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Effect of fungal enzymes on decolorization of textile dyeing industry effluent

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Abstract

Enzyme technology has been receiving increased attention in the latest development of biological remediation. The present study is aimed to explore the potential of the fungal species to decolorize the dyes from textile dyeing industry effluent.The white rot fungi *Phanerochaetechrysosporium*and *Ganodermalucidum* were cultured in the selected substrates like wheat bran and sugarcane bagasse. The fungal enzymes were extracted and purified. The raw effluent (undiluted) and the diluted effluent (25%, 50% and 75% dilution with MSM) were taken for the study. Both diluted and undiluted effluent were adjusted to varying pH ranging from 2.0 to 8.0 and inoculated with *Phanerochaetechrysosporium* and *Ganodermalucidum*under controlled conditions and kept at room temperature for observation. Appropriate amount of the precipitated enzymes were added and kept in shaker for incubation at room temperature. Percentage decolorization was noted every 24 hours upto 7 days and calculated. From the results it was noted that the decolorization by the fungal species occurred effectively in 50% and 25% diluted effluent at pH 8.0. Whereas enzymatic decolorization occurred effectively in 75% diluted effluent at pH 5.0. It was noted that *Phanerochaetechrysosporium*and *Ganodermalucidum*could decolorize up to 90.6% and 85.7% of textile dyeing industry effluent respectively. Whereas, the enzymes extracted from *Phanerochaetechrysosporium*and*Ganodermalucidum* could decolorize up to 90.8% and 88.8% respectively. Hence, both the organisms can be exploited for the decolorization of various dyes that are released from textile dyeing industry.

Key words : Fungal enzymes (xylanase and laccase), *Phanerochaetechrysosporium* and *Ganodermalucidum*, MSM- Minimal Salt Medium.

INTRODUCTION

Rapid growth and urbanization with new technology in various industries have been contaminating the existing water resources by discharging effluent. Many dyes and pigments are hazardous and toxic for human as well as for aquatic life in the concentration at which they are being discharged to receiving water bodies [19]. Further, the untreated effluent discharged into the environment, causes severe contamination of surface and underground water resulting in adverse effects on flora, fauna and the general health of the residents around the area [9]. Safe and effective disposal of toxic effluent is a challenging task for industrialists and environmentalists [17]. Most of the conventional methods need high capital cost, which are not suitable for small-scale industries. Recently, biological treatment plays a prominent role in bioremediation [10]. Enzyme technology has been receiving increased attention in the latest development of biological remediation [6]. It was found that the isolated white-rot fungi have the potential to discolour different dyes [1].Laccases are blue copper oxidases, found in some plants and secreted by a wide range of ligninolytic fungi. These enzymes are well known for their ability in oxidizing several organic compounds, mainly phenolics and aromatic amines, at the expenses of molecular oxygen. Therefore, they could find application in the field of enzymatic bioremediation of many industrial wastewaters, and in particular to bleach and/or detoxify dye-containing effluents. Not all industrial dyes behave as laccase substrates, but this limitation is often overcome by the judicious use of redox mediators. These could substantially widen the application range of laccases as bioremediation tools [7][12]. The present study is aimed to explore microbial populations, having the potential to decolorize and degrade dyes from textile dyeing industry effluent. It is also aimed to find out the physiological conditions necessary for the organisms, under which they decolorize the dyes.

MATERIALS AND METHODS

Collection and maintenance of the fungal species: The fungal cultures *Phanerochaetechrysosporium* and *Ganodermalucidum* were collected and maintained in Rose Bengal Chloramphenicol and Sabouraud's Dextrose Broth medium [15].

Selection of substrates: Substrates selected for the study were wheat bran and sugarcane bagasse[14].

Culturing of the selected microorganisms: 10g of wheat bran and sugarcane bagasse were taken in 2 different conical flasks with 70 per cent moisture content[11]. Autoclaved and inoculated with the selected microorganisms and kept at room temperature for a period of 7 days.

Enzyme extraction: The fermented cultures were mechanically ground using 0.2 M phosphate buffer (pH 7.2) in the ratio of 1:5 (5 ml/g) and filtered through Whatman No 1 filter paper and centrifuged at 10,000g at 4˚C for 10 minutes. The supernatant obtained was used for the estimation of protein and enzyme activity [9].

Determination of protein content: The protein content of the extract was determined using Lowry's method (1951)[8].

Assessment of enzyme activity: Laccase: Laccase activity was determined using DMP (2, 6-Di Methoxy Phenol) as a substrate, and is estimated by the method of de Jong[3].

Xylanase: Xylanase activity was determined by the method of Bailey [2].

Purification of enzymes: 1. Ammonium sulphate precipitation 2. Dialysis

Collection of textile dyeing industry effluent: The effluent was collected from a textile dyeing industry and stored at room

temperature.

Effect of *Phanerochaetechrysosporium* and *Ganodermalucidum* on decolorization :The raw effluent (undiluted) and the diluted effluent (25%, 50% and 75% dilution with minimal salt medium (MSM)) were taken for the study [18]. Each set of diluted and undiluted samples were adjusted to varying pH ranging from 2.0 to 8.0 and inoculated with *Phanerochaetechrysosporium* and *Ganodermalucidum* under controlled conditions and kept at room temperature for observation. The extent of decolorization was measured spectrophotometrically and the percentage of decolorization is determined using the formula

$$\text{Decolorization (i)} = \frac{\text{Initial OD (0)} - \text{Final OD (i)}}{\text{Initial OD (0)}} \times 100$$

where decolorization (i) is the color removal efficiency until day 'i', Initial OD (0) is the optical density determined immediately after inoculation, Final OD (i) is the optical density determined in the i^{th} day.

Effect of enzymes extracted from *Phanerochaetechrysosporium* and *Ganodermalucidum* on decolorization :The enzymes precipitated with ammonium sulphate were used for the study. The effluent was diluted (25%, 50% and 75% dilution) with MSM. The undiluted and diluted effluents were adjusted to varying pH ranging from 2.0 to 8.0. Appropriate amount of enzymes were added and kept in a shaker for incubation at room temperature. Percentage decolorization was noted every 24 hours upto 7 days and calculated as before.

RESULTS

Hence the present study was undertaken to investigate the decolorization efficiency of white-rot fungi and their enzymes on textile dyeing industry effluent. The effect of various operational parameters on the maximum percentage decolorization was investigated.

The activity of laccase and xylanase in both fungal species: The results showed that the combination of wheat bran and *Ganodermalucidum* was found to be best for the production of selected enzymes at pH 5 (Fig 1 and Fig2). The activity of

Table 1: Activity of laccase and xylanase in purified samples

Organisms	Enzymes	Activity	Crude extract	Ammonium sulphate precipitation	Dialysis
Phanerochaete chrysosporium	Laccase	Activity (U/g)	110.7	142.5	133.5
		Specific activity (U mg^{-1}protein min^{-1})	0.483	2.24	2.15
		Purification fold	-	4.6	4.5
	Xylanase	Activity (U/g)	171.3	334	291.5
		Specific activity (U mg^{-1}protein min^{-1})	1.02	2.91	2.68
		Purification fold	-	2.8	2.6
Ganodermalucidum	Laccase	Activity (U/g)	134	76.5	71.5
		Specific activity (U mg^{-1}protein min^{-1})	0.82	1.26	1.24
		Purification fold	-	1.5	1.5
	Xylanase	Activity (U/g)	236.2	262.5	234.9
		Specific activity (U mg^{-1}protein min^{-1})	1.31	2.389	2.318
		Purification fold	-	2.2	1.7

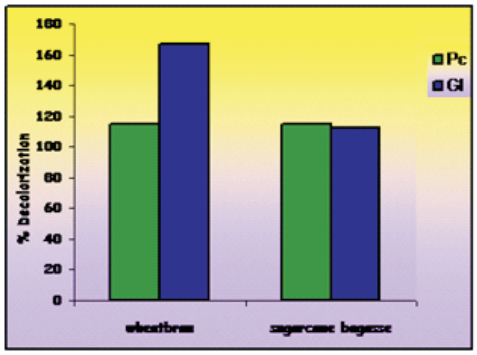

Fig 1 : Laccase production

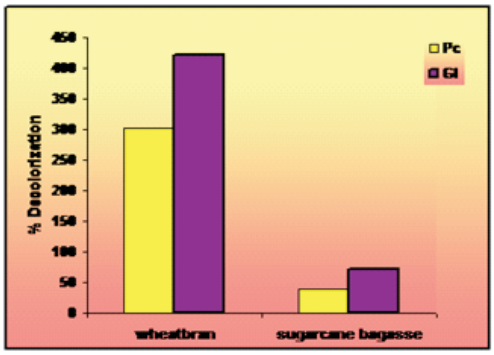

Fig 2 : Xylanase production

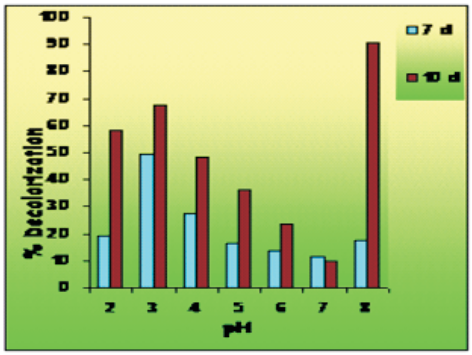

Fig 3 : Effect of *P.chrysosporium* in decolorizing the 50 per cent diluted textile dyeing industry effluent

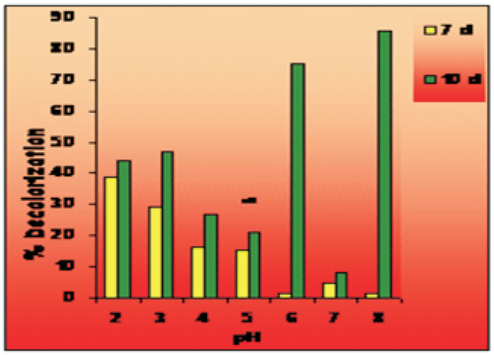

Fig 4 : Effect of *G.lucidum* in decolorizing the 25 per cent diluted textile dyeing industry effluent

enzymes in both species and both substrates is shown in Table 1. The enzyme activities were found to increase after ammonium sulphate precipitation followed by dialysis.

Purification of laccaseand xylanase : Enzymes extracted from wheat bran and sugarcane bagasse cultured with *Phanerochaetechrysosporium* were mixed. Similarly, the substrates cultured with *Ganodermalucidum* were mixed. The combined extract was subjected to ammonium sulphate precipitation and dialysis. The activity, specific activity and purification fold of both the enzymes are presented in Table 1. There was an increase in the specific activity of enzymes after ammonium sulphate precipitation and dialysis, when compared with crude extract.

Decolorization efficiency of fungal species on the effluent: From the results obtained, it can be noted that decolorization efficiency of the organisms are not consistent andchanged with varying pH and dilutions of the effluent. The highest decolorization (90.6%) was exhibited by *Phanerochaetechrysosporium* in 50 per cent diluted effluent at pH 8.0, followed by *Ganodermalucidum* which showed maximum decolorization (85.7%) at pH 8.0 in 25 per cent diluted effluent.

Decolorization efficiency of fungal enzymes on the effluent: The diluted (25 %, 50 % and 75 %) and undiluted effluent adjusted to varying pH ranging from 2.0 to 8.0 were treated with the enzymes and their decolorization efficiency was observed every 24 hours for 7 days. From the results, it is clear that, decolorization by the enzymes extracted from both the organisms was effective at pH 5.0 in 75 per cent diluted effluent.

It was noted that decolorization by the fungal species occurred effectively in 50 per cent and 25 per cent diluted effluent at pH

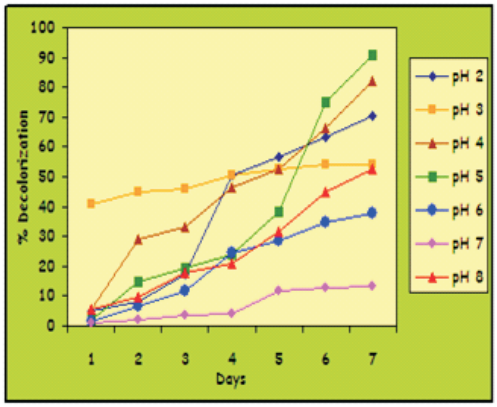

Fig 5 : Decolorization of 75 per cent diluted effluent using enzyme extracted from *P.chrysosporium*

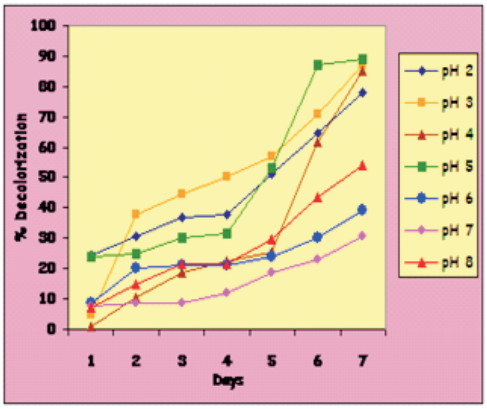

Fig 6 : Decolorization of 75 per cent diluted effluent using enzyme extracted from *G.lucidum*

8.0. Whereas enzymatic decolorization occurred effectively in 75 per cent diluted effluent at pH 5.0. Thus the dilution of the effluent required for the enzymatic decolorization is more (75% dilution) when compared with the decolorization using fungal species (50 % and 25% dilution).

Thus from the study it is clear that both *Phanerochaetechryso -sporium* and *Ganodermalucidum* could serve as a good source of laccase and xylanase. *Phanerochaetechrysosporium* and *Ganodermalucidum* could decolorize up to 90.6 per cent and 85.7 per cent of textile dyeing industry effluent respectively. Whereas, the enzymes extracted from *Phanerochaetechrysosporium* and *Ganodermalucidum* could decolorize the effluent up to 90.8 and 88.8 per cent respectively. Hence, both the organisms can be exploited for the decolorization of various dyes that are released through textile dyeing industry effluent.

DISCUSSION

Laccases are common enzymes in nature especially in plants and fungi. Besides fungal laccases, which are most frequently studied form of laccases, bacterial laccases from *Pseudomonas putida*F6, *Pseudomonas* sp. LBC1 and *Escherichia coli* have also been purified and characterized. Most of the laccases studied are of fungal origin especially from the classes of White rot fungi. Fungal laccases play an important role in pigment production, plant pathogenesis and degradation of lignocellulosic materials [4] [23]. Xylan degrading enzymes were induced when *Phanerochaetechrysosporium* was grown at 30°C in shake media containing xylan Avicel PH 102, or ground corn stalks. The highest xylanase activity was produced in the corn stalk medium, while the xylan- based fermentation resulted in the lowest induction [3].

Decolorization of textile azo dye, orange II by white rot fungi, *Phanerochaetechrysosporium*. Orange II (85%) was removed in 7 days (optimum decolorization on 5th day at 28- 30°C and pH 5.0) in liquid cultures under shaking aerobic condition using *Phanerochaetechrysosporium*. Higher dye concentration in simulated dye showed inhibitory effects on decolorization. Decolorization ability of fungus was correlated to lignolytic enzyme activity [22].Treatment of textile dye waste water was carried in a batch reactor using *Ganodermalucidum*. Under optimum temperature (36.5 °C) pH 6.6, and agitation speed 200 rpm and dye waste water concentration 1: 2, the maximum decolorization and COD reduction were found to be 81.4 and 90.3 per cent [21].

CONCLUSION

The decolorization efficiency of *Phanerochaetechryso-sporium* and *Ganodermalucidum* to diluted and undiluted effluent were not consistent. The extent of decolorization changed with varying pH and dilutions of the effluent. The highest percentage decolorization (90.6 %) was exhibited by *Phanerochaetechryso-sporium* in 50 per cent diluted effluent at pH 8.0, followed by *Ganodermalucidum*, which showed decolorization (85.7%) at pH 8.0 in 25 per cent diluted effluent. It was found that decolorization of effluent using the enzymes extracted from *Phanerochaetech-rysosporium* as 90.8 per cent and *Ganodermalucidum* as 88.8 per cent at pH 5.0 in 75 per cent diluted effluent. It was noted that the decolorization by the fungal species occurred effectively in 50 per cent and 25 per cent diluted effluent at pH 8.0. Whereas enzymatic decolorization occurred effectively in 75 per cent diluted effluent at pH 5.0. Thus the dilution of the effluent required for the enzymatic decolorization is more (75% dilution) when compared with the decolorization using fungal species (50 % and 25% dilution). Thus, from the study, it can be concluded that *Phanerochaetechrysosporium* and *Ganodermalucidum* could serve as a good source for the enzymatic decolorization of textile dyeing industry effluent.

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