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Kinetic and thermodynamic study of acid red 131 dye decolourization by *Aspergillus niger*

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ABSTRACT

The aim of this work was to determine the optimal conditions and to obtain thermodynamic data for the decolorization of Acid Red 131 using an enzymatic system. The effect of physic-chemical parameters on decolorization was studied. The enzyme (laccase) activity was found to be 2.277 U/ µg. The decolorization of Acid red 131 by *Aspergillus niger* were obtained at three different temperatures. The reaction followed first order kinetics. For enzyme catalyzed bio-degradation, reaction were found to be less enthalpy-driven (high positive ΔH values), with negative entropy (ΔS) indicating that in the transition state, more complex ternary dye structures were formed. The negative values of ΔG explain the fast reaction rates at the transition state during biodegradation of the Acid Red dye, which is exoergonic by nature.

Key words: Laccase, Decolorization, Physico-chemical parameters, Thermodynamic data.

INTRODUCTION

The two major reasons for release of industrial dyes into the environment are through effluents from synthesis plants and from dye-using industries. Due to the complex structure and synthetic nature, dye degradation is a laborious process (Sosath, 1999). These dyes have stability towards light, temperature, microbial attack and the color affects the photosynthetic activity (Couto et al., 2009). Certain pollutants persist in the environment for long durations are considered to be recalcitrant pollutants (Ambatkar mugdha & Mukundan usha, 2012). Because of stricter government legislation concerning the release of effluents into the environment, an increase in research towards the field of industrial waste water treatment has raised (Couto et al., 2013). To treat dye containing wastewater several physico-chemical methods includes adsorption, coagulation/flocculation, membrane filtration, precipitation and oxidation have been used but these are expensive and produce large amount of sludge which requires safe disposal (Franciscon et al., 2009). There is an enormous need to develop an economic and cost effective way of dealing with dyeing waste in face of ever increasing production activities. Biological processes have received a growing interest as a viable alternative owing to their cost effectiveness ability to produce less sludge and environmental friendliness (Banat et al., 1996; Dubrow et al., 1996; Park et al., 2003). A wide variety of microorganisms including *Pseudomonas luteola, Aeromonas hydrophila, Aspergillus niger, Trametes versicolor, Aspergillus terricola, P.chrysosporium, Saccharomyces cerevisiae, Candida tropicalis, C. lipolytica, Spirogyra species* (Ravikumar et al., 2012) have been reported and are capable of biological degradation of synthetic dyes. Enzymatic systems have been developed for the decolorization and mineralization of azo dyes under certain environmental conditions (Pandey et al., 2007). Extracellular lignin-modifying enzymes (LMEs) of certain white-rot fungi are responsible for the treatment of dye containing waste water (Pointing, 2001) and detoxify (Kumar et al., 2007) xenobiotic compounds under aerobic conditions (Mohorcic et al., 2006). Laccase is a part of larger group of enzymes termed the multicopper oxidases (MOC), (Komori et al., 2009) belonging to the group of blue-copper proteins (Minussi et al., 2007). Fungal laccases have been confirmed for their capability to degrade several azo dyes (Tauber et al., 2005; Husain, 2006). Many variables/factors that affect the enzyme production and decolorization are expressed by different taxa and culture conditions. The ability of microbes to carry out dye decolorization has received much attention, either individually or in consortia (Verma & madamwar, 2003; Moosvi et al., 2007).

The aim of the present study was to investigate the kinetics and thermodynamics of effective Acid Red 131 dye decolorization by enzyme laccase achieved from *Aspergillus niger*.

MATERIALS AND METHODS

Chemicals: All the chemicals were purchased from HI- Media. Dye (Acid red 131) used was gifted by KH SHOES, Vellore.

Organism and culture condition: The microorganism used in this study was *Aspergillus niger*, was gifted by Marina labs, Chennai and was maintained in PDA (Potato Dextrose Agar) slants at 4^0C. The pure culture was grown on 250mL Erlenmeyer flask containing 100mL of Nutrient broth (pH 7.4±0.2). The fungi were grown aerobically for 24hrs at room temperature under shaking condition [5]. The 24hr culture was kept at 4^0C and used as a seed inoculum.

Enzyme and protein assay: Laccase enzyme activity was done using 1mM guaiacol as a substrate in 0.1 M sodium acetate buffer (pH 6.5) with a total volume of 5mL as described in Mansur et al., 2003. 1 U of enzyme activity was defined as the amount of enzyme that elicited an increase in A465 (ε = 26,600 M^{-1} cm^{-1}) of 0.1 absorbance unit per minute. Enzyme activity (equation 01) was calculated by using the formula,

$$\text{Enzyme activity}\left({}^{\text{U}}/_{\text{ml}}\right) = \frac{[\text{A}_\text{t} - \text{A}_0] \times \text{Dilution factor} \times \text{Total volume} \times 1000}{\text{M. E. C} \times \text{Enzyme volume} \times \text{Time}} \qquad (01)$$

Where

A_0 is the initial absorbance immediately after the enzyme addition

A_t is the final absorbance of reaction mixture after incubation

M.E.C is the molar extension coefficient of guaiacol.

Protein assay was performed using standard Folin-Ciocalteau method (1951) using bovine serum albumin as a standard protein.

Dye decolorization: *A.niger* from preserved culture was grown for 24 hrs under shaking conditions at room temperature in 250 mL Erlenmeyer flask containing 100 mL nutrient broth (pH 7.4±0.2). The reaction mixture for all the decolorization studies consists of 100mg/L (w/v) dye in 100ml of 24 hr old culture. 5mL of sample was aliquoted at every 60 minutes and centrifuged at 1000g for 10 min (Surwase et al., 2012). Decolorization was studied by monitoring the decrease in the absorbance at 550nm from the clear supernatant. The percentage of decolorization was calculated using the following equation (Equation 02).

$$\text{A} = \frac{(\text{Initial absorbance}) - (\text{Final absorbance})}{\text{Initial absorbance}} \times 100 \qquad (02)$$

Effect of Physicochemical parameter on dye decolorization: The effect of pH on dye decolorization was studied at different pH range (pH 5, 6, 7, 8 & 9). The effect of temperature was studied at optimum pH at a temperature range (30, 40 & 50^0C). The effect of dye concentration towards decolorization of dyes was determined by incubating the reaction mixture at dye concentrations (25, 50, 75, 100mg/L) at optimum pH and temperature. Kinetics and thermodynamic parameters such as Entropy (ΔS), Enthalpy (ΔH) and Gibbs free energy (ΔG) of dye decolorization was calculated using Eyring-Polanyi plot.

RESULTS AND DISCUSSION

Enzyme activity: The protein concentration found using Folin-Ciocalteau method (Lowry et al., 1951) was 990.268(µg/mL). The specific activity of crude laccase enzyme was found using guaiacol as a standard substrate to yield 2.277(U/ µg).

Effect of Physicochemical parameter on dye decolorization: The decrease in the spectral absorbance during decolorization of Acid Red 131 was recorded using UV-Visible Spectrophotometer (Elico, India). The optimum pH at which maximum decolorization occurred was found to be pH 7. The optimum temperature towards decolorization of Acid Red 131 reported maximum decolorization at 30^0C. *Proteus mirabilis* showed maximum decolorization at optimum pH and temperature between 6.5-7.5 and 30-35^0C. This shows that decolorization occurred at neutral pH and 35^0C (Govindwar et al., 2010). Dye concentration (mg/L) towards the decolorization of Acid Red 131 exhibited maximum decolorization at 75mg/L after 60 minutes incubation. The rate of decolorization was constant at different concentration, but with higher loads of dye demand more time to be degraded. Ren et al., 2006 reported that decreased decolorization ability of *Aeromonas hydrophila t*o Crystal Violet was observed, when its concentration was increased.

Thermodynamic studies on dye decolorization: The order of the reactions was determined by fitting the best straight line obtained to rate equations for zero-order, first-order and second-order reactions. Acid Red 131 decolorization/degradation followed first-order kinetics. The graphs showed that the kinetics for the degradation reactions followed straight lines with the best values of R (correlation coefficient) for first-order kinetics (Table 1). It was observed that the dye kinetic constant (k) values decreased as the temperature increases from 30-50^0C. The decreases in the rate of degradation could be because of structural changes in the active site of the dyes that prevent the formation of the enzyme–dye complexes, or because of the absence of some enzyme mediator acting together with the laccase. By applying the Eyring–Polanyi equation (03) to the Acid Red 131 data, the thermodynamic data and kinetic constants at three temperatures were obtained.

$$Ln\frac{k}{T} = \frac{-\Delta H}{R} \times \frac{1}{T} + \ln\frac{k_E}{h} + \frac{\Delta S}{R} \qquad (03)$$

The straight line obtained for dye degradation followed the equation: y = 1616x-5.991 (0.759) the thermodynamic data were obtained by using the Eyring- Polanyi equation, where ΔH is the enthalpy of activation, ΔS is the entropy of activation, R is the gas constant, and h is Planck's constant. The Gibbs energy of activation (ΔG) was calculated by use of the equation ΔG = ΔH – TΔS.

The ΔH and ΔS values were found to be 13435.424 and -247.350 J mol^{-1} K^{-1}, respectively (Table 2). The positive value of enthalpy explains the increasing rate of reaction with increasing temperature and is endothermic decolorization process. The negative entropy indicates that the more ordered complex formed than the reactants. The high positive values of ΔG explain the low reaction rates during biodegradation of the reactive dyes (Afonso et al., 2012). Results indicate that the reaction is exoergonic by nature, where ΔG of the reaction is negative, and supports fast reactivity at the transition state.

Table.1.Equations for the linear best fit and rate constants (k) obtained using the kinetic equation for first-order kinetics at 30, 40, 50 and 60^{0}C for the biodegradation of Acid Red 131

Temperature(^{0}C)	Linear Equation	R	kI × 10^{-2} h^{-1}
30	-1.845x + 2.845	1	184.5
40	-1.088x + 2.088	1	108.8
50	-1.118x + 2.118	1	111.8

Table.2.Thermodynamic data ΔH, ΔS, and ΔG for the bio- degradation of dye

Thermodynamic data	Acid Red 131
ΔH(J/mol)	13435.424
ΔS(J/mol)	-247.350
ΔG (kJ/mol)	-61.116

CONCLUSION

Crude laccase enzyme from *Aspergillus niger* was used as a biocatalyst (enzyme) for the decolorization of the Acid Red 131. The effect of various process parameters such as temperature, Incubation time and dye concentration towards the decolorization of Acid Red 131 was studied. The thermodynamic studies on decolorization follows first order kinetics and the reaction is exoergonic. RSM was successfully applied to determine the optimal operational conditions for maximum decolorization**.** This suggest that laccase from *A.niger* has proved to be an efficient and eco-friendly for the decolorization of synthetic dyes.

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REFERENCES

Ambatkar mugdha and Mukundan usha, Enzymatic treatment of wastewater containing dyestuffs using different delivery systems. Sci. Revs. Chem. Commun, 2(1), 2012, 31-40.

Banat IM, Nigam P, Singh D, Marchant R, Microbial decolorization of textile dye containting effluents: A review. Bioresour Technol., 58, 1996, 217-27.

Couto SR, Dye removal by immobilised fungi. Biotech Adv., 27, 2009, 227-235.

Dalel Daassi, Tahar Mechichi, Moncef Nasri, Rodriguez-couto, Decolorization of the metal textile dye Lanaset Grey G by immobilized white-rot fungi. Journal of Environmental Management, 129, 2013, 324-332.

Dawkar VV, Jadhav UU, Tamboli DP, Govindwar SP, Efficient industrial dye decolorization by *Bacillus sp*. VUS with its enzyme system. Ecotoxicol Environ Saf, 73, 2010, 1696–1703.

Dubrow SF, Boardman GD, Michelsen D L, Chemical pretreatment & Aerobic – Anaerobic degradation of textile dye waste water. John wiley & son's publishers, Newyork, 1996.

Franciscon E, Zille A, Garboggini FF, Silva IS, Paulo AC, Durrant LR, Microaerophilic-aerobic sequential decolourization/biodegradation of textile azo dyes by a facultative *Klebsiella sp*. strain VN-31. Process Biochem, 44, 2009, 446-52.

Gaanappriya Mohan, Logambal K, Ravikumar R, Investigation on the removal of direct red dye using *Aspergillus niger* and *Aspergillus flavus* under static and shaking conditions with modeling. International Journal of Science, Environment and Technology, 1(3), 2012, 144 – 153.

Husain Q, Potential applications of the oxidoreductive enzymes in the decolorization and detoxification of textile and other synthetic dyes from polluted water: A review. Crit. Rev. Biotechnol., 26, 2006, 201- 221.

Komori H, Miyazaki K, Higuchi Y, X-ray structure of a two- domain type laccase: a missing link in the evolution of multi-copper proteins. FEBS Lett, 583, 2009, 1189-1195.

Kumar K, Devi SS, Krishnamurthi K, Dutta D, Chakrabarti T, Decolorization and detoxification of Direct Blue-15 by a bacterial consortium. Bioresour. Technol., 98, 2007, 3168-3171.

Lowry OH, Rosbrough NJ, Farr AL, Randall RJ, Protein measurement with the folin phenol reagent. J. Biol. Chem., 193, 1951, 265.

Mansur M, Arias ME, Copa-Patiño JL, Flardh M, González AE, The white-rot fungus *Pleurotus ostreatus* secretes laccase isozymes with different substrate specificities. Mycologia, 95(6), 2003, 1013–1020.

Minussi RC, Pastore GM, Duran N, Laccase induction in fungi and laccase/N–OH mediator systems applied in paper mill effluent. Bioresour. Technol., 98, 2007, 158-164.

Mohorcic M, Teodorovic S, Golob V, Fiedrich J, Fungal and enzymatic decolorization of artificial textile dye baths. Chemosphere, 63, 2006, 1709-1717.

Moosvi S, Kher X, Madamwar D, Isolation characterization and decolorization of textile dyes by a mixed bacterial consortium JW-2. Dye. Pigmen., 74, 2007, 723-729.

Pandey A, Singh P, Iyengar L, Bacterial decolorization and degradation of azo dyes. Int Biodeter Biodegrad, 59, 2007, 73-84.

Park C, Lee Y, Kim T, Lee M, Lee B, Lee J, Kim S, Enzymatic decolorization of various dyes by *Trametes versicolor* KCTC 16781. Korean J Biotechnol.Bioeng., 18, 2003, 398-403.

Pointing SB, Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol, 57, 2001, 20–33.

Ren S, Guo J, Zeng G, Sun G, Decolorization of triphenylmethane, azo, and anthraquinone dyes by a newly isolated *Aeromonas hydrophila* strain. Applied Microbiology and Biotechnology, 72(6), 2006, 1316-1321.

Sosath F, Biologisch-chemische Behandlung von Abwässern der Tex- tilveredelung mit ReaKtivfarbstoffen, VDI Fortschrittsberichte, Reihe Umweltverfahrenstechnik. 1999, 209.

Swati V, Surwase, Krutika K, Deshpande, Swapnil S, Phugare, Jyoti P, Jadhav, Biotransformation studies of textile dye Remazol Orange 3R'. 3 Biotech, 2012, DOI 10.1007/s13205-012-0093-1.

Tauber M, Gubitz GM, Rehorek A, Degradation of azo dyes by laccase and ultrasound treatment. Appl. Environ. Microbiol., 71, 2005, 2600- 2607.

Verma P, Madamwar D, Decolorization of synthetic dyes by a newly isolated strain of *Serratia marcescens*. World J Microbiol Biotechnol, 19, 2003, 615-618.

Wagner J. Barreto, Suely M. O. Doi, Renata Afonso, Nathalia D. Bernardino, A kinetic study of the biodegradation of textile dye mixtures by *Pleurotus ostreatus* fungus. Monatsh Chem., 143, 2012, 1389–1395.