

Dye decolorization using alginate gel entrapped laccase from *Cercospora* sp.**Chand D., Thakur V., Kumari A., Lata J. and Kumar P.**

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INTRODUCTION

Laccases (E.C.1.10.3.2), p-diphenols dioxygen oxidoreductase are part of a group of enzymes termed the multicopper enzymes. The use of molecular O₂ as oxidant and fact that H₂O is only byproduct are very attractive catalytic features rendering laccases as an excellent 'Green Catalyst' (Riva 2006).

Current technologies such as coagulation, zonation and activated carbon can efficiently remove only restricted classes of dyes (Hassan and Hawkyard., 2002). So combination of physical, chemical and biological processes are most efficient for dye decolorization but can be expensive (Hai et al., 2007). Activated carbon is used to remove acidic dyes but not reactive dyes (Robinson et al., 2001). Electro coagulation has been used to remove dyes and pigments aggregates from textiles wastewater (Zidane et al., 2001).

Worldwide, waste water from textile industries are discharged in large quantities into natural water bodies on a daily basis since are visible at low concentration of 0.005 ppm (mg/ml) (O' Neill at al., 1999). It is estimated that approximately 50,000 different types of dyes are lost to the environment worldwide and 30 % of reactive dye stuffs are lost and discharged with the effluents (Lewis 1999). Several dyes, particularly azo dyes are mutagenic as parent molecule or when they are metabolized (Moaward et al., 2003).

Here, we describe, a bench scale optimization of process parameters for decolorization of methyl orange dye using alginate gel entrapped laccase from *Cercospora* sp.

MATERIALS AND METHODS

Microorganism, culture conditions and preparation of alginate beads The fungal isolate *Cercospora* sp. has been procured from the culture collection of the Department of Biotechnology, H.P. University, Shimla-5, India. Laccase producing *Cercospora* sp. was prepared by point inoculation of culture from the master plates on Potato dextrose agar (PDA) plates containing 20% infusion from potato, 2% Dextrose and 1.5% agar (pH 6.2) at 30 °C, for 96 h. These 96 h precultures were used in production medium for laccase enzyme.

1mM of veratryl alcohol was added as inducer before inoculation. Fungal preculture disc (6mm) from growing edge of mycelium was taken and eight discs were transferred in 100ml of production medium containing 1.25% soya meal, 1.8% fructose, 1% yeast extract, 3µm CuSO₄.5H₂O, 10µm CaCl₂.2H₂O, 10µm

MgCl₂.2H₂O (pH 6.0) and was kept at 30 °C at 150 rpm for 108 hrs. After incubation medium was filtered with Whatman No. 1 filter paper. The laccase was found to be extracellular in nature and hence the filtrate was used for the enzyme assay.

Assay of laccase activity Laccase activity was determined by oxidation of ABTS (2,2'- Azino-bis (3-ethylbenzenethiazoline-6-sulphonic acid)) as described by Nagai et al., (2003). One unit of laccase activity is defined as the amount of enzymes that catalyzed oxidation of 1 µmol of substrate in one minute under standard assay condition i.e. µmol/min.

Immobilization of laccase on calcium alginate beads 3% sodium alginate and 0.2 M calcium chloride was prepared. 5 ml of crude enzyme was mixed with 5 ml of sodium alginate and this mixture was added drop wise using syringe in calcium chloride solution in stirring conditions. Beads was left for 30 minutes, washed with distilled water and used for further experiments.

Optimization of reaction parameters for dye decolorization using alginate bead entrapped laccase

The decolorization of methyl orange dye was carried out using alginate gel entrapped laccase from *Cercospora* sp. in four different buffers (Citrate phosphate, Sodium acetate, Sodium citrate, and sodium phosphate) at different pH values ranging from 2.5 to 8 at temperature range from 25 °C to 55 °C with varied concentration of dye (20 mM to 200 mM) as well as enzyme dose of 3.09 to 18.09 IU.

Incubation time ranging from 10 min to 360 min and effect of different mediators such as ABTS, HOBt, PABA and Hydroquinone at varied concentration from 20 mM to 200 mM was used to estimate optimum time and mediator concentration for dye decolorization.

Decolorization of dye using alginate bead entrapped laccase by batch and fed batch mode

In 1.5 liter compact Bio Flo Neo Brunswick Scientific Co. Inc. fermenter using a 500 ml reaction mixture at 25°C, dye decolorization was performed in batch and fed batch mode. The reaction was allowed to proceed for 30 min, repeatedly to show the reusability of entrapped laccase.

RESULTS**Optimization of reaction parameters for dye decolorization using alginate bead entrapped laccase**

The maximum decolorization of dye was recorded in

0.025 M citrate phosphate buffer at pH 2.5 and at temperature of 25°C. Alginate beads (20 Nos.) with 12.36 IU showed maximum decolorization after 30 min of incubation in pyrogallol as a mediator. These optimized conditions was used at 50 ml scale where 73.38% and 76.50% dye decolorization was recorded in batch and fed batch mode supply of dye respectively.

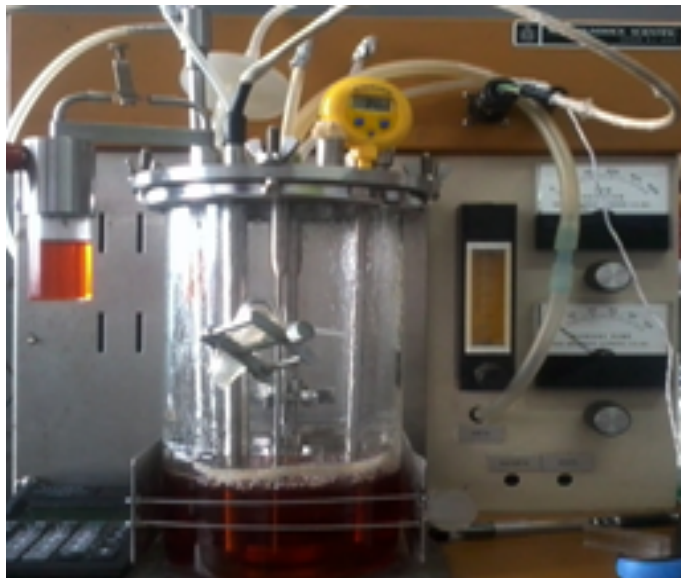


Fig. 1 Fermenter using alginate entrapped laccase for dye decolorization

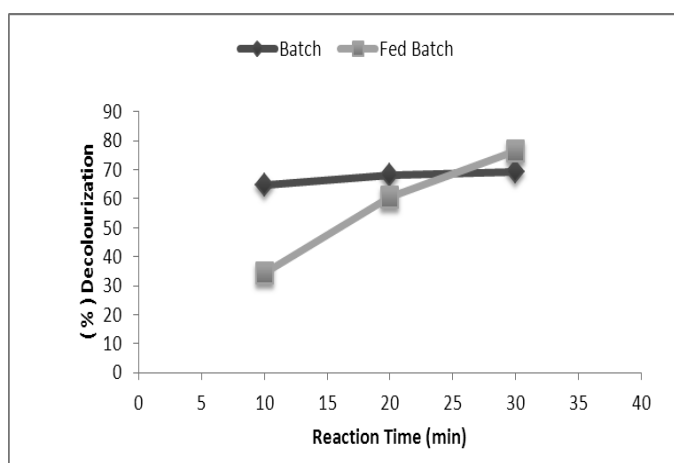


Fig. 2 Dye decolorization in batch and fed batch mode in fermenter

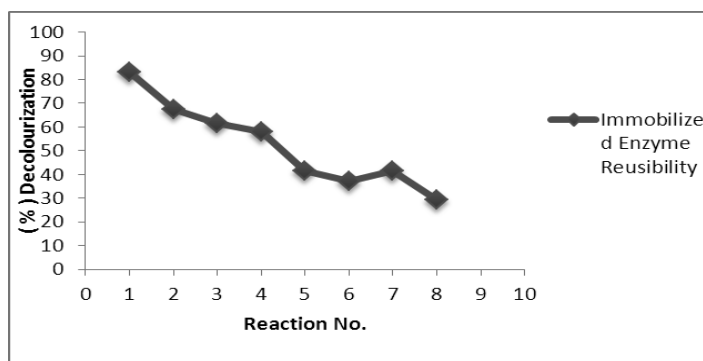


Fig. 3 Reusability of alginate entrapped laccase

Decolorization of dye using alginate bead entrapped laccase by batch and fed batch mode Dye

decolorization was performed in batch and fed batch mode in fermenter using a 500 ml reaction mixture at 25°C (Fig. 1). In batch mode a single feed of 25 ml of dye with enzyme dose of (1.36 IU x 6000 beads) IU in citrate phosphate buffer of 0.025 M at pH 2.5 in 30 min, whereas in fed batch mode, the dye were added in 3 feeding of 8.33 ml each with time interval of 10 min. Batch and fed batch showed 68% and 72% decolorization respectively (Fig. 1). The reaction was allowed to proceed for 30 min, repeatedly to show the reusability of entrapped laccase (Fig. 3).

CONCLUSIONS

Laccase was immobilized by entrapment method in calcium alginate. Moreover the advantage of using immobilized enzyme was its reusability and it found to retain above 50% dye decolorization efficiency upto 7th cycle.

REFERENCES

- Hai F, Yamamoto K, Fukushi K (2007). *Hybrid treatment system for dye wastewater*. Critical Reviews in Environm Sc & Technol 37 (4): 315-377.
- Hassan M.M., Hawkyard C.J. (2002). *Decolorization of aqueous dyes by sequential oxidation, treatment with ozone and fenton's reagent*. Journal of Chemical Technology & Biotechnology 77: 834-841.
- Lewis D (1999). *Coloration in the next century*. Review of Progress in Coloration and Related Topics 29(1):23-28.
- Moawad H, El-Rahim W, Khalafallah M (2003). *Evaluation of biotoxicity of textile dyes using two bioassays*. J. of Basic Microbiology 43 (3): 218-229.
- Nagai M, Kawata M, Watanabe H, Ogawa M, Saito K, takesawa T, Kanda K, Sato T (2003). *Important role of fungal intracellular Laccases for melanin synthesis: purification and characterization of an intracellular laccases from Lentinula edodes fruit bodies*. Microbiology 149: 2455-2462., 5: 219-225.
- O'Neill C, Hawkes F, Hawkes D, Lourenco N, Pinheiro H, Delee W (1999). *Color in textile effluents- sources, measurement, discharge consents and simulations: A review*. J. of Chem Technol & Biotechnol 74 (11).
- Riva S (2006). *Laccases: Blue enzyme for green chemistry*. Trends in Biotechnology.
- Robinson T, McMullan G, Marchant R and Nigam P (2001). *Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative*. Bioresource Technology 77 (3): 247-255.
- Robinson T, Nigam P.S. (2001). *Remediation of textile dye waste water using a white rot fungus Bjerkandera adusta through solid-state fermentation (ssf)*. Applied Biochemistry and Biotechnology 151 (23): 618-628.
- Zidane F, Drogui P, Lekhlif B, Bensaid J, Blais J.F., Belcadi S and Kacemi K.E. (2008). *Decolorization of dye containing effluent using mineral coagulant produced by electrocoagulation*. Journal of Hazardous Matter 161 (23): 974-981.