

Short Communication

Redox Mediators Assisted-degradation of Direct Yellow 4

Shazia Nouren¹, Haq Nawaz Bhatti², Munawar Iqbal^{3*}, Ismat Bibi⁴, Nosheen Nazar⁴, Dure Najaf Iqbal³, Qudsia Kanwal³, Abida Kausar⁵, Fida Hussain⁶

¹Department of Chemistry, Govt. College Women University, Sialkot, Pakistan

²Department of Chemistry, University of Agriculture, Faisalabad, Pakistan

³Department of Chemistry, University of Lahore, Lahore, Pakistan

⁴Department of Chemistry, Islamia University Bahawalpur, Pakistan

⁵Department of Chemistry, Government College Women University, Faisalabad, Pakistan

⁶Department of Botany, Qurtuba University of Science and Information, Technology & Islamia College, Peshawar, Pakistan

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Abstract

Direct yellow 4 degradation was investigated in the presence of various mediators (*p*-coumaric acid, 1, hydroxybenzotriazole (HOBT), syringaldehyde, vanillin, syringic acid, veratryl alcohol, and pyrocatechol) at pre-optimized conditions of process variables, i.e., pH 5.0, temperature 50°C, enzyme dose 24 U/mL, and 0.25 mM H₂O₂ concentration. *Citrus limon* peroxidase (CL-POD) was used for degradation of DY4 dye. In the absence of mediators, the DY4 degradation was 60%, whereas mediators enhanced the POD biodegradation efficiency up to 87%. Among mediators investigated, syringaldehyde showed promising efficiency. We investigated syringaldehyde concentrations in the range of 0.0125-0.5 mM, and 0.025 mM was optimum for maximum dye degradation, which revealed that mediators could be used to enhance the biodegradation of dyes.

Keywords: *Citrus limon* peroxidase, decolorization, direct yellow 4, redox mediators

Introduction

Dyes are widely used in the food, cosmetics, leather, textile, paper, and plastic industries, and subsequently discharged into the environment. Other than toxicity of dyes [1-3], they also alter water colour, cause eutrophication, and deplete oxygen, which ultimately

disturbs aquatic life [1, 4-6]. So far, dye remediation is compulsory for avoiding environmental pollution issues [7-10]. Among wastewater treatment methods, biodegradation has received considerable attention due to its eco-friendly, economical, and efficient nature. In the biodegradation process, different enzymes are utilized, and among enzymes, POD are reported to be efficient for the degradation of diverse types of pollutants [6, 11-12], i.e., POD from horseradish [13], soybean [14], and *Cucubita pepo* [15] are reported to be highly efficient for the degradation of dyes. Pakistan is a citrus-producing

*e-mail: bosalvee@yahoo.com

country and the huge amount of waste peels that are generated is an efficient POD source [16-17].

The present study was conducted to apprise the CL-POD-mediated degradation of DY4 dye. The effect of various mediators was studied in detail on DY4 degradation using optimum conditions of pH, temperature, incubation time, enzyme dose, H₂O₂ amount, and dye concentration.

Materials and Methods

Chemicals and Reagents

The chemicals and reagents used were of analytical grade, i.e., dipotassium phosphate (99%), potassium dihydrogen phosphate (99%), ammonium sulphate (99%), sodium carbonate (99%), and hydrogen peroxide (30%) – all of which were purchased from Sigma-Aldrich Chemical Co. USA. DY4 was obtained from local textile industries in Faisalabad, Pakistan. Ultra-pure water (18.2 Ω.cm) from Milli-Q system (Millipore) was used throughout the study for solution preparation.

Decolourization of DY4

To study the effect of mediators (*p*-coumaric acid, 1, hydroxybenzotriazole (HOBt), syringaldehyde, vanillin, syringic acid, veratryl alcohol, and pyrocatechol) on dye degradation, pH 5.0, temperature 50°C, enzyme dose 24 U/mL, H₂O₂ concentration 0.25 mM, and DY4 concentration 18.75 mg/L was kept constant. The DY4 solution was mixed with respective amounts of mediators and placed in a shaker (Model PA 9/250 U). After a stipulated time period, samples were drawn and kept in water bath for 10 min to stop the reaction, which was then centrifuged (10,000 rpm for 5 min) and absorbance was recorded along with control (untreated dye sample) at λ_{max} 402. The percentage dye degradation was calculated using the relationship shown in Eq. 1:

$$\text{Decolorization (\%)} = \left[\frac{A_i - A_f}{A_i} \right] \times 100 \quad (1)$$

...where A_i and A_f are the absorbance values of un-treated and treated dyes.

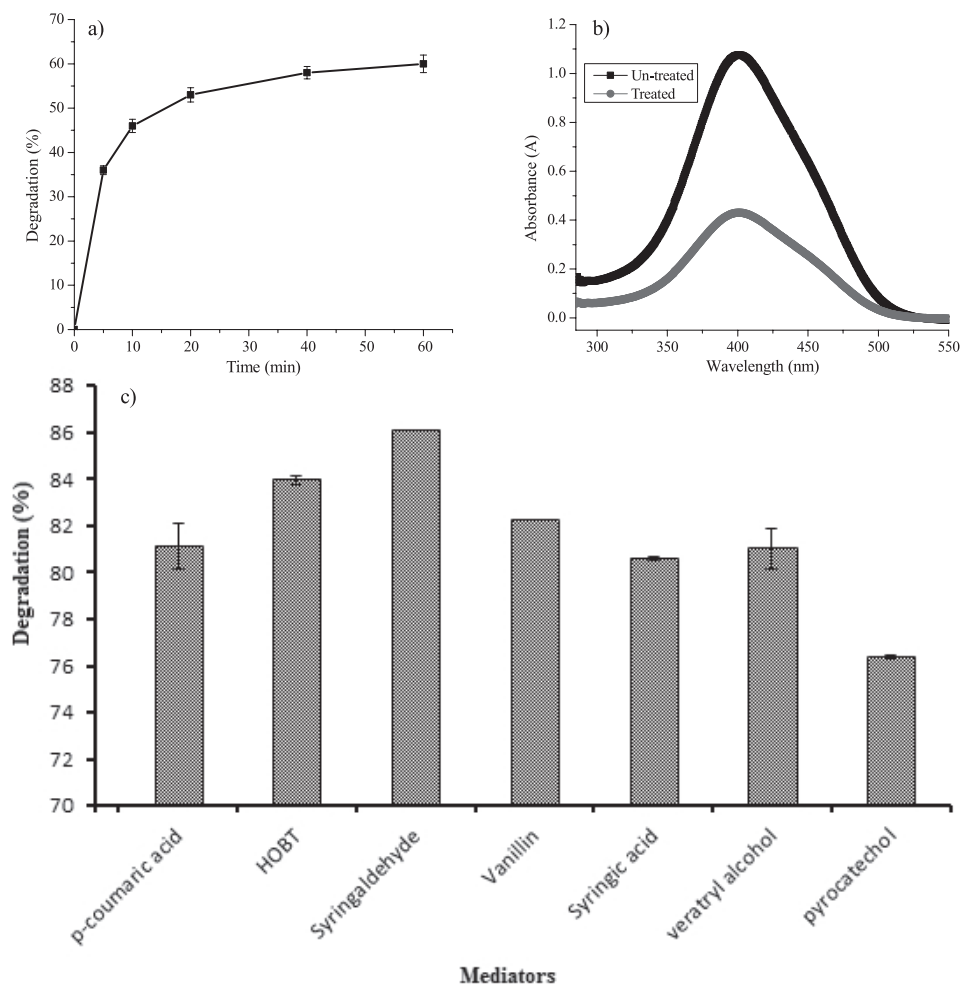


Fig. 1. a) Bio-degradation of direct yellow 4 (DY4) dye without mediator (pH 5.0, 50°C, enzyme dose 24 U/mL, H₂O₂ concentration 0.25 mM, incubation time 30 min, and DY4 concentration 18.75 mg/L), b) UV-Vis spectrum of DY4 dye in the absence of mediator, and c) effect of various redox mediators on *Citrus limon* peroxidase (CL-POD) efficiency on DY4 degradation.

Results and Discussion

CL-POD-mediated decolourization of DY4 in the presence of various mediators was investigated. The effect of mediators was studied by degrading DY4 using pre-optimized conditions of process variables, i.e., pH 5.0, 50°C, enzyme dose 24 U/mL, H₂O₂ concentration 0.25 mM, and DY4 concentration 18.75 mg/L. Without a mediator, DY4 degradation was 60% (Figs 1a-b). However, mediators (i.e., *p*-coumaric acid, 1, HOBT, syringaldehyde, vanillin, syringic acid, veratryl alcohol and pyrocatechol) enhanced DY4 degradation significantly (Fig. 1c). *p*-coumaric acid, HOBT, syringaldehyde, vanillin, syringic acid, veratryl alcohol, and pyrocatechol showed the dye degradation up to 81.1%, 84.2%, 86.3%, 82.05%, 80.3%, 81.5%, and 77.3%, respectively. Degradation response of dye revealed that *p*-coumaric acid, HOBT, syringaldehyde, vanillin, syringic acid, veratryl alcohol, and pyrocatechol enhanced the DY4 degradation in the range of 17 to 25% versus control. It is well known that under typical conditions, the enzymes interact with substrate and the catalytic site of the enzyme abstracts electrons from the substrate and transformed it into a byproduct. The mediators oxidized by the enzyme oxidize the dye molecule, which results in the substrate being converted into oxidized product. Therefore,

mediators may offer more possibilities, i.e., either indirectly oxidizing the dye molecule/enabling the enzyme to attack substrate, thereby leading to enhanced oxidation of the target compound [18-19]. Among mediators under investigation, syringaldehyde efficiency was promising in enhancing dye degradation. In subsequent experiments, various concentrations of syringaldehyde were studied and results thus obtained are shown in Fig. 2a). The different concentrations of syringaldehyde showed a variable effect on dye degradation, which indicates that syringaldehyde is very effective in enhancing dye bio-degradation. The concentration of 0.025 mM showed the highest dye degradation ability. The variable effect of mediators on degradation can be correlated with different natural mediators and their electron-donating ability. Degradation is governed by redox potential of the mediator and oxidation mechanism of the substrate. So far, different mediators showed variable behaviour for dye degradation. Figs 2(b-c) depict the effect of time of incubation on degradation of DY4 in the presence of 0.025 mM syringaldehyde (0.025 mM), which showed degradation of 89% within a very short time interval. These findings are in line with maximum dye degradation being achieved using suitable mediators [20].

Previous studies also revealed that redox mediators enhanced the dye degradation efficiently [21-22].

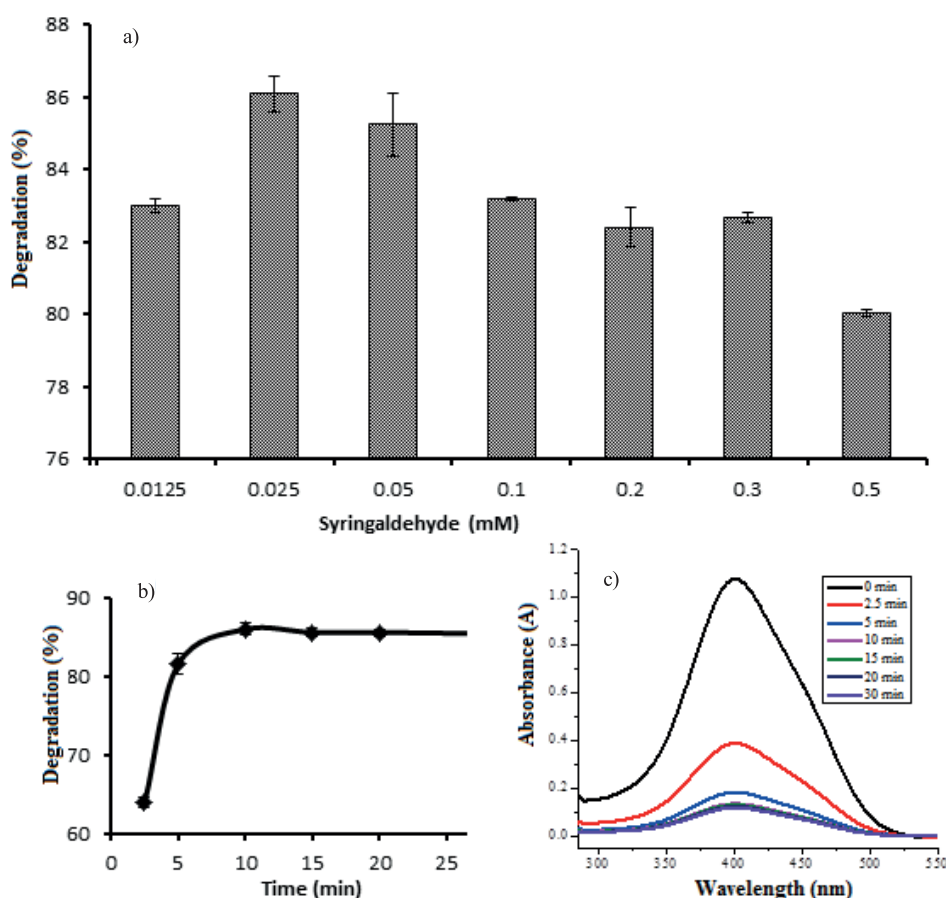


Fig. 2. a) Effect of different concentrations of syringaldehyde (mediator) on DY4 bio-degradation by CL-POD, b) effect of incubation time on degradation of DY4 in the presence of syringaldehyde (0.025 mM), and c) UV-Vis spectrum of DY4 in the presence of syringaldehyde.

Hydroxybenzotriazole proved to be an efficient mediator for plant-based POD-mediated biodegradation. Decolorization of effluents by *T. foenum-graecum* seeds [23] and *M. charantia* POD [24] and mixtures of acidic dyes treated by turnip POD also showed promising efficiency [25]. In the present investigation, syringaldehyde (0.0125-0.5 mM) was studied and maximum degradation of DY4 was achieved at 0.025 mM concentration level, and beyond this concentration the degradation rate decreased. The same trend was observed previously for violuric acid as a redox mediator for turnip POD effect on degradation of dispersed dyes. The dye degradation enhancing effects of mediators have been correlated with dual role mediators. However, lower mediator concentration was found to be efficient and at higher concentration low efficiency was reported, which was due to the competitive inhibition of active sites, thus inhibiting enzyme activity at higher concentrations [26]. Therefore, optimizing the mediator is important for effective biodegradation of dyes. So far, based on results, the mediators proved to be efficient in enhancing POD dye degradation capability. Biodegradation is an eco-friendly method [27-42] that could be used to enhance bio-degradation of dye and textile wastewater to avoid environmental pollution [43-68].

Conclusions

DY4 degradation was investigated in the presence of various redox mediators at pre-optimized conditions of pH, temperature, enzyme dose, and H₂O₂ concentration. In the presence of redox mediators, up to 89% degradation was achieved (including 60% without mediators). *p*-coumaric acid, HOBT, syringaldehyde, vanillin, syringic acid, veratryl alcohol, and pyrocatechol efficiency were lower than for syringaldehyde and significantly higher than control. The low concentration of mediators was more effective as compared to higher concentrations. In view of the promising efficiency of mediators, these could possibly be used to enhance the bio-degradation efficiency of enzymes.

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