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By-product identification and phytotoxicity of biodegraded Direct Yellow 4 dye

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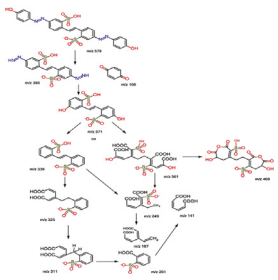
HIGHLIGHTS

- Direct Yellow 4 was undergone to biodegradation using *C. limon* peel peroxidase.
- Statistical tools was used for optimization of degradation conditions.
- Metabolites were identified and a degradation mechanism was proposed.
- At optimum conditions, up to 89% degradation of DY4 was achieved.
- Metabolites were found less toxic than the original dye.

GRAPHICAL ABSTRACT



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ABSTRACT

Citrus limon peroxidase mediated decolourization of Direct Yellow 4 (DY4) was investigated. The process variables (pH, temperature, incubation time, enzyme dose, H_2O_2 amount, dye concentration, co-metal ions and surfactants) were optimized for maximum degradation of dye. Maximum dye decolourization of 89.47% was achieved at pH 5.0, temperature 50 °C, enzyme dose 24 U/mL, H_2O_2 concentration 0.25 mM and DY4 concentration 18.75 mg/L and incubation time 10 min. The co-metal ions and surfactants did not affect the dye decolourization significantly. Response surface analysis revealed that predicted values were in agreement with experimentally determined responses. The degradation products were identified by UPLC/MS analysis and degradation pathway was proposed. Besides, phytotoxicity assay revealed a considerable detoxification in response of biodegradation of DY4 dye. *C. limon* showed promising efficiency for DY4 degradation and could possibly be used for the remediation of textile effluents.

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1. Introduction

Dyes are widely used in cosmetics, textile, leather, food, paper and plastic industries and in view of toxicity of dyes; the environment is under the threat. Dyes contamination alter the water colour, enhance eutrophication, deplete oxygen and ultimately, aquatic life is disturbed to great extent (Ullah et al., 2013; Iqbal et al., 2015; Bilal et al., 2016a, 2016b; Iqbal, 2016; Mushtaq et al., 2016; Nadeem et al., 2016; Rashid et al., 2016; Saeed et al., 2016a; Tahir et al., 2016). Azo dyes contain (−N═N−) functional group, which make them persistent under natural environmental conditions. Recent studies also profiled the toxicity (carcinogenic, cytotoxic, mutagenic etc) of azo dyes (Steter et al., 2014). Azo dyes are used commonly in textile (60–70% of total dyes consumption in textile) and a considerable amount is lost in the environment due to low dye exhaustion (Bilal et al., 2016b; Iqbal, 2016). Since synthetic dye are stable, exits in the environment for longer time and in response of partial dyes degradation under natural conditions, more toxic by-products are produced (Bhatti et al., 2013; Adeel et al., 2015; Gulzar et al., 2015; Qureshi et al., 2015; Bouatay et al., 2016; Muneer et al., 2016; Saeed et al., 2016a, 2016b). Therefore, dye remediation is must for safe and healthy environment. To date, various methods (adsorption, coagulation, filtration, ozonation, photo-Fenton process, UV/NaOCl, electrochemical oxidation, ultrasonic irradiation and UV/H_2O_2 etc.) are in practice for dye remediation. However, these methods are either inefficient to degrade dye completely or cause secondary pollution issues (Manzoor et al., 2013; Iqbal and Bhatti, 2015; Iqbal and Khera, 2015; Iqbal and Nisar, 2015; Jamal et al., 2015; Qureshi et al., 2015; Sayed, 2015; Ukpaka et al., 2015; Babarinde et al., 2016; Babarinde and Onyiaocha, 2016; Iqbal et al., 2016a; Jafarinejad, 2016; Majolagbe et al., 2016; Peter and Chinedu, 2016; Shindy, 2016).

Biodegradation received considerable attention since advanced biological methods have been developed for remediation, which are also regarded as eco-friendly, low cost and more efficient. In bio-process, different enzymes are utilized for dyes remediation and among enzymes, peroxidases (POD) are reported to be efficient for the degradation of various organic pollutants (Bilal and Asgher, 2015b, a; Iqbal and Khera, 2015; Bilal et al., 2016b; Ukpaka, 2016b; Ukpaka, 2016d, c, a). In biodegradation process, POD (EC 1.11.1.7) degrades the target compounds and in response of multi-step reaction, harmless end products are produced as presented in reactions R1-R3 (Dunford and Stillman, 1976).

$$\text{heme[Fe(III)]}_{(\text{Peroxidase})} + H_2O_2 \rightarrow \text{heme[O═Fe (IV)- R}^+\text{]}_{(\text{Compound I})} + H_2O \quad \text{(R1)}$$

$$\text{heme[O═Fe(IV)- R}^+\text{]}_{(\text{Compound I})} + S \rightarrow \text{heme[(O═Fe (IV)]}_{(\text{Compound II})} + S \quad \text{(R2)}$$

$$\text{heme[O═Fe(IV)]}_{(\text{Compound II})} + S \rightarrow \text{heme[Fe (III)]}_{(\text{Peroxidase})} + S \quad \text{(R3)}$$

POD have been employed for dyes degradation i.e., hoseradish POD (Onder et al., 2011), soybean POD (Ali et al., 2013) and *Cucubita pepo* (Boucherit et al., 2013). Therefore, in view of POD efficiency, researchers have explored various POD sources and their efficiency for organic pollutant degradation and nevertheless, POD estimation and efficiency is explored from citrus species native to Pakistan. Pakistan is among 10 largest citrus producing countries in the world and as a results of citrus processing, huge amount of peel is produced, which is discarded as waste in spite of presence of various bioactive compounds (Kamal et al., 2013) and source of POD (Mohamed et al., 2008; Nouren et al., 2013). Therefore, to explore the citrus POD efficiency for remediation of dye will be of great importance since a huge amount of citrus peel is available, free of cost in Pakistan. Moreover, biodegradation is eco-friendly for the remediation of pollutants versus conventional treatment methodologies (Saroj et al., 2014; Bautista et al., 2015; Liu et al., 2016; Martínková et al., 2016; Palli et al., 2016; Ventura-Camargo et al., 2016).

Present study was conducted to apprise the *C. limon* (POD) mediated decolourization of DY4 dye. The biodegradation experiments were run under central composite design (CCD) and process variables were optimized through response surface analysis. Degradation by-products were identified using advance techniques and DY4 dye degradation mechanism was proposed. Moreover, *Zea mays* phytotoxicity assay was performed of treated and un-treated dye solution in order to evaluate the bio-efficiency of treatment method.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals and reagents used were of analytical grade i.e., dipotassium phosphate (99%), potassium dihydrogen phosphate (99%), ammonium sulfate (99%), sodium carbonate (99%), hydrogen peroxide (30%) were purchased from Sigma-Aldrich Chemical Co. USA. Direct Yellow 4, Congo red, Crystal violet, Fuchsin acid, Fuchsin basic, Malachite green, Methyl green, Methylene blue and Rodamine B were obtained from local textile industries, Faisalabad, Pakistan. Ultra-pure water (18.2 Ω cm) from Milli-Q system (Millipore) was used throughout the study for solution preparation.

2.2. Isolation and determination of peroxidase from lemon peel

C. limon peel (10 g) homogenized with 100 mL of 100 mM phosphate buffer (pH 7.0) in blender and mixture was passed through Whatman filter paper (grade 42). The filtrate was centrifuged at 10,000 rpm for 15 min, supernatant was pooled and the residue was re-extracted. The solution was subjected to salt fractionation by adding 80% (w/v) ammonium sulfate. The solution was placed overnight at 4 °C and precipitates were separated by centrifugation. Then, precipitates were dissolved in 100 mM sodium acetate buffer (pH 7.0) and dialyzed against 25 mM of same buffer (Bhatti et al., 2006). POD activity was determined spectrophotometerically (CE Cecil 7200, UK) following the formation of tetraguaiacol (λ_{max} = 470 nm, ε = 26.6 mM^{-1} cm^{-1}) (Liu et al., 1999).

2.3. Screening of synthetic dyes

Nine synthetic dyes i.e., Direct Yellow 4 (λ_{max} 402), Congo red (λ_{max} 499), Crystal violet (λ_{max} = 590), Fuchsin acid (λ_{max} = 543), Fuchsin basic (λ_{max} = 542), Malachite green (λ_{max} = 617.5), Methyl green (λ_{max} = 631.5), Methylene blue (λ_{max} 664.5) and Rodamine B (λ_{max} = 554) were screened in initial trials. Dye solution (12.5 mg/L)