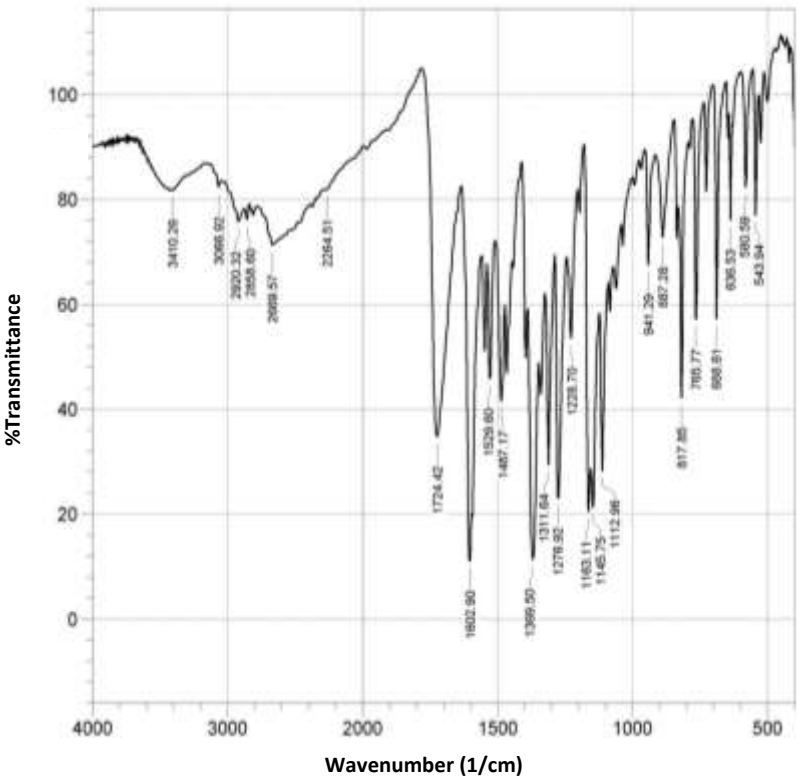

Figure 1a. FTIR spectrum of methyl red dye untreated by any laccase enzyme (Control).

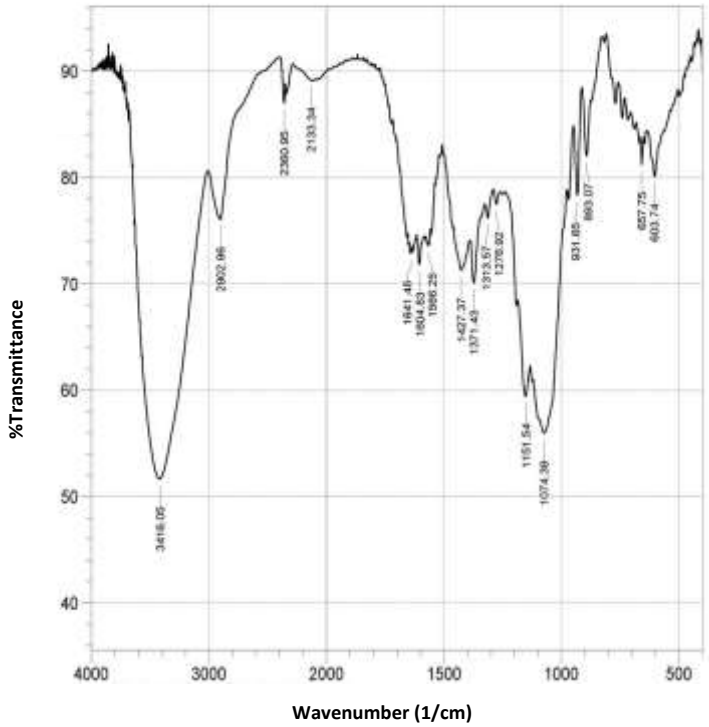

Figure 1b. FTIR spectrum of methyl red dye decolourized by the purified laccase enzyme of *Kluyveromyces dobzhanskii* strain DW1.

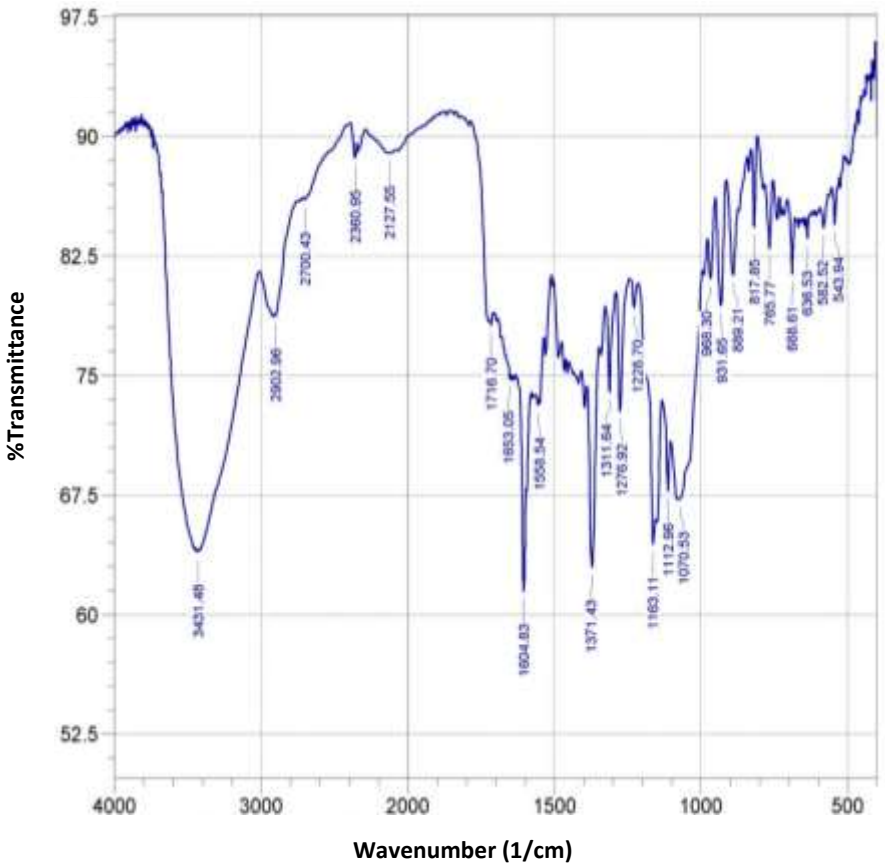

Figure 1c. FTIR spectrum of methyl red dye decolourized by the crude laccase enzyme of *Pichia manshurica* strain DW2.

laccase enzyme of *P. manshurica* exhibited sharp and intense peaks majorly between the wavelength regions of 500 and 2000 cm^{-1} with the exception of 2902.96 and 2360.95 cm^{-1} (which are unsaturated aliphatics). From the dye spectrum, a broad and intense band was observed at wavenumber 3431.48 cm^{-1}. The spectrum showed a total of 25 peaks and the presence of aliphatic halogen, alkane, alkene, ether, imine, carbonyl, alkyne and amine chemical groups.

The FTIR spectrum of malachite green: untreated by laccase enzyme, treated by the immobilized laccase enzyme of *K. dobzhanskii* DW1 and treatment by the crude laccase enzyme of *P. manshurica* DW2 are as shown in Figure 2a to c, respectively. From Figure 2a, the spectrum of untreated malachite green (control dye) shows the changes in percentage transmission at different wavelengths and the spectrum was collected within a scanning range of 4000 to 400 cm^{-1}, with peaks observed precisely from 420.5 to 3736.24 cm^{-1}. From the control dye spectrum, prominent peaks were observed mostly in the wavelength region of 500 to 2000 cm^{-1}, while moderate peaks were observed between wavenumbers 2000 and 4000 cm^{-1}. The FTIR spectrum of malachite green dye before decolourization (control) by laccase enzyme as shown in Figure 2a revealed 25 peaks belonging to the chemical groups like disulfide, aliphatic halogen, alkane, thio ether, amide, sulfone, imino, amine, alkene, alkyne and alcohol.

Figure 2b shows the spectrum of malachite green decolourized by the immobilized laccase enzyme of *K. dobzhanskii* DW1 with peaks recorded precisely from 489.94 to 3419.9 cm^{-1}. The FTIR spectrum exhibits prominent peaks around wavenumbers 1000 to 4000 cm^{-1} inclusive of the broad band peak at 3419.90 cm^{-1}. A total of 17 peaks showing the presence of aliphatic halogen, alkane, alcohol, aromatic alkane, amide, sulfone, carbonyl, alkyne and amine chemical groups were observed.

Figure 2c shows the spectrum of malachite green decolourized by the crude laccase enzyme of *P. manshurica* DW2 with peaks observed within a scanning range of 500 to 4000 cm^{-1} precisely from 536.23 to 3427.62 cm^{-1}. The FTIR spectrum of malachite green decolourized by the laccase enzyme of *P. manshurica* DW2 exhibited prominent peaks around wavenumbers 1000 to 4000 cm^{-1} inclusive of the broad band peak at 3419.90 cm^{-1}. The FTIR spectrum revealed chemical groups like disulfide, alkane, thio ether, alcohol, aromatic

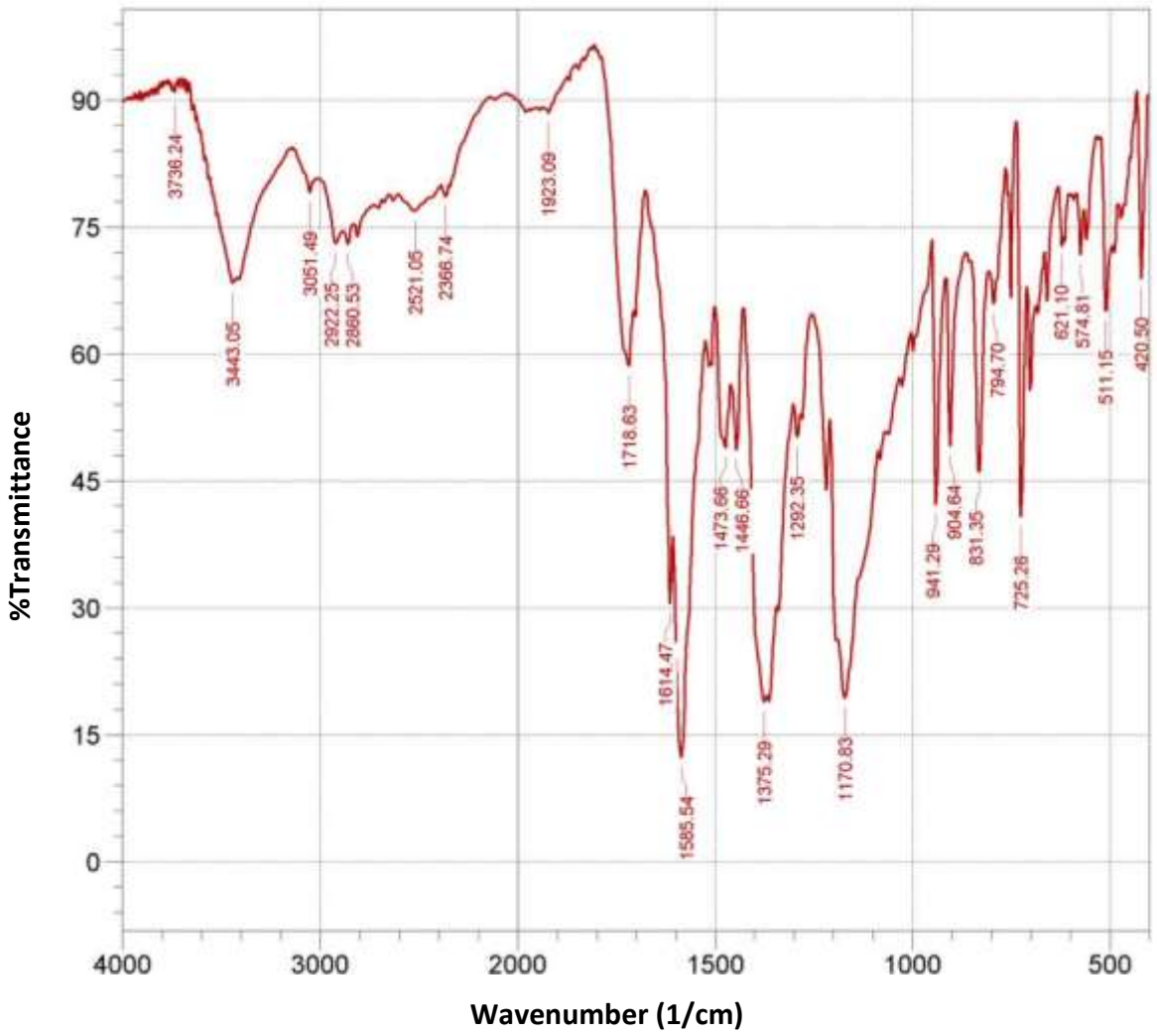

Figure 2a. FTIR spectrum of malachite green dye untreated by any laccase enzyme (Control).

alkane, amide, sulfone, carbonyl, alkyne and alcohol/ phenol. The exhibited prominent peaks in the degraded dyes may be an indication on degradation of alkynes and aromatic groups to alkanes

The percentage decolourization range of both dyes (malachite green and methyl red) by the forms (crude, purified and immobilized) of laccase of *K. dobzhanskii* DW1 and *P. manshurica* DW2 was between 2.2 and 87.5%. The highest percentage decolourization for malachite green by laccase enzyme of *K. dobzhanskii* was 81.5% at 0.05 g/l concentration of the dye while the highest percentage decolourization for methyl red by laccase enzyme of *K. dobzhanskii* was 87.5% at 0.05 g/l dye concentration. Also, the highest percentage decolourization for malachite green by laccase enzyme of *P. manshurica* was 84.4% at 0.1 g/l concentration of the dye while the highest percentage decolourization for methyl red by laccase enzyme of *P. manshurica* was 76.89% at 0.1 g/l dye concentration. Taguchi et al., (2018) similarly reported 82% decolorization of Orange G (an azo dye) at 0.3 mM concentration in the presence of iodide by laccase enzyme of *Iodidimonas* sp. Q-1

Similar result of 4.1 to 91.5% was observed by Zouari-Mechichi et al., (2006) when purified and crude laccase enzymes of *Trametes trogii* strain B6J was used to decolourize 0.05 g/l of azo dyes; Neolane blue, Neolane pink, Neolane yellow and Maxilon blue and the indigoid dyes Basacryl yellow and Bezaktiv S-BF turquoise. Ravikumar et al., (2013) reported that the purified laccase of *Hypsizygus ulmarius* showed maximum amount of decolourization in Remazol Brilliant Blue R (85%) followed by methyl orange (75%), Alizarin Red (73%), methyl violet (72%) and congo red (69%) without any additional redox mediator although at a reduced dye concentration of 0.025 g/l. Furthermore, with the addition of a redox mediator, 0.1 mM iodide was found to be sufficient for 71 to 99% decolorization of the azo and indigoid dyes, while 1 mM iodide was required for 78%

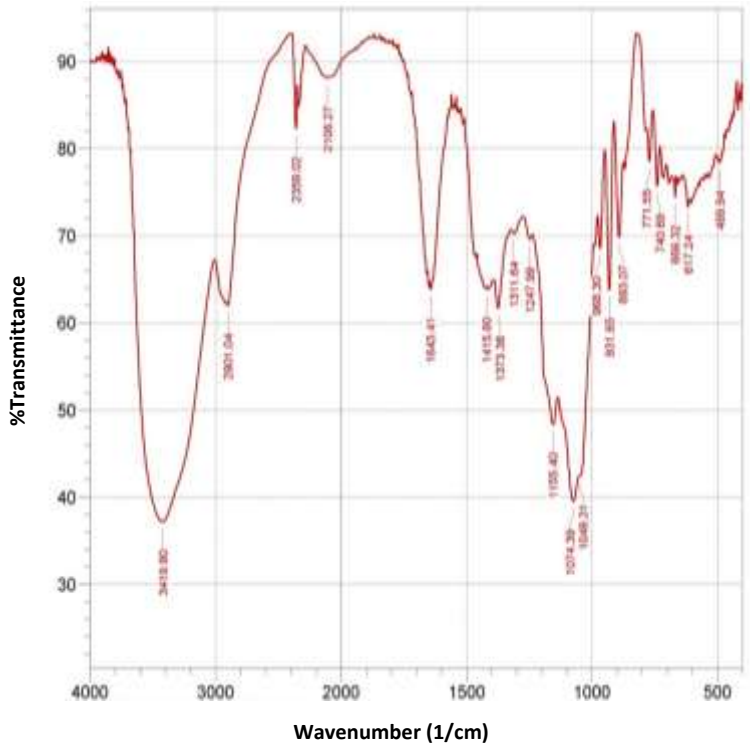

Figure 2b. FTIR spectrum of malachite green decolourized by the immobilized laccase enzyme of *Kluyveromyces dobzhanskii* DW1.

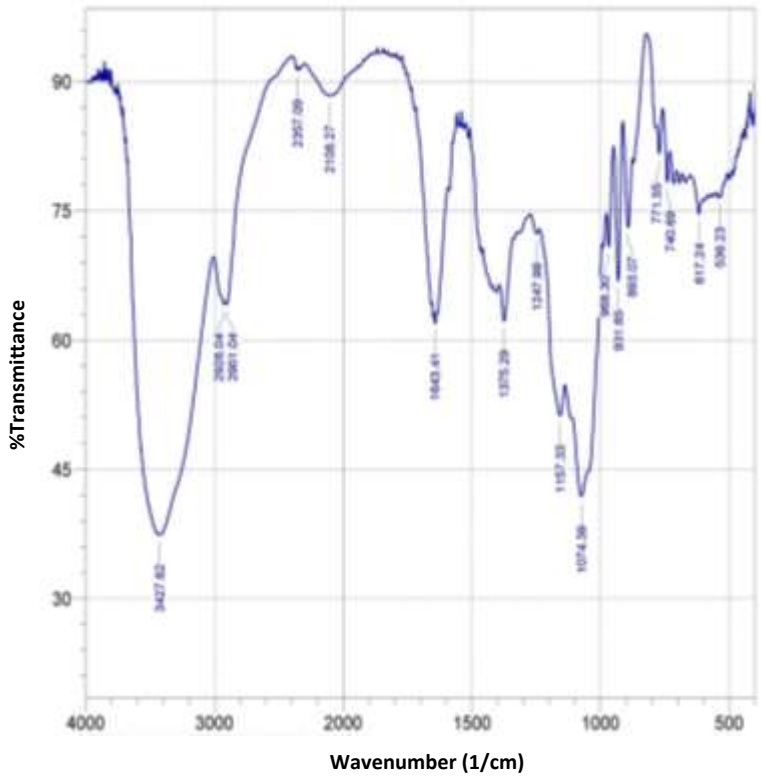

Figure 2c. FTIR spectrum of malachite green decolourized by the crude laccase enzyme of *Pichia manshurica* DW2.

decolorization of RBBR (Taguchi et al., 2018).

Conclusion

In this study, laccase enzyme from two yeast strains (*P. manshurica* DW2 and *K. dobzhanskii* DW1) were applied for the decolourisation of two synthetic dyes (Methyl red and Malachite green) and the enzyme forms (that is, crude, purified or immobilized) significantly affect the percentage decolorisation. The reduction in the peak numbers of the FTIR spectra of the decolourised dyes indicated the degradation or removal of some reactive functional groups. The removal of the main components (azo chemical group) of the dyes proved their effectiveness in decolourisation and bioremediation of the textile wastes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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