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First Report on the Biodegradation of Direct Flavine 5-G and Reactive Red S3B Textile Dyes by *Piptoporus betulinus*

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Abstract. The current study focuses on the biodegradation of selected synthetic dye by using a brown rot fungus, *Piptoporus betulinus*. Response Surface Methodology (RSM) under Central Composite Design (CCD) was employed to optimize the biodegradation of two synthetic dyes named direct flavine 5-G (Direct-1) and reactive red S3B (Reactive-1). The biodegradation process was optimized by study of the effect of pH, temperature, time period and dye concentration. Further, carbon and nitrogen sources were added to enhance the biodegradation process. Ligninolytic enzymes including laccase, lignin peroxidase and manganese peroxidase were also studied during biodegradation experiments to check their role in the biodegradation process. The results showed that there was 81.17 % biodegradation of reactive-1 and 75.91 % of direct-1 after optimization of parameters i.e. pH, temperature, time period and dye concentration through RSM. The biodegradation of reactive-1 dye was increased up to 97.29 % after the addition of various carbon and nitrogen sources during the biodegradation process, while the biodegradation of direct-1 dye increased upto 93.03 % after the addition of carbon and nitrogen sources. These readily available nutrients enhance fungal growth and enzyme production that causes more biodegradation. There was positive interaction among most of the parameters studied, for the biodegradation of above two dyes. The ligninolytic enzymes were actively involved in the process with activities 1638.05 IU/mL for LiP, 1443.01 IU/mL for MnP and 258.88 IU/mL for laccase at the optimized conditions. The optimal conditions for biodegradation of both dyes were pH 6, inoculum size 6 mL, incubation period 5 day and dye concentration 0.03 % at temperature 32.5 °C. Lip was the most efficient enzyme involved in the biodegradation process.

Keywords: biodegradation, *Piptoporus betulinus*, textile dyes, response surface methodology, ligninolytic enzymes

Introduction

Dye is any natural or synthetic substance which is used to colour different materials. In other words dye is any coloured substance which is capable to bind the material on which it is applied (Ekta *et al*., 2005). Natural dying is not a new method, it is an ancient method. As early as 180000 years ago, Neanderthal tribe made paintings in caves with yellowish and reddish iron oxide, black manganese dioxide and white clay. The value of natural dye was lowered after the invention of first synthetic dye in 1856. The synthetic dyes got popularity due to extensive use in different fields such as food cosmetics, nonlinear optical activity and in textile industries (Vijayalakshmidevi *et al*., 2015). But in the last few decades, value of synthetic dye is lowered due to its hazardous effect on both health and environment. The most common health problems of synthetic dyes are allergy and cancer and they are non-biodegradable (Mahmood *et al*., 2017). Nowadays, industrial effluents are one of the leading causes of environmental pollution though out the world. . Reactive and direct dyes (both are azo dyes) used in this study have a stable structure (Mahmood *et al*., 2015). Reactive dyes are water soluble and its functional group attached to active site of cellulose in textile fibre (Bhatti *et al*., 2011; Zarkogianni *et al*., 2011). These reactive dyes are hydrolyzed during dying process and are released into water, causing sever contamination. Dyes which are obtained from the natural sources are environmentally friendly because in nature all the processes are completed by natural ways. But

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after using of natural dyes in dying process they are released into environment which produces aesthetic problems and also during their extraction they produces a huge sludge (Mussak and Bechtold., 2009). Today, the environmental regulation authorities become stricter towards environment pollution so, we should learn the importance of natural dyes to stop polluting our world (Singh *et al*., 2015; Riley *et al*., 2014). Basidiomycetes fungi are the important fungi involved in wood decaying process and it is of nearly 30% of wood decaying fungus (Mahmood *et al*., 2016). The wood decaying fungus classified as Brown rot fungi and White rot fungi due to their property of digesting lgnin along with cellulose and hemicellulose (Mussak and Bechtold., 2009). Enzymes produced by fungi are used to degrade different organic and inorganic and aromatic compounds that is why they are used in this study (Wolfenden and Willson 1982). Four dyes including two synthetic and two natural dyes were used to study decolourization from BRF. Objective of this study was to check the capability of BRF to decolrourize these dyes and to develop environmental friendly waste water treatment procedure for future. Oxidative enzymes study refers to degradation. This study would help to protect the environment from harmful and un-aesthetic waste produced by textile industry either by using synthetic or natural dyes (Mahmood *et al*., 2017).

Materials and Methods

Collection of *Piptoporus betulinus.* The fungus was collected from Industrial and Environmental Biotechnology Laboratory (IEBL), Department of Biochemistry, PMAS Arid Agriculture University Rawalpindi. It was then isolated on malt extract agar media (MEA) having pH 5.5. Fungal inoculum was prepared in malt extract broth media for the use in biodegradation process (Mahmood *et al*., 2017).

Collection of dyes. Two synthetic textile dyes, including direct flavine 5-G (direct-1) and reactive red S3B (reactive-1), commonly used in the textile industries of Pakistan were purchased from Delta Lab Scientific Company, Lahore.

Dyes decolourization. Process of decolourization was carried out in 250 mL flacks. Initially decolourization was performed by adding 90 mL of dye solution (0.02 %) in dH_2O_2, 2 mL fungal inoculum and 8 mL of broth media, mixture was put in shaking incubator at 120 rmp and 28 °C temperature for specific days. Absorbance was noted at time zero and after every 24 h. For taking absorbance 2 mL of mixture was withdrawn, centrifuged at 12,000 rmp for 15 min (Mahmood *et al*., 2017; Mahmood *et al*., 2015). Percentage decolourization was then calculated by following formula:

$$\% \text{ Decolourization} = \frac{(A_{ini} - A_{fin})}{\text{Aini}}$$

where;

A_{ini} = Intial absorbance of effuent before incubation
A_{fin} = final absorbance of effluent after incubation

Effect of nutritional source on dye decolourization. Response Surface Methodology (RSM) was used to design the experiments for the optimization of different parameters including pH, time period, temperature and dye concentration. Effect of different carbon and nitrogen sources on the biodegradation of dyes was also checked. All the experiments were designed and results were analyzed by using JMP software. Experiments were performed in duplicate by the following the procedure mentioned above (Mahmood *et al*., 2015).

Study of ligninolytic enzyme system. In order to check the role of ligninolytic enzymes in the biodegradation of dyes, enzymatic analysis also performed during optimization process. Three ligninolytic enzymes including laccase, manganese peroxidase (MnP) and ligin peroxidase (LiP) were studied and their activities were determined by standard methods. The laccase enzyme assay was performed by method of (Wolfeden and Wilson, 1982) by using 1 mM ABTS 2, 2 azinobis (3-ethylbenzthiazoline-6 sulphonate) as substrate in 50 mM malonate buffer to maintain pH at 4.5. The absorbance was noted at 436 nm at time zero and after 10 min. The Manganese peroxidase (MnP) activity assay was performed by the method described by (Wariishi *et al*., 1992) by using 1mM $MnSO_4$ as substrate in 50 mM sodium malonate buffer to maintain pH at 4.5. The absorbance was noted at 279 nm after 10 min interval. The lignin peroxidase (LiP) activity assay was performed by method of (Tien and Kirk, 1984) by using 4mM veratryl alcohol (3,4-dimethoxybenzylalcohol) as substrate in 100 mM sodium tartarate buffer to maintain pH at 3. The absorbacne was checked at 310 nm after the interval of 10 min.

Results and Discussion

Biodegradation of dyes by *P.betulines*. Fungi with the help of different secreted enzymes, able to decolourize the dyes present in the surrounding by the oxidation process. Presence of suitable conditions like pH, temperature, fungal spores, dyes concentration and different carbon and nitrogen sources enhance the biodegradation process by more fungal growth and higher secretion of enzymes (Mahmood *et al*., 2017; Mahmood *et al*., 2015).

Biodegradation of reactive-1 textile dye. Azo dyes are extensively used synthetic dyes in the textile industries and enter into the environment as effluents. reactive-1 textile dye is an azo dye with huge industrial utilization. In current study there was 81.17 % biodegradation achieved at pH 6, 6 mL inoculum, at 32 °C temperature after 5 days (Fig. 1 a-c). The 3D response surface graphs obtained shows that there was positive interaction between most of the parameters which lead to enhanced biodegradation of direct-1 dye. Any change

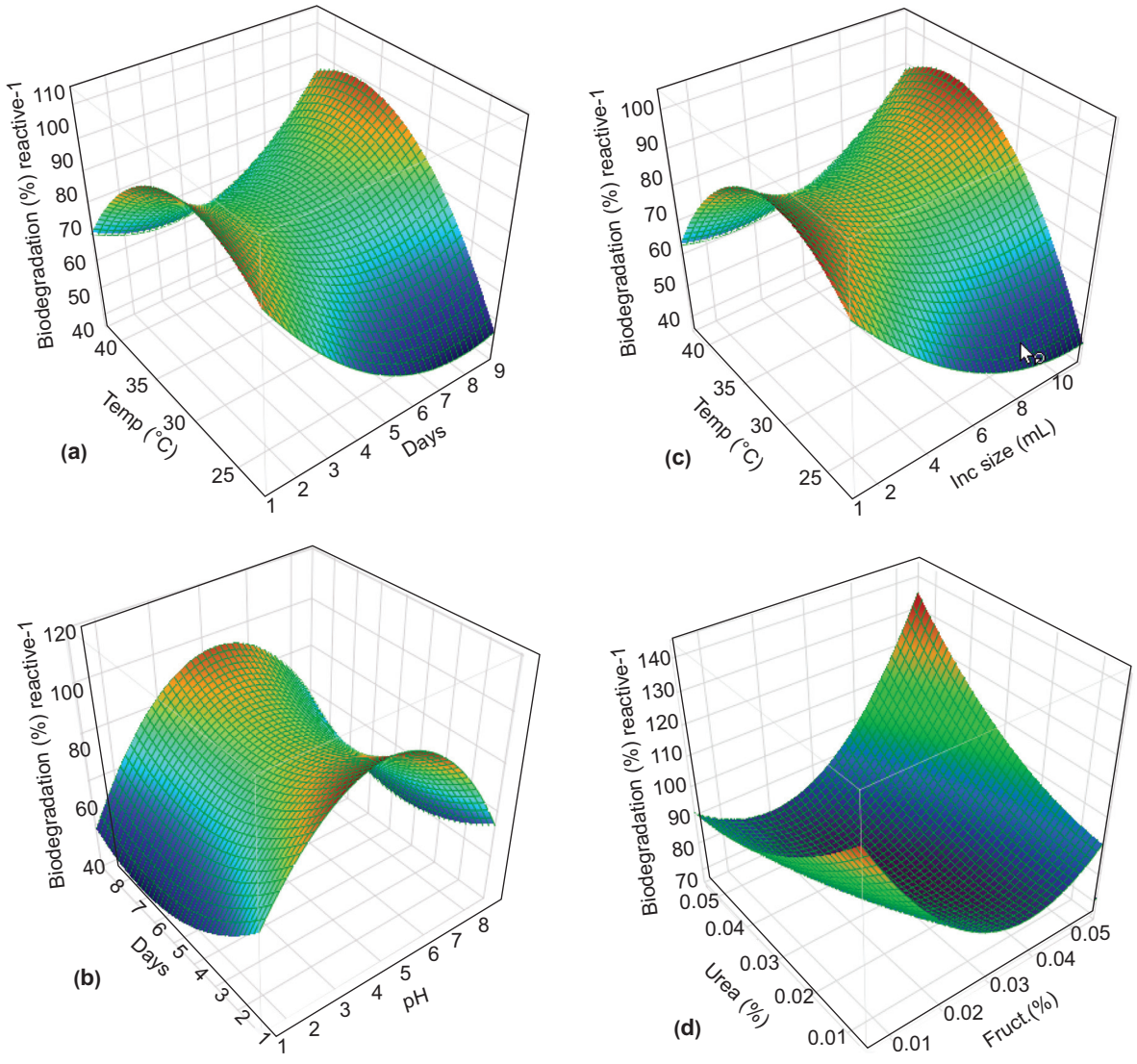

Fig. 1. 3D response graphs showing interaction between different parameters during the biodegradation of reactive-1 textile dye by *P. betulinus* (**a**) Temperature and days (**b**) Days and pH (**c**) Temperature and Incoulum size (**d**) Urea and fructose

in conditions from optimized values will lead to decrease in biodegradation. The biodegradation of reactive-1 dye was increased up to 97.29 % (16.12 %) after the addition of carbon and nitrogen sources (Fig.1d). The readily available nutrients enhance the microbial mediated processes by increasing microbial growth and enzyme production.

Another study reported 85 % decolourization of Azo dyes (50 mg/L) e.g reactive red KE-3B and reactive brilliuant blue K-GR within 36 h. Variation in result is due to higher dye concentration (Singh *et al*., 2015; Mahmood *et al*., 2015).

Biodegradation of direct-1 textile dye. There was 75 % biodegradation of direct-1 dye at pH 5, dye concentration 0.03 % and temperature 28 °C after 5 days. Most of the parameters interact positively for biodegradation of direct-1 dye, while there was negative interaction between some parameters (Fig. 2 a-c). The addition of carbon and nitrogen sources shows that the biodegradation enhanced upto 93.03 % (18 %) (Fig. 2d). This is due to more fungal growth and secretion of ligninolytic enzymes in the presence of these nutrients.

These results are in accordance to study conducted by (Hadibarata *et al*., 2013) for decolourization of azo

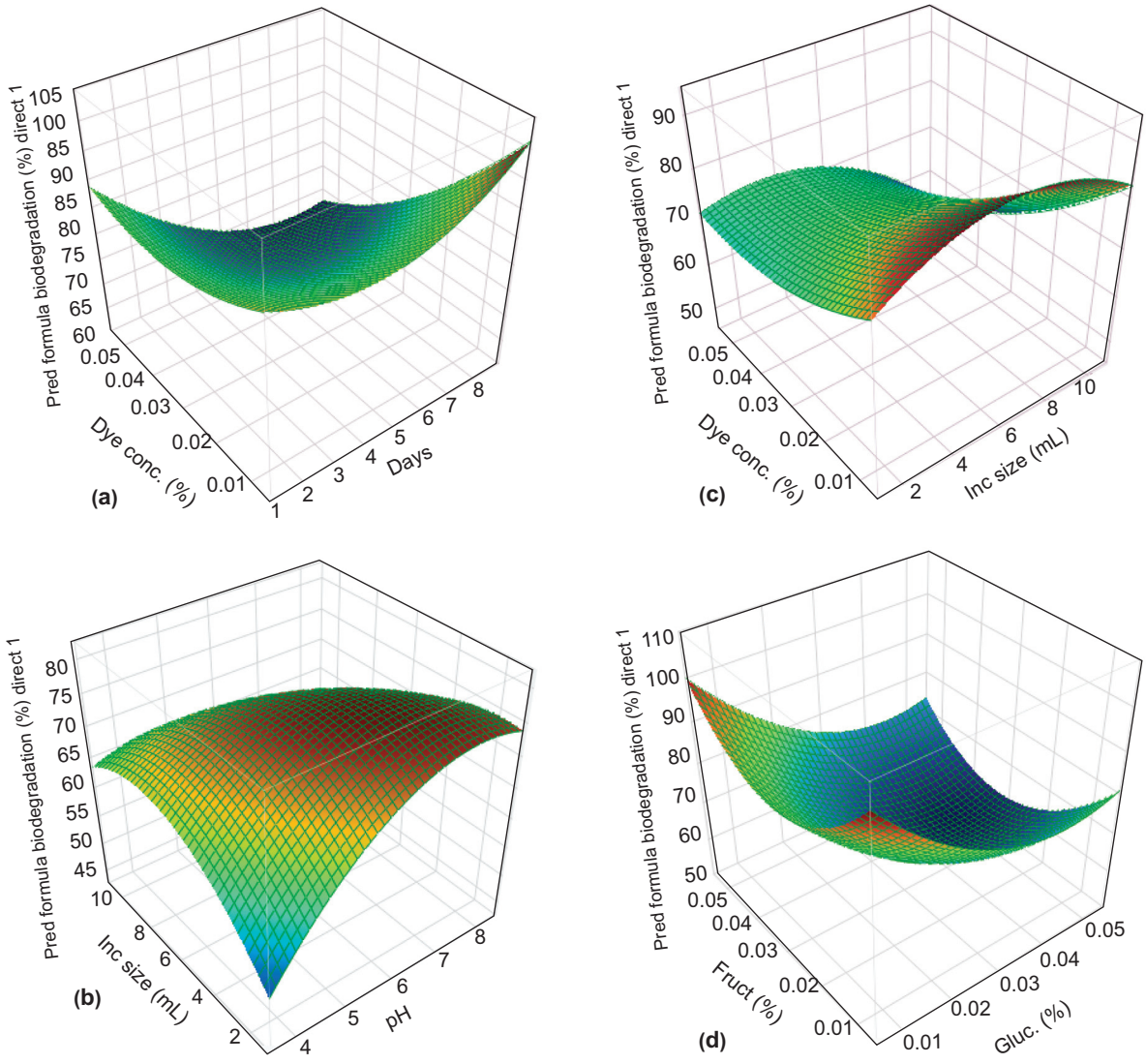

Fig. 2. 3D response graphs showing interaction between different parameters during the biodegradation of reactive-1 textile dye by *P. betulinus* (**a**) Dye conc. and days (**b**) Inoculum size and pH (**c**) Dye conc. and Incoulum size (**d**) Glucose and fructose

dyes by *Pleurotus eryngii* F032. They had performed degradation analysis under similar conditions with addition of agitation and surfactant i.e. Tween 80. They got 93.57 % dye biodegradation after 72 h of shaking incubation under acidic conditions. Further, ligninolytic activities increased under optimum conditions along with higher biodegradation.

Extracellular ligninolytic enzymes. Fungus released extracellular enzymes to oxidize the nutrients and other chemicals in the surrounding medium. The aromatic synthetic dyes are oxidized by ligninolytic enzymes including LiP, MnP and Laccase. During current study it was observed that biodegradation process positively linked with enzymes production. Maximum enzymatic activities were obtained at conditions, where there was higher biodegradation of dyes. The result shows there was 1266.52 IU/mL activity of Lip, 1443.01 IU/mL of MnP and 258.88 IU/mL for laccase at maximum biodegradation of dyes. There are many previous studies showing similar results, enhanced biodegradation linked with higher ligninolytic activities (Mahmood *et al*., 2017; Singh *et al*., 2015). These findings confirmed the positive role of these enzymes in the biodegradation of both dyes.

On the basis of results obtained during the study it can be concluded that *P. betulinus* can be used for the biodegradation of various dyes and dyes containing effleunts. Further, it is active producer of three types of ligninolytic enzymes including LiP, MnP and laccase. manganese peroxidase (MnP) is the most active extracellular enzyme produced by the *P. betulinus* (Singh *et al*., 2015; Mussak and Bechtold., 2009). These brown rot fungi could be potential candidates for the treatment of wastewater (Mahmood *et al*., 2015).

Conflict of Interest. The authors declare they have no conflict of interest.

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