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Development of novel enzymatic bioremediation process for textile industry effluents through response surface methodology

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ABSTRACT

Crude ligninolytic enzymes extract from *Schyzohyllum commune* IBL-06 having high activities of LiP (2186.02 U mL^{-1}), MnP (1676.75 U mL^{-1}) and laccase (259.07 U mL^{-1}) was used for bioremediation of textile industry effluents. Response Surface Methodology (RSM) was adopted using D-optimal design to optimize the effluent decolorization process. The effects of different mediators on effluent decolorization were also investigated under preoptimized conditions. The optimum conditions for maximum decolorization of the effluents collected from Khyber Textile (KHT), Ishaq Textile (IST), Kalash Textile (KAT) and Masood Textile (MAT) industries of Faisalabad, Pakistan were: initial pH 4.5; temperature, 35 °C; effluent concentration, 180 ppm and veratryl alcohol (mediator), 1 mM. It was noted that values of water quality parameters including pH, color, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total organic carbon (TOC) and formaldehyde for enzyme treated effluents were within permissible limits set by the National Environmental Quality Standards (NQS). Analysis of peaks in HPLC profiles for treated and untreated effluents confirmed the degradation of residual dyes by ligninolytic enzymes extract of *S. commune* IBL-06.

1. Introduction

Synthetic dyes are the chemical pollutants that originate mainly from textile and plastic industries and serious health hazards to animals and human beings. Synthetic dyes are recalcitrant chemicals that are resistant to biodegradation. Decolorization of dyes by traditional physicochemical methods including adsorption, precipitation and chemical treatment has economical and environmental disadvantages. The development of an eco-friendly treatment technology for such dyes and textile dye containing effluents is still a major environmental concern faced by the modern world. In this regard, white rot fungi (WRF) have been reported to secrete ligninolytic enzymes that are capable of catalyzing the degradation of dyes and have potential applications in bioremediation of dye containing industrial effluents (Asgher et al., 2008). However, direct application of WRF on industrial scale gives rise to difficulties like satisfying the growth requirements on a large scale, long incubation times and adsorption of dyes on fungal mycelia (Casas et al., 2009).

The *In vitro* treatment with ligninolytic enzymes from WRF can help addressing such issues due to their versatile catalytic properties but use of purified and/or immobilized enzymes increases the cost of industrial processes (Wang et al., 2008). In recent past, some efforts have been made to investigate the potential of cell-free crude enzyme extracts of WRF for *In vitro* large-scale industrial and bioremediation applications (Asgher et al., 2008). The process control of these enzymatic applications is simplified by the absence of living organisms and has advantages, such as the ability to operate over a wider range of pollutant concentrations, pH and temperatures. Moreover, the biodegradative capacity of non-specific ligninolytic enzymes is not affected by toxic compounds that could otherwise inhibit fungal growth. The un-purified crude enzyme extract may also provide the enzymes with substances that mediate the catalytic cycle of WRF peroxidases and laccase that are otherwise eliminated during enzyme purification.

In recent years, efforts have been made for the development of dye biodegradation process using WRF but concerted efforts are still required to establish the biological systems for industrial scale bioremediation of industrial effluents (Asgher et al., 2012a). Reducing the costs of treatment processes by optimizing the process parameters is the ultimate target of basic research for industrial applications (Lee et al., 2011). The conventional Classical Optimization Strategy (COS) of varying one-factor-at-a-time cannot guarantee the determination of true physical and nutritional optimum conditions as it does not involve interactions among different variables/parameters. However, Response surface methodology (RSM) involving more than one factors at a time at different

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Table 1
Characteristics of textile industry effluents used for decolorization by crude enzymes extract.

Textile industry	Color	λ_{max} (nm)	Initial pH	Types of dyes used
Khaibar Textile (KHT)	Burgundy	515	9.82	Not known
Ishaq Textile (IST)	Red	510	8.64	Not known
Masood Textile (MAT)	Green	410	10.86	Not known
Kalash Textile (KAT)	Blue	660	10.87	Not known

levels considers the interactions between two or more parameters and provide reliable optimization results (Rengadurai et al., 2012). The current study was focused at developing an effective bioremediation process for textile industry effluents using crude enzyme extract from *Schyzophylum commune* IBL-06.

2. Materials and methods

All the experimental and analytical work was carried out in Industrial Biotechnology Laboratory, Department of Chemistry & Biochemistry, University of Agriculture Faisalabad, Pakistan.

2.1. Fungal culture and inoculum development

For inoculums development, the indigenously isolated *S. commune* IBL-06 strain was grown in Kirk's basal nutrient medium (Tien and Kirk, 1988) in Erlenmeyer flask (500 mL) that was supplemented with Millipore filtered 1% glucose. The medium was adjusted at pH 4.5 with M NaOH/M HCl and sterilized for 15 min at 121 °C and inoculated with spores of *S. commune* IBL-06 from slant culture. The inoculated flask was incubated (120 rpm) at 30 °C for 5–7 days to get homogenous spore suspension of the fungus (1×10^6–10^8spores/mL) that was used as inoculums.

2.2. Production of ligninolytic enzymes extract under optimum conditions

Triplicate flasks containing pre-optimized SSF medium of corn stover were autoclaved and inoculated with 5 mL homogenous inoculums (Yasmeen et al., 2013). The optimum conditions were: initial pH, 4.5; moisture, 60%; temperature, 35 °C; inoculum size, 4 mL and incubation time, 144 h using the combination of glucose (1%) and urea (0.2%) as carbon and nitrogen sources; tween-80 (1 mM), 0.2 mL; 1 mM H_2O_2 1 mL; and Ca^{2+} 1 mM. The SSF flasks were harvested after 72 h by adding 100 mL of 10 mM sodium succinate buffer (pH 4.5) and shaking (120 rpm) for 30 min. The contents were filtered through Wattman No.1 filter paper and the filtrates were centrifuged at 3000 × *g* for 10 min at 4 °C to remove mycelia pellets and cell debris. The clear supernatants were assayed for ligninolytic enzymes including lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. The crude enzyme extract was stored at 4 °C in refrigerator for further use in bioremediation studies.

2.3. Ligninolytic enzyme assays

2.3.1. Laccase assay

Laccase activity in the crude enzyme extracts was determined by the method of Shin and Lee (2000) by monitoring the oxidation of 2, 2 azinobis (3-ethylbenzthiazoline-6 sulphonate (ABTS) at 436 nm (ϵ_{436} = 36,000 M^{-1} cm^{-1}). The reaction mixture contained 1 mL of 1 mM ABTS in 1 mL of 50 mM malonate buffer (pH 4.5) and 100 μL of culture supernatant. The mixture was incubated at 25 °C and absorbance was read after 10 min interval using UV/vis spectrophotometer (T60, PG Instruments, UK).

2.3.2. Manganese peroxidase assay

MnP was assayed following the method of Wariishi et al. (1992). The assay mixture contained 1 mL of 1 mM $MnSO_4$ in 1 mL of 50 mM sodium malonate buffer (pH 4.5) in the presence of H_2O_2 and culture supernatant. It was incubated at 25 °C and absorbance was recorded at 270 nm after 10 min interval (ϵ_{436} = 11,590 $M^{-1}cm^{-1}$).

2.3.3. Lignin peroxidase assay

LiP activity of the culture supernatant was determined by the method of Tien and Kirk (1988). The H_2O_2 dependent oxidation of veratryl alcohol to veratraldehyde was monitored at 25 °C. The reaction mixture contained 1 mL tartrate buffer (100 mM) of pH 3, 1 mL of 4 mM veratryl alcohol, 500 μL of 0.2 M H_2O_2 and 100 μL of culture supernatant. The activity of mixture was measured against a reagent blank at 310 nm (ϵ_{310} = 9300 M^{-1} cm^{-1}).

2.4. Decolorization of industrial effluents by crude enzyme extracts

2.4.1. Effluents collection

Practical textile industry effluents were collected from Khaibar Textile (KHT), Ishaq Textile (IST), Kalash Textile (KAT) and Masood Textile (MAT) industries of Faisalabad, Pakistan. The scanning UV/vis spectrophotometer was used to identify the wavelengths resulting in maximum absorbance (λ_{max}) for each effluent. The information on pH, color and wavelength of maximum absorbance (λ_{max}) for the effluents is given in Table 1. The industries did not disclose any information on the dyes being used by them due to their business secrets. However, all the industries informed that they were using mixtures of different types of dyes to develop different color shades.

2.4.2. Optimization of decolorization process by Response Surface Methodology (RSM)

RSM is a statistical experimental technique applied under appropriate experimental design to resolve multi-variable equations (Kotzamanidis et al., 2013) and determines the relationship among the complaisant contributing factors (parameters) and the responses obtained. In comparison to the orthodox mathematical or one factor optimization at a time methods, RSM is more time saving and economical. The D-Optimal experimental design was applied under RSM using DOE 8.0.7.1 Trial Version software. Three factors at four different levels were used in triplicate. Four different concentrations of effluents (60,120, 180, 240 ppm), varying temperatures (30, 35, 40, 45 °C) and pH (4.5, 5, 5.5, 6) were selected as the critical variables and nominated as A, B and C, respectively (Table 2). A total number of 20 runs (Table 3) were carried out to estimate the coefficients for the decolorization of textile effluents. The data were subjected to Analysis of Variance (ANOVA) and 3D response surface graphs were constructed using DOE 8.0.7.1 Trial Version software and MINITAB 15 programs to study the responses (% decolorization of effluents) and interactions between variables. The quality of the fit of this model was expressed by the coefficient of determination (R^2) in the same program (Tavares et al., 2009).