CHAPTER-8

Synthesis of Laccase Nanoparticle
And Its Application
In
Dye Decolorization
And
Biotransformation

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8.1 Introduction

Synthesis of nanoparticle of various shape and sizes for the immobilization of biomolecules has attracted increasing scientific attention due to their usefulness in many applications. Nanoparticles have been used in chemical synthesis (Kumar et al. 2003), optoelectronics (Wang et al. 1991), single-electron transistors, light emitters (Klein et al. 1991) biosensors (Kim et al. 2006), catalysis (Gole et al. 2001), *in vivo* imaging (Dubertret et al. 2002), etc. Various nanoparticles like gold nanoparticle, Fe$_3$O$_4$ magnetite nanoparticle, silver nanoparticle, silica nanoparticle have been developed and possess properties like small size and large specific surface area for adequate binding of protein and biological interactions. Enzyme immobilized on to this nanoparticle by adsorption, cross-linking or by covalent attachment, have high sensitivity, stability, reusability and high surface-to-bulk ratio (Dyal et al. 2003; Xu et al. 2004; Huang et al. 2003).

Laccase oxidize various aromatic and non-aromatic compounds by a radical catalyzed reaction mechanism using molecular oxygen. The wide reaction capabilities and broad substrate specificities of laccase makes them a promising enzyme in various applications like textile dye bleaching, pulp bleaching, bioremediation of soils and water, polymer synthesis, food improvement and for the development of biosensors or biofuel cells (Balardin, 2006). The effective utilization of enzyme immobilization of laccase on silica nanoparticle is one such strategy, which allows good catalytic properties to recover and reuse of enzyme for several reaction cycles. Silica nanoparticle has been widely used for the bioactive molecule immobilization including protein, peptides, enzymes, antibodies and nucleic acids (Avnir et al. 1994; Gill, 2001). Silica nanoparticle can also accommodate diverse dimensions of enzyme without disturbing their biological activity and can be considered as a suitable support for the immobilization of enzyme (Galliker et al. 2010). Silica nanoparticle was synthesized by the hydrolysis of tetraethylorthosilicate (TEOS) in an ethanolic medium in the presence of ammonia. This silica nanoparticle was further cross-linked with enzyme by a cross-linking agent like glutaraldehyde in order to form an enzyme nanoparticle. Enzyme immobilized nanoparticle have been used as a model nanoparticle in a large number of experimental investigations. Wang et al. (2010) studied fabrication of magnetic mesoporous silica nanoparticle for the adsorption of laccase produced from *Trametes versicolor*. Salis et al. (2009) studied immobilization of *Pleurotus sajor-
caju laccase on functionalized SBA-15 mesoporous silica for the oxidation of a mixture of phenolic compounds. Galliker et al. (2010) studied immobilization of laccase on modified silica nanoparticle for the transformation of phenolic compounds. In view of importance of laccase nanoparticle as an effective biocatalyst, the objectives of the present study are as follows:

1. To synthesize the silica nanoparticle for the cross-linking of purified laccase produced from *Ganoderma cupreum* AG-1.

2. Characterization of laccase nanoparticle by TEM, FTIR and DLS spectroscopy.

3. To compare purified and laccase nanoparticle in terms of its biochemical and kinetic properties.

4. Application of laccase nanoparticle for the decolorization of Reactive violet 1 dye, Bisphenol A and transformation of certain chemicals.
8.2 Results and discussion

8.2.1 Effect of different concentration of protein (purified laccase enzyme) on the immobilization of laccase on silica nanoparticle

The immobilization reaction involves the nucleophilic addition between the amino group of laccase and the carbonyl group of glutaraldehyde on glutaraldehyde activated silica nanoparticle (Galliker et al. 2010). In order to have maximum amount of protein (i.e. Purified laccase enzyme) to be immobilized on silica nanoparticle, various concentration of protein (100 - 800 µg/ml) were allowed to cross-link with 100 mg of silica nanoparticle. From the amount of protein detected in the elutes, it was inferred that, as the concentration of protein increased from 100 to 300 µg/ml, the immobilization yield increased from 71% to 85% with a maximum yield of 92% was obtained when 400 µg/ml of protein was cross-linked with silica nanoparticle. The laccase activity obtained was 824 U/gm, when 400 µg/ml protein was used to immobilize on silica nanoparticle (Fig. 8.1). However, the concentration of protein when increased above 400 µg/ml the immobilization yield decreased with laccase activity remaining constant.

Fig. 8.1 Effect of different protein concentration on the immobilization yield

This may be due to the formation of a dense protein layer, which cause a stearic hindrance and diffusion barrier for the assessment of the enzyme on the immobilization carrier, resulting in a low immobilization yield. Similar results have also been reported by Abdulkareem et al. (2002), who reported 1 mg/ml protein as
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an optimum concentration for the immobilization on DEAE-Granocel support. However, Huang et al. (2006) reported that increase in laccase concentration from 0.25 to 2.0 mg/ml results in an increase in laccase activity for the immobilization of laccase on CuTAPc-Fe3O4 composite. Bayramoglu et al. (2011) reported adsorption capacity of CHX-g-p(IA) and CHX-g-p(IA)-Cu(II) membranes for laccase increased as the protein concentration increased from 0.1 mg/ml to 1.0 mg/ml and any further increase in protein concentration up to 2.0 mg/ml. laccase adsorption on CHX-g-p(IA) and CHX-g-p(IA)-Cu(II) membranes remains constant.

8.2.2 Characterization of laccase nanoparticle

The cross-linking of laccase onto silica-nanoparticle was characterized using FTIR, DLS and TEM analysis. The FTIR spectrum of silica nanoparticle and laccase nanoparticle are shown in Fig. 8.2 (a) and (b).

![FTIR analysis of (a) silica nanoparticle and (b) laccase nanoparticle](image)

The FTIR spectrum of both the nanoparticle show peak at 1099 cm⁻¹, 958 cm⁻¹ and 796 cm⁻¹ which corresponds to the asymmetric vibration of Si-O, Si-OH and symmetric vibration of Si-O in silica nanoparticle, the absorption band between
3300 cm\(^{-1}\) and 3500 cm\(^{-1}\) is assigned to O-H stretching and H-bonded water, which are the characteristic of silica nanoparticle (Aldona et al. 2004). In order to synthesize glutaraldehyde activated silica nanoparticle, glutaraldehyde is used as a cross-linker, where in the aldehyde group of the cross-linker reacts with the amino group of silica nanoparticle. The incubation of glutaraldehyde activated silica nanoparticle with laccase allows the covalent binding of laccase with silica nanoparticle, thereby the laccase nanoparticle are synthesized. The FTIR spectrum of laccase nanoparticle (Fig. 8.2 b) shows a peak at 2935 cm\(^{-1}\) for C-H stretching, confirming the cross-linking of laccase with silica-nanoparticle by glutaraldehyde molecule. The TEM image of silica nanoparticle shows smooth spherical surface (Color plate 8.1 a), while the TEM image of laccase nanoparticle (Color plate 8.1 b) shows rough spherical surface.
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The DLS analysis of silica nanoparticle and laccase nanoparticle are shown in Fig. 8.3. An increase in the diameter of silica nanoparticle after immobilization of laccase is observed from 220 nm to 620 nm.

8.2.3 Effect of pH on the activity of purified laccase and laccase nanoparticle

The determination of optimum pH for the oxidation of ABTS by both purified laccase and laccase nanoparticle were carried out in the pH range of 3 – 8. The optimum pH of purified laccase and laccase nanoparticle was found to be 3 and 4, respectively (Fig. 8.4). The results obtained showed one unit shift in the optimum pH towards the higher value after the cross-linking of laccase on silica nanoparticle. This may be attributed to the change in the micro environment of the enzyme created by the cross-linking of laccase with the silica nanoparticle and also due to the ionic interaction between the enzyme and the charged surface of the silica nanoparticle.

![Fig 8.4 Effect of pH on the activity of purified laccase and laccase nanoparticle](image)

Fig 8.4 Effect of pH on the activity of purified laccase and laccase nanoparticle

Laccase nanoparticle shows more than 90% residual activity in the pH range of 3 - 5, while purified laccase shows a comparative decrease in the residual activity within the same pH range. Kalkan et al. (2011) reported 0.5 and 1.5 unit shift of optimum pH after covalent immobilization of laccase on Fe₃O₄-CS-CCn & Fe₃O₄-CS-EDAC support, respectively. Wang et al. (2010) also reported a shift in the optimum pH for the oxidation of catechol upon immobilization of laccase on magnetic mesoporous silica nanoparticle.
8.2.4 Effect of temperature on the activity of purified laccase and laccase nanoparticle

The effect of temperature on the activity of purified laccase and laccase nanoparticle were determined in the range of 30 - 70 °C for the oxidation of ABTS. The result obtained, show a shift in the optimum temperature towards the lower value, after cross-linking of laccase on the silica nanoparticle. The optimum temperature of purified laccase was found to be 55 °C while the optimum temperature of laccase nanoparticle was found to be 50 °C for the oxidation of ABTS (Fig. 8.6). The alteration in the optimum temperature after immobilization of enzymes on various support materials depends on the immobilization method and interaction between the support and the enzyme.

Fig 8.5 Effect of temperature on the activity of purified laccase and laccase nanoparticle

A shift of optimum temperature towards the lower temperature values have also been reported by Kalkan et al. (2011) wherein, the free laccase showed 40°C as the optimum temperature and laccase immobilized on Fe₃O₄-CS-EDAC nanoparticle shows 30°C as the optimum temperature. Huang et al. (2006) reported 55°C and 45°C as the optimum temperature for free and CuTAPc-Fe₃O₄ nanoparticle laccase, respectively for the oxidation of ABTS.

8.2.5 Temperature stability of purified laccase and laccase nanoparticle

Temperature stability of the purified laccase and laccase nanoparticle were assessed by incubating the purified laccase and laccase nanoparticle in a temperature
range of 30-70°C for 24 h in acetate buffer (pH 5, 50 mM). Fig 8.6 (A-E) depicts the activity of laccase nanoparticle decreases slowly as compared to the purified laccase.

Fig. 8.6 Temperature stability of purified laccase and laccase nanoparticle at (A) 30°C, (B) 40°C, (C) 50°C, (D) 60°C and (E) 70°C
The residual activity of the purified laccase and laccase nanoparticle after 24 h of incubation was almost similar at an incubation temperature of 30°C. The purified laccase showed 41%, 31% and 6% residual activity at an incubation temperature of 40°C, 50°C and 60°C respectively, after 24 h of incubation. Whereas, laccase nanoparticle showed 66%, 57% and 32% residual activity at an incubation temperature of 40°C, 50°C and 60°C respectively, after 24 h of incubation. The results obtained show that the cross-linking of laccase on silica nanoparticle increases the stability by 25 - 30% in the temperature range of 40 - 60°C. However, at 70°C after 4 h of incubation purified laccase loses 75% of its initial activity, while laccase nanoparticle lost only 25% of its initial activity. Longer incubation period of 24 h at 70°C results in a complete loss of the activity of the purified laccase, whereas laccase nanoparticle could retain only 14% of its initial activity. This loss of activity may be attributed to the change in the conformational structure due to the breakage of bonds thus leading to the decreased activity. However, enzymes when immobilized on support materials, it creates a kind of protection and provides a resistance against the conformational changes of the enzyme structure, which results in an increased thermal stability of laccase nanoparticle (Cabana et al. 2011). Lu et al. (2007) reported laccase immobilized on alginate-chitosan microcapsule retains 68.8% residual activity as compared to 22.7% residual activity of free laccase after 6 h of incubation at 60°C. Hu et al. (2007) reported that laccase immobilized on nanoparticle and kaolinite was more stable as compared to the free laccase when incubated in a temperature range of 40-70°C. Huang et al. (2007) also reported that *Pycnoporous sanguineus* laccase immobilized on Zn nanoparticle shows more resistance to thermal inactivation than the free laccase at 55°C.

### 8.2.6 pH stability of purified laccase and laccase nanoparticle

In order to check the pH stability both purified laccase and laccase nanoparticle laccase were incubated in a pH range of 3 - 5 (Fig. 8.7 A - C) at 30°C for 24 h and the residual activity were measured periodically at an interval of 4 h. The results obtained showed that the laccase nanoparticles were well stabilized when kept in a pH range of 3 - 5 for 24 h. The laccase nanoparticles show more than 55% residual laccase activity at all the tested pH values after 24 h of incubation. The laccase nanoparticle shows 58% residual activity after 24 h of incubation at pH 3.
while, purified laccase shows only 28% residual activity at the same pH after 24 h of incubation.

![Graph A](image1)
![Graph B](image2)
![Graph C](image3)

**Fig. 8.7 pH stability of purified laccase and laccase nanoparticle at (A) pH 3, (B) pH 4 and (C) pH 5**

Laccase nanoparticle shows 5 - 10% improvement in pH stability as compared to the purified laccase at pH 4 and 5. The improvement in pH stability may be observed due to the multipoint attachment of laccase on the immobilization support which makes the laccase less prone to pH induced conformational changes (Mohidem and Mat, 2009). Similar results have been reported by Abdulkareem et al. (2002) and Irshad et al. (2012) for the improvement of pH stability of laccase after immobilization on DEAF-Granocel 500 and SOL-GEL matrix, respectively within the pH range of 2 to 7.
8.2.7 Storage stability of purified laccase and laccase nanoparticle

In order to store the catalyst for a long time period, the storage stability is an important parameter. Thus the purified laccase and laccase nanoparticle were stored at 4°C and -20°C in acetate buffer (50 mM, pH 5) up to 40 days and the residual activity was determined periodically. The results of storage stability of purified laccase and laccase nanoparticle at storage temperature of 4°C and -20°C are presented in Fig. 8.8. The result shows that the storage at 4°C for 40 days, the residual activity of laccase nanoparticle was found to be 90%, whereas purified laccase could retained 71% of its initial activity. However, when storage temperature was -20°C the residual activity of laccase nanoparticle and purified laccase was found to be 92% and 85%, respectively after 40 days of storage. The results indicate that nanoparticle laccase exhibits improved storage stability as compared to the purified laccase at 4°C and -20°C. The improved stability of laccase might be observed due to the stabilization of active conformation of enzyme by multipoint interactions with the support used for immobilization. Huang et al. (2006) reported 85% residual activity of CuTAPc-Fe3O4 laccase nanoparticle as compare to the 30% residual activity of free laccase after 1 month storage at 4°C. Bayramoglu et al. (2011) reported 37% residual activity of laccase immobilized on CHX-g-p(1A)-Cu(II) membrane after 5 week of storage at 4°C as compared to the complete loss of the activity of free laccase.

Fig. 8.8 Storage stability of purified laccase and laccase nanoparticle at (A) 4°C and (B) -20°C
8.2.8 Kinetic study of purified laccase and laccase nanoparticle

The enzyme kinetic parameters i.e. \(K_m\) and \(V_{\text{max}}\) for the oxidation of ABTS by purified laccase and laccase nanoparticle was determined by taking different concentrations of ABTS ranging from 0.5 - 100 mM ABTS. The Michaelis - Menten (\(K_m\)) and Velocity maximum (\(V_{\text{max}}\)) of the purified laccase and laccase nanoparticle have been calculated from line weaver-burk plots (Fig. 8.9 A and B).

![Fig. 8.9 Line weaver-burk plot of (A) purified laccase; (B) laccase nanoparticle](image)

The \(K_m\) value of laccase nanoparticle was found to be 0.23 mM, which is 21.05% higher than the \(K_m\) value of purified laccase (0.19 mM). The \(V_{\text{max}}\) value of the laccase nanoparticle was found to be \(3.58 \times 10^4\) mM/min, which is only 29.01% of the \(V_{\text{max}}\) value of the purified laccase (12.34 \(\times 10^4\) mM/min). The results obtained shows that the catalytic efficiency of the laccase nanoparticle was lower than the purified laccase. The lower catalytic efficiency of the laccase nanoparticle may be due to the rigid conformation of enzyme in nanoparticle form, which will not allow any changes in the conformation of the active site in order to accommodate the substrates. Also, when enzyme cross-linked upon nanoparticle the enzyme active centers may be stearically hindered by the neighboring enzyme molecules and thus they are not available to the substrate which in turn lowers the catalytic efficiency of the laccase nanoparticle (Sari et al. 2006). A lower catalytic efficiency of laccase crystal had been observed by Roy et al. (2005) who, reported that the \(V_{\text{max}}\) of the CLEC laccase is only 12% than the \(V_{\text{max}}\) of the native laccase. Huang et al. (2006) also reported lower catalytic efficiency of CuTAPc-Fe\(_{3}\)O\(_4\) nanoparticle laccase as compared to the free laccase, who reported 88.88% higher
Km and 43.33% lower Vmax value of CuTAPc-Fe$_3$O$_4$ nanoparticle laccase as compare to the free laccase.

### 8.2.9 Reusability of laccase nanoparticle

Laccase is an expensive biocatalyst; the reuse of the catalyst makes the enzymatic process economically viable in order to cut down the production cost. The reusability of the laccase nanoparticle was investigated for the oxidation of ABTS at 30°C for 20 subsequent cycles. The results obtained showed that more than 90% of the activity of laccase nanoparticle was retained till the 13th cycle. Thereafter a gradual decrease in laccase was observed after each cycle of ABTS oxidation and almost 79% of residual activity was retained till 20th cycle (Fig. 8.10), thus showing that laccase cross-linked with silica nanoparticle was reasonably stable when repeatedly used for the catalysis of ABTS oxidation. This property of laccase nanoparticle is beneficial for the application in a batch or in a continuous mode. Bayramoglu et al. (2011) reported 81% residual activity of laccase immobilized on CHX-g-p(IA)-Cu(II) membrane, after 10th cycle of syringaldazine oxidation. Liu et al. (2012) reported 50% residual activity of laccase immobilized on CMMC support after 10th cycle of ABTS oxidation. Whereas, Huang et al. (2006) reported 80% residual activity of laccase immobilized on magnetic Fe$_3$O$_4$ nanoparticle after five consecutive operational cycles.

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**Fig. 8.10 Reusability of laccase nanoparticle for ABTS oxidation**
8.2.10 Decolorization of Reactive violet 1 dye using purified laccase and laccase nanoparticle

The application of purified laccase and laccase nanoparticle was studied for the decolorization of textile dye Reactive violet-1. The results obtained shows that 60% of the dye was decolorized with in 4 h by laccase nanoparticle (Fig. 8.11). While, purified laccase shows 54% decolorization with in 4 h. However, after 12 h of catalytic action, 92% and 90% decolorization of reactive violet-1 was observed when treated with laccase nanoparticle and purified laccase, respectively.

![Decolorization of Reactive violet 1 dye using purified laccase and laccase nanoparticle](image1)

Fig. 8.11 Decolorization of Reactive violet 1 dye using purified laccase and laccase nanoparticle

![UV-visible spectrum of Reactive violet-1 dye and degraded sample](image2)

Fig. 8.12 UV-visible spectrum of Reactive violet-1 dye and degraded sample
Fig 8.12 shows the UV-visible spectrum of control dye and decolorized dye samples. The UV-visible spectrum of Reactive violet-1 dye shows a sharp peak at 560 nm, while the decolorized samples by treatment with purified laccase and laccase nanoparticle shows a decrease in absorbance at visible region and an increase in absorbance in UV region. Upon comparison of purified laccase and laccase nanoparticle for the decolorization efficiency, it was observed that purified laccase and laccase nanoparticle shows almost similar decolorization efficiency for Reactive violet-1 dye. Valle-Vigon and Furtes, (2011) reported that 80% dye degradation of Acid green 25 and Remazol Brilliant Blue dye by laccase immobilized nanoparticle. Bayramoglu et al. (2011) reported 48%, 37% and 19% decolorization of Methyl Orange, Cibcron Blue F3GA and Reactive Black 5 dyes by Trametes versicolor laccase immobilized on CHX-g-p(1A)-Cu(II) membrane. Makas et al. (2010) reported 35% decolorization of Methyl Orange dye after 6 h treatment with laccase entrapped in semi-IPNs prepared from k-carrageenan.

8.2.10.1 Effect of pH on the decolorization of Reactive violet 1 dye by laccase nanoparticle

The effect of pH on the decolorization of Reactive violet 1 dye by laccase nanoparticle was examined in the pH range of 2 – 7. A bell shaped pH dependence on the decolorization of Reactive violet 1 dye was observed by laccase nanoparticle (Fig. 8.13).
The laccase nanoparticle shows more than 75% decolorization of Reactive violet 1 dye with a pH range of 3 – 6. The optimum pH for dye decolorization was obtained at pH 4 with 96% decolorization of Reactive violet 1 dye. However, decolorization of Reactive violet 1 dye was less than 30% at neutral (pH 7) and acidic pH (pH 2). A similar bell shaped pH dependence profile of laccase electrode has been observed by Tortolini et al. (2012). Usluoglu and Arabaci, (2013) reported pH 4 as the optimum pH for maximum decolorization of acid and metal complex dyes by phenol oxidase immobilized on alginate beads. Mogharabi et al. (2012) reported pH 8 as the optimum pH for the decolorization of dye solution of various textile dyes (coomassie blue, bromthymol blue, amido black 10B, methyl red, Eosin, and malachite green) by laccase immobilized on alginate gelatin gel.

8.2.10.2 Effect of Temperature on the decolorization of Reactive violet 1 dye by laccase nanoparticle

The effect of temperature on the decolorization of Reactive violet 1 dye by laccase nanoparticle was studied by incubating the reaction mixture at a temperature between 20 - 60°C (Fig. 8.14).

![Fig. 8.14 Effect of Temperature on the decolorization of Reactive violet 1 dye by laccase nanoparticle](image-url)

The optimum temperature for the maximum decolorization (96%) of Reactive violet 1 dye by laccase nanoparticle was obtained at an incubation temperature of 30°C. The decolorization of reactive violet 1 dye by laccase nanoparticle was also comparable in the incubation temperature range of 25°C -
50°C. However, further increase in temperature results in decreased decolorization of Reactive violet 1 dye. The decrease in decolorization might be observed due to the unfolding or degradation of laccase at high temperature. Similar results have been observed by Mogharabi et al. (2012) for the decolorization of crystal violet dye by laccase in alginate-gelatin mixed gel. In contrast, Reyes et al. (1999) reported maximum decolorization of textile dye solution at 60°C by laccase immobilized on agarose gel Affi-Gel 15.

8.2.10.3 Effect of dye concentration on the decolorization of Reactive violet 1 dye by laccase nanoparticle

The effect of dye concentration on the decolorization of reactive violet 1 dye by laccase nanoparticle was evaluated by incubating various concentration (25 to 200 mg/ml) of reactive violet 1 dye with laccase nanoparticle at 30°C for 12 h.

Fig. 8.15 Effect of dye concentration on the decolorization of Reactive violet 1 dye by laccase nanoparticle

Fig. 8.15 shows that the decolorization (95 – 96%) of reactive violet 1 dye was almost similar as the concentration of reactive violet 1 dye was increased from 25 – 125 mg/l. However, further increase in dye concentration up to 200 mg/l concentration results in decrease decolorization of reactive violet 1 dye till 77%. At higher concentration of dye, less number of enzyme encounter the dye molecule results in decreased decolorization efficiency. Similar results have been reported by other researchers (Young and Yu, 1997; Silva et al. 2011).
8.2.11 Biotransformation of Ferulic acid and Vanillin

The biotransformation of ferulic acid and vanillin by laccase nanoparticle was characterized by UV-Visible spectrum, TLC, and FTIR analysis. The UV-Visible spectrum of ferulic acid shows two major peaks at 318 and 334 nm, while the biotransformed product shows a single peak at 325 nm (Fig. 8.16 A). There was an appearance of different spots in the biotransformed product with decreased $R_f$ values of 0.85 as compared to the $R_f$ value (0.93) of original ferulic acid, which confirms the biotransformation of ferulic acid (Fig. 8.16 B). The FTIR spectrum of ferulic acid showed peaks at 3300 cm$^{-1}$ (carboxylic acid O-H stretching), 1647 cm$^{-1}$ (carboxylic acid C=O stretching), 1275 cm$^{-1}$ (carboxylic acid C-O stretching) and 1515; 1551 cm$^{-1}$ (aromatic C=C) confirms the skeleton of ferulic acid (Fig. 8.16 C). The FTIR spectrum of biotransformed product (Fig. 8.16 D) showed sharp peaks at 1734 cm$^{-1}$, 1372 cm$^{-1}$ (-COO and C=O stretching) which may arise due to the oxidation of ferulic acid causing an esterification reaction. A single sharp peak also appears at 1234 cm$^{-1}$ due to CH bending. However, a decrease in peaks at 3300 cm$^{-1}$ (carboxylic acid O-H stretching) observed which may be due to the removal of -OH group from ferulic acid skeleton. Thus the above analysis predicts the biotransformation of ferulic acid by laccase nanoparticle with the formation of some aster like derivative of ferulic acid.

The UV-Visible spectrum of vanillin shows three major peaks at 278, 310 and 335 nm, while the biotransform product showed peaks at 295 and 335 nm (Fig. 8.17 A). There was an appearance of different spots in the biotransformed product with decreased $R_f$ values of 0.85 as compared to the $R_f$ value (0.95) of control vanillin, which confirms the biotransformation of vanillin (Fig. 8.17 B). The FTIR spectrum of vanillin (Fig. 8.17 C) showed peaks at 3343 cm$^{-1}$ (carboxylic acid O-H stretching), 1664 and 1253 cm$^{-1}$ (stretching vibration of C=O), the peak around 1450, 1404, and 939 cm$^{-1}$ belongs to the benzene ring of vanillin, while the weak absorption peaks at 2832 – 2944 cm$^{-1}$ were attributed to C-H in methyl and methylene groups of vanillin structure. The FTIR spectrum of biotransform product (Fig. 8.17 D) showed removal of peaks in the spectrum at 1664 and 1253 cm$^{-1}$ (stretching vibration of C=O) which indicate the removal of C=O group from the vanillin structure.
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Fig. 8.16 Characterization of Ferulic acid and its biotransformation product

(A) UV-Visible spectrum (B) TLC (C) FTIR of Ferulic acid (D) FTIR of biotransformation product.
Fig. 8.17 Characterization of Vanillin and its biotransformation product
(A) V-Visible spectrum (B) TLC (C) FTIR of Vanillin (D) FTIR of biotransformation product
From the above analysis, a confirmed reaction was not predicted, but we can say that a new biotransformed product of vanillin is generated by laccase nanoparticle reaction.

**8.2.12 Degradation of Bisphenol A by laccase nanoparticle**

The degradation of bisphenol A by laccase nanoparticle was analyzed by UV-Visible, TLC, and HPLC analysis. The UV-Visible spectrum of bisphenol A shows two major peaks at 230 and 280 nm, while the degraded product showed loss of peak at 230 nm (Fig. 8.18 A). The TLC analysis showed appearance of different spot in the degraded product with decreased Rf value of 0.81 as compared to the Rf value (0.95) of control bisphenol A (Fig. 8.18 B). HPLC analysis showed complete disappearance of bisphenol A from laccase nanoparticle catalyzed bisphenol A sample. In the HPLC analysis, the peak of bisphenol A was obtained at the retention time of 12.75 min (Fig. 8.18 C). while, the degraded sample shows peak at retention time of 3.18, 3.60 and at 3.81 min (Fig. 8.18 D). Thus, HPLC analysis confirms the degradation of bisphenol A by laccase nanoparticle. Laccase nanoparticle mediated oxidation of bisphenol A has been reported by Galliker et al. (2010). Liu et al. (2012) reported the degradation of phenol and p chlorophenol by magnetic mesoporous carbon. Okazaki et al. (2002) reported degradation of bisphenol A in the presence of 1- hydroxybenztriazole (HBT) by laccase reverse micelles system. The degradation of phenolic compounds by laccase mediated system has also been reported by several other researchers (Michzoe et al. 2005; Li et al. 2010; Unal et al. 2011).
Fig. 8.18 Characterization of Bisphenol A and its degraded product by laccase nanoparticle (A) UV-Visible spectrum (B) TLC (C) HPLC of Bisphenol A (D) HPLC of Bisphenol A degraded product.
Conclusion

In the present study silica nanoparticle were synthesized using stober method and cross-linked with purified laccase produced from *Ganoderma cupreum* AG-1 by cross-linking agent glutaraldehyde. The produced laccase nanoparticle was characterized by TEM, FTIR and DLS spectroscopy. The laccase nanoparticle and purified laccase showed optimum temperature of 50°C and 55°C respectively and optimum pH 4 and 3, respectively for the oxidation of ABTS. The laccase nanoparticle showed high temperature, pH and storage stability as compared to the purified laccase. The laccase nanoparticle shows 79% residual activity till 20th cycle for the oxidation of ABTS. The laccase nanoparticle was further used for the decolorization of Reactive Violet–1 dye, biotransformation of ferulic acid and vanillin and degradation of endocrine disrupting chemical bisphenol A.