Chapter 10 - Applications: Phenolic Effluent Treatment
10.1. Introduction

Most of the hazardous pollutants are phenolic in nature and persists in the environment. The ability of laccases to oxidize phenolic compounds and reduce molecular oxygen to water has led to intensive studies of these enzymes. Therefore the fungal strains with high laccase activity and substrate affinity that can tolerate harsh environmental conditions have a potential for biotechnological applications.

Salt tolerant laccase secreting fungi can be utilized in treatment of saline and phenolic rich industrial effluents such as coir effluent and textile effluent that needed to be diluted several fold before microbial treatment.

In order to increase the potential use of laccase in industrial effluent treatment processes, their immobilizations are necessary for biochemical stability and reusability (Couto et al. 2004; Delanoy et al. 2005; Peralta-Zamora et al. 2003). If the immobilized laccase is to find commercial use it must retain its activity over several reuses.

10.1.1. Objectives of the study

The present study was focused to eliminate the phenolic contents from effluents utilizing *Trichoderma* sp NFCCI-2745 as well as its laccase in immobilized form.
10.2. Materials and Methods

10.2.1. Chemicals, Culture Conditions and Enzyme Immobilization

As described earlier in section 3.2 and section 9.2

10.2.2. Industrial Effluents

Coconut husk retting liquor was generated by soaking the coconut husk in a tank with estuarine water for 3 months. Textile effluent was collected from Textile Yarn dyeing industry, Panunda, Thalassery, Kerala, India and wood processing effluent was collected from Rubco Wood Industries, Chirrakara, Thalassery, Kerala, India.

10.2.3. Analysis of phenolic content in the effluent

The phenolic compounds in the effluent were determined spectrophotometrically using a slightly modified method of Bray and Thorpe (1954). To 1 ml of the culture supernatant at given time point, 500 μl of 20 % Na₂CO₃ and 200 μl of diluted Folin-Ciocalteaeu reagent (1:1) were added. The final volume of the reaction mixture was made to 3 ml with distilled water. The tubes were incubated at room temperature for 5 minutes. Blanks were also treated similarly. Absorbance was measured at 725 nm. The phenol content of the culture was estimated using a standard graph prepared from catechol.
10.2.4. Treatment of Phenolic content of the effluents by Trichoderma viride Pers. NFCCI-2745

Since the fungus was isolated from coconut husk retting zone with high salinity phenol content, the strain was utilized in treating various saline phenolic effluents such as coir ret liquor, textile and wood treating and processing effluent at initial concentrations of 28, 22 and 29 mg L\(^{-1}\). The effect of these effluents on the enzyme production of the fungal isolate and the subsequent reduction of phenolic content was checked by preparing the medium with 25% respective effluents. The filter sterilized effluent was added to a 48 hour old culture. Laccase assay was done every 24 hours of incubation for 7 days. The reduction in the added phenolic contents in the culture filtrate was determined spectrophotometrically using a slightly modified method of Bray and Thorpe (1954).

10.2.5. Assessing the Efficiency of Immobilized Laccase in the treatment of Phenolic Industrial Effluents

If the immobilized laccase is to find commercial use it must retain its activity over several reuses. Cu Alginate, Cu-Ba Alginate, Cu-Sr Alginate and Cu-Ni Alginate were used in the present study. Phenolic effluents such as coir processing effluent, textile and wood processing effluent were selected for studying the efficiency of immobilized alginate laccase in removing phenolic content of the effluents. In order to carry out a continuous process for further implementation in industry, the effects of immobilization conditions on the removal of phenolic content from the effluent were checked.
by comparing the performance of the immobilized preparations in three repeated batches. Phenolic contents in the effluent were determined spectrophotometrically using a slightly modified method of Bray and Thorpe (1954).

Twenty five ml of the phenolic effluents at initial concentrations of 28, 22 and 29 mg L\(^{-1}\) for coir processing effluent, textile and wood processing effluent, respectively were continuously pumped into a pack-bed immobilized enzyme beads in a 10 mL-syringe (total laccase activity 10 U) at room temperature (~30±3°C) at the first cycle. The beads were tested for its ability to detoxify the phenolic contents by monitoring the change in the respective specific absorbance maxima and estimating the phenol content of the effluent every 15 minutes over a period of 6 hours.

10.3. Result and discussion

10.3.1. Effectiveness of Trichoderma sp NFCCI-2745 in Treating Saline Phenolic Effluents

Since the fungus was isolated from coconut husk retting zone with high phenolic content (Paulmurugan et al. 2004), the effect of phenolic industrial effluent like coir ret liquor, textile and wood processing effluent on growth of the fungal isolate was tested. As expected, the strain could effectively remove the phenolic content from the media. Also, all the effluents enhanced laccase production by *T. viride* NFCCI-2745. It was observed that the cultures showed enhanced enzyme activity after the addition of
the effluents to the media at the 48\textsuperscript{th} hour of incubation and reached its maximum within 96 hours of incubation. Of the different effluents such as coir ret liquor, textile and wood processing effluent tested at 25 \% for laccase production in \textit{T. viride}, wood processing effluent supported a maximum laccase activity of 15.61 U ml\textsuperscript{-1} (Figure 10.1). The strain could also effectively remove the phenolic content from the media.

\textbf{Figure 10.1:} Effectiveness of \textit{Trichoderma} sp NFCCI-2745 in Treating Saline Phenolic Effluents
10.3.2. Treatment of Phenolic Industrial Effluents by Immobilized Laccase

All the tested Immobilized enzymes displayed almost similar effectiveness in removing the phenolic content of the effluents in the first cycle but Cu-alginate and Cu-Ba alginate displayed reusability in the 2<sup>nd</sup> and 3<sup>rd</sup> cycle tested.

For coir processing effluent treatment by the immobilized Cu alginate enzymes, a rapid phenolic removal by about 75 % within 90 min was observed and 90 % removed within 150 minutes (Figure. 10.2a). The immobilized beads were well reusable in the second and the third cycles with the phenolic removal percentages of 85 and 75 %, respectively (Figure. 10.2a).

![Figure 10.2a: Cu Alginate Enzyme in treating Industrial Effluents](image-url)
In case of wood processing effluent, about 60 % was achieved within the first 30 min, after that phenolic removal gradually increased until reached to 98 % in 120 min (Figure. 10.2a). The immobilized beads were well repeatable in the second and the third cycles with the highest treatment percentages of 95 and 90 %, respectively (Figure. 10.2a). For textile effluent treatment by the immobilized alginate enzymes, phenolic removal by about 75 % was observed within 120 min and 90 % removed within 180 minutes of treatment (Figure. 10.2a). The immobilized beads were able to reuse, but phenolic removing efficiency for cycle-2 and 3 were decreased to 70 and 45 %, respectively (Figure. 10.2a).

For coir processing effluent treatment by the immobilized Cu-Ba alginate enzymes, a rapid phenolic removal by about 70 % within 90 min was observed and 90 % removed within 150 minutes (Figure. 10.2b). The immobilized beads were well reusable in the second and the third cycles with the phenolic removal percentages of 80 and 70 %, respectively (Figure. 10.2b). In case of wood processing effluent, about 65 % was achieved within the first 30 min, after that phenolic removal gradually increased until reached to 98 % in 120 min (Figure. 10.2b). The immobilized beads were well repeatable in the second and the third cycles with the highest treatment percentages of 90 and 85 %, respectively (Figure. 10.2b). For textile effluent treatment by the immobilized alginate enzymes, phenolic removal by about 75 % was observed within 120 min and 95 % removed within 210
minutes of treatment (Figure. 10.2c). The immobilized beads were able to reuse, but phenolic removing efficiency for cycle-2 and 3 were decreased to 70 and 40 %, respectively (Figure. 10.2b)

Figure 10.2b: Cu-Ba Alginate Enzyme in treating Industrial Effluents

For coir processing effluent treatment by the immobilized Cu-Sr alginate enzymes, a rapid phenolic removal by about 75 % within 90 min was observed and 90 % removed within 150 minutes (Figure. 10.2c). The immobilized beads were able to reuse, but phenolic removing efficiency for cycle-2 and 3 were decreased to 65 and 55 %, respectively (Figure. 10.2c). In case of wood processing effluent, about 65 % was achieved within the first 30 min, after that phenolic removal gradually increased until reached
to 95% in 120 min (Figure. 10.2c). The immobilized beads were repeatable in the second and the third cycles with the highest treatment percentages of 80 and 75%, respectively (Figure. 10.2c). For textile effluent treatment by the immobilized alginate enzymes, phenolic removal by about 75% was observed within 120 min and 90% removed within 180 minutes of treatment (Figure. 10.2c). The immobilized beads were able to reuse, but phenolic removing efficiency for cycle-2 and 3 were decreased to 60 and 35%, respectively (Figure. 10.2c).

![Figure 10.2c: Cu-Sr Alginate Enzyme in treating Industrial Effluents](image)

For coir processing effluent treatment by the immobilized Cu-Ni alginate enzymes, a rapid phenolic removal by about 70% within 90 min was observed and 90% removed within 150 minutes.
(Figure. 10.2d). The immobilized beads were able to reuse, but phenolic removing efficiency for cycle-2 and 3 were decreased to 65 and 50 %, respectively (Figure. 10.2d). In case of wood processing effluent, about 60 % was achieved within the first 30 min, after that phenolic removal gradually increased until reached to 98 % in 120 min (Figure. 10.2d). The immobilized beads were repeatable in the second and the third cycles with the highest treatment percentages of 75 and 65 %, respectively (Figure. 10.2d). For textile effluent treatment by the immobilized alginate enzymes, phenolic removal by about 75 % was observed within 120 min and 90 % removed within 210 minutes of treatment (Figure. 10.2d). The immobilized beads were able to reuse, but phenolic removing efficiency for cycle-2 and 3 were decreased to 50 and 30 %, respectively (Figure. 10.2d).

**Figure 10.2d:** Cu-Ni Alginate Enzyme in treating Industrial Effluents
It was reported earlier that Cu-alginate laccase from *Pleurotus ostreatus*, decolorizing a maximum of 70 % dye in batch operations (Palmieri *et al*. 2005), Cu-alginate laccase from *Lentinus polychrous*, decolorizing indigo carmine in the 3rd cycle (Phetsom *et al*. 2009) and immobilized laccase from *Ganoderma* sp. decolorizing 100 % Indigo carmine in 6 cycles (Teerapatsakul *et al*. 2008).

In conclusion the enhanced laccase secretion by *Trichoderma viride* NFCCI-2745, in the presence of phenolic industrial effluents is an added advantage and can be utilized in the treatment of saline and phenolic rich industrial effluents such as coir/coconut husk retting effluent and textile effluent that needed to be diluted several fold before microbial treatment. In our study, we were successful in removing the phenolic content of the effluent (Figure. 10.2), with simultaneous decolorization (Data not shown) with beads immobilized in different combinations of metal ions - Copper sulphate cross linkers. To our knowledge this is the first study describing the immobilization of laccase with different cation cross linkers and their combinations with Copper sulphate. The enzyme showed high efficiency in eliminating the phenolic content of the effluents when entrapped in Cu–Ba and Cu-alginate beads. The stability exhibited by the immobilized laccases towards a broad range of pH and temperature and its effectiveness in phenolic effluent treatment signify its substantial potential in biotechnological applications.
Since industrialization will doubtlessly continue to be accompanied by the generation of hazardous pollutants, it is necessary to develop efficient strategies for environment conservations. Thus at present microbial enzymes may be considered as a reliable tool in managing environmental pollutants.