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Immobilization of Laccase in poly (Vinyl Alcohol)-Calcium Alginate Beads

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Abstract. Laccase enzyme (L) obtained from *Tramates versicolor* was entrapped into polyvinyl alcohol–calcium alginate (PVA-CaAlj) beads. Michaelis-Menten constant (Km) and maximum reaction rate (Vmax) values were found to be $1.70x10^{-2}$ mM and $2.08x10^{-3}$ mM.min^{-1} for free enzyme respectively. Km and Vmax values were found as $2.87x10^{-2}$ mM and 5.30×10^{-3} mM.min^{-1} for entrapped enzymes respectively. Optimum pH was determined as 5.0 and 6.0 and optimum temperature determined as 40ºC and 45ºC for free laccase and entrapped laccase respectively. After 30 days of storage at 4 ºC free laccase retained 60 % of its original activity. Also after 30 days of storage at 4 ºC, entrapped enzymes were retained 85 % its original activity. Immobilized enzyme was used repeatedly 10 times, were retained 75% of its original activities.

***Keywords*:** Laccase, immobilization, polyvinyl alcohol–calcium alginate, entrapment.

Lakkazın poli (Vinil Alkol) -Kalsiyum Aljinat Kürelerine İmmobilizasyonu

Özet. Tramates versicolor' dan elde edilen lakkaz enzimi (L), polivinil alkol-kalsiyum aljinat (PVA-CaAlj) kürelerine hapsedildi. Michaelis-Menten sabiti (Km) ve maksimum reaksiyon hızı (Vmax) değerleri sırasıyla serbest enzim için $1.70x10^{-2}$ mM ve $2.08x10^{-3}$ mM.dak^{-1} olarak bulundu.İmmobilize enzim için Km ve Vmax değerleri de sırasıyla $2.87x10^{-2}$ mM ve 5.30×10^{-3} mM.dak^{-1} olarak bulundu. Optimum pH değerleri serbest enzim için 5.0 ve immobilize enzim için 6.0 olarak belirlendi. Optimum sıcaklık sırasıyla serbest lakkaz ve immobilize lakkaz için 40ºC ve 45ºC olarak belirlendi. 4 ºC tutulan serbest lakkazın 30 günlük depolama sonrasında orijinal aktivitesinin % 60'ı koruduğu bulunurken aynı koşullarda tutulan immobilize enzimin ise orijinal aktivitesinin% 85'ini koruduğu bulundu. İmmobilize enzimin 10 kez tekrar kullanım sonrasında orijinal aktivitesinin % 75'ini koruduğu bulunmuştur.

***Anahtar Kelimeler*:** Lakkaz, immobilizasyon, polyvinil alkol–kalsiyum aljinat, tutuklama.

1. INTRODUCTION

Enzyme catalysed reactions are very important in biotechnology. Immobilizations of enzymes are useful techniques that provide continuous and repeated use in enzymatic processes and applications. [1-3]. Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2), a type of multicopper oxidase which is a biocatalyst. Laccase can able to catalyse reduction of oxygen to water [4-6]. Fungi, plants and some bacteria are laccase production resources [7-10].

Several supports have been used for immobilization of laccase by entrapment method, such as entrapment in polymeric gels [11], entrapment on polyacrylamide semi-interpenetrating polymer networks [12], entrapment on Semi-interpenetrating polymer networks [13], entrapment on Polyacrylamide-based semi-interpenetrating networks [14], entrapment into carrageenan based semi-interpenetrating polymer Networks [15] .

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In this study laccase was immobilized polyvinyl alcohol–calcium alginate (PVA-CaAlj) beads by entrapment. Effects of immobilization on enzyme activity, kinetic parameters, storage stability and reuse capability of the laccase were investigated.

2. MATERIALS AND METHODS

Materials

Laccase (EC 1.10.3.2.) from *Trametes versicolor* were supplied from Fluka. Sodium alginate and syringaldazine were supplied from Sigma. Polyvinyl alcohol, Calcium chloride $CaCl_2$, Citric acid and sodium hydroxide were supplied from Merck. Ammonium persulphate (APS) was supplied from Analar. Phosphoric acid and Ethyl alcohol were supplied from Riedel-de Haen. All chemicals used were analytical grade in this study.

Immobilization of laccase into polyvinyl alcohol–calcium alginate (PVA-CaAlj) beads

Laccase was immobilized into (poly (vinyl alcohol)-Ca alginate) beads by entrapped method. 50 mL, 1% w/w Sodium alginate dissolved in distilled water and 50 mL, 2% w/w poly (vinyl alcohol) dissolved in distilled water were thoroughly mixed. Then Laccase enzyme was dissolved in phosphate buffer (pH: 6.5, 0.04 M) were added to the solution and mixed well. Fallowing this resulting homogenous mixtures were added drop wise from a burette to the 0.3 M $CaCl_2$ solution. When Na-alginate was contacted with $CaCl_2$, enzyme molecules were immobilized in water-insoluble Ca-alginate poly (vinyl alcohol) spheres due to sodium-calcium exchange. The resulting polymeric spheres were then stored in distilled water at 4 °C for later use.

Determination of laccase activity

Determination of laccase activity for free and immobilized form was assayed spectrophometrically as defined by Leonowicz and Grzywnowicz [16].

Spectrophotometric measurements were carried out with Shimadzu UV-Visible spectrophotometer UV/Vis. 1800 at 530 nm and enzyme activities were determined with the help of syringaldazine calibration curve.

Effect of Temperature and pH

Effects of Temperature and pH for free and immobilized laccase activities were determined under different pH and temperatures range.

Storage Stability

The storage stability of the free and entrapped laccase was measured in 30 days. Free and entrapped laccase were kept in Phosphate buffer at 4 °C.

Kinetic Studies

Kinetic parameters of Michaelis−Menten equation K_m and Vmax were determined for free and immobilized laccase at constant temperature and pH for various syringaldazine concentrations.

Repeated use of Immobilized Laccases

Immobilized laccase enzyme was used 10 times in a day. Temperature, pH and substrate concentration were kept constant in each reaction.

3. RESULTS AND DISCUSSION

Effects of Temperature and pH

The activity of the free and immobilized laccase at different pH values is shown in Figure 1. Optimum pH was determined as 5.0 and 6.0 for free laccase and entrapped laccase respectively. Similar results have been reported for free laccase related laccase sources and substrates [12, 17-19]. For immobilized laccase optimum similar pH values have been reported previously [12-15, 17-19]. The pH value in this study was found to be relevant with previous studies. The activity of the free and immobilized laccase at different temperature values is in the Figure 2. The optimum temperature was determined as 40°C and 45°C for free laccase and entrapped laccase respectively. Optimum temperature of entrapped laccase value was found higher than optimum temperature value of free

laccase. Similar result have been reported in previous studies [12-15].

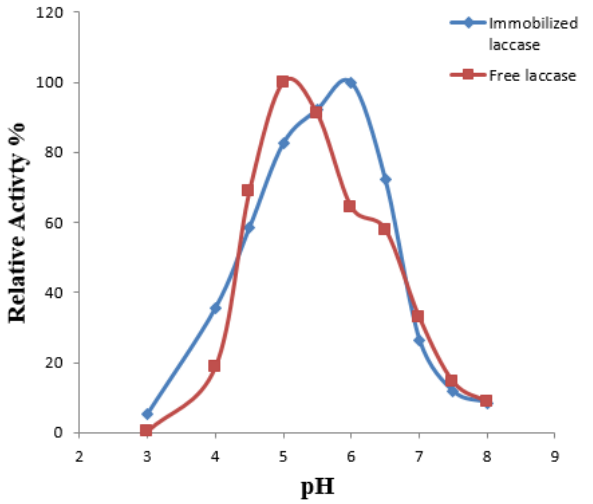

Figure 1. Effect of pH on the activity of free and immobilized enzyme.

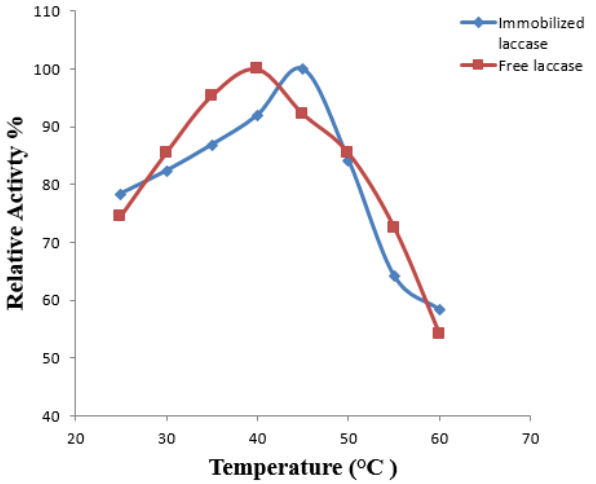

Figure 2. Effect of temperature on the activity of free and immobilized enzyme.

Storage Stability

The free and entrapped laccases were kept at 4 °C and the activities of free and entrapped enzyme were measured periodically over duration of 30 days. The entrapped laccase retained 85% of its starting activity while free laccase retained 60% of starting activity when stored in buffer solution for 30 days. The results are shown in Figure 3. In literature, glaceraldehyde crossed laccase on magnetic chitosan nanoparticles maintained 85% of activity after 30 days [20]. When laccase was immobilized with covalent attachment to DEAE-Granocel 500, CM-Granocel and acrylic carrier, it was reported to retain 90% of the activity of the immobilized laccase when stored at 4 °C for 4 months [18]. When laccase was immobilized with glutaraldehyde crosslinker by adsorption on chitosan microspheres and Fe^{+3} transition metal chelates, the enzyme immobilized on the chelate at 4 °C for 3 months and the water-soluble chitosan [21]. That the enzyme immobilized on the microspheres preserves 90% of the activity of the enzyme immobilized thereon, whereas the enzyme immobilized on the microspheres retains 85% of the activity. Our results are consistent with the results in the literature.

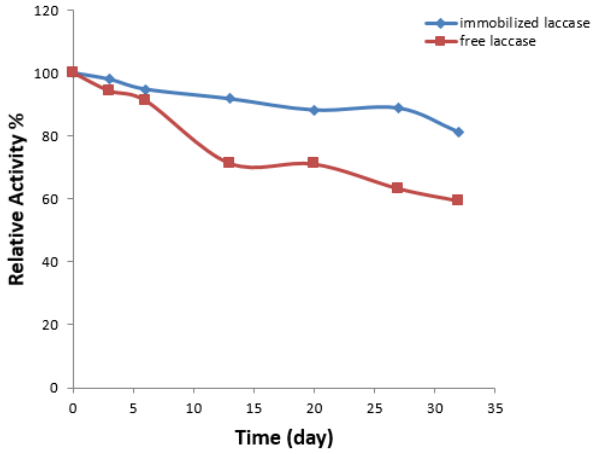

Figure 3. Effect of storage on the activity of free and immobilized enzyme.

Kinetics Parameters

The kinetic parameters results of free and immobilized laccase are presented in Table 1. Michaelis-Menten constant (Km) and maximum reaction rate (Vmax) values were estimated from the Lineweaver–Burk plots [22]. Km and Vmax values were found as 1.70×10^{-2} mM and 2.08×10^{-3} mM.min^{-1}, for free enzyme respectively. Km and Vmax values were found as 2.87×10^{-2} mM 5.30×10^{-3} mM.min^{-1}, for entrapped enzymes respectively (Figure 4.). In the light of the results of the experiment, it is seen that Km and Vmax values are increased by immobilization of the enzyme. The value of Km is increased by immobilization of the

enzyme this behaviour can be caused micro-environmental effects. In literature, When Laccase immobilized on magnetic chitosan nanoparticles Km value was reported to 31.1 μM [20]. When Eupergite was immobilized, they found that the Km was 0.150 mM and the Vmax value was 7.6 $x10^{-3}$ mM $.min^{-1}$ [5].

Table 1. Kinetic parameters of free and immobilized enzymes.

	K_m (mM)	V_{max} (mM.min^{-1})
Free enzyme	$1.70x10^{-2}$	$2.08x10^{-3}$
Immobilized enzyme	$2.87x10^{-2}$	5.30×10^{-3}

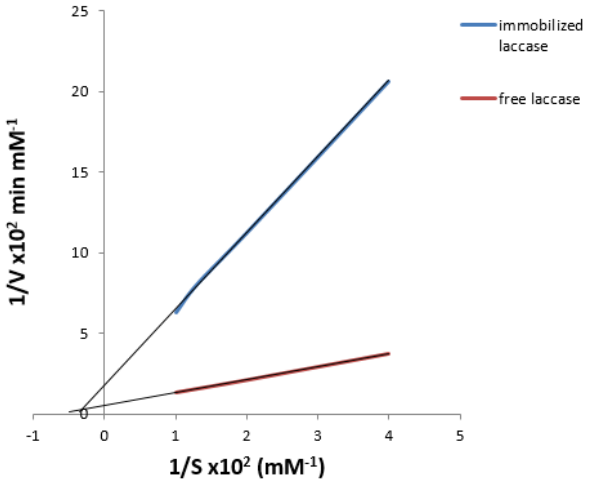

Figure 4. Lineweaver-Burk plots for free and immobilized enzyme.

Repeated use of immobilized enzymes

In this study, entrapped laccase into (poly (vinyl alcohol)-Ca alginate) beads were repeated in 10 cycles in a day. The immobilized enzymes were protected 75% of its original activities. Repeated use effect of immobilized laccase activity was presented in Figure 5.

In literature, it has been found that glaceraldehyde-crosslinked laccase on magnetic chitosan nanoparticles maintains about 85% of activity after 10 times used [20]. Laccase was immobilized on the amine-terminated nanocomposites (Cu TPAc) - Fe_3O_4 (copper tetraamin phthalocyanine) with gulutaraldehyde crosslinker and was found to retain 80% of activity after 5 times of use [23]. The enzyme laccase immobilized by covalent attachment on activated PVA has been found to retain 60% of its activity after 10 times used [24].

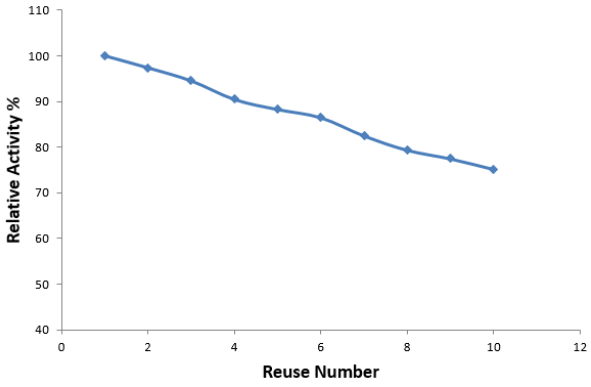

Figure 5. Effect of reuse on the activity of immobilized enzyme.

4. CONCLUSIONS

Laccase enzyme was immobilized by entrapment method into polyvinyl alcohol–calcium alginate (PVA-CaAlj) beads and the immobilization process was optimized.

It has been found that optimum pH and temperature values are better when compared to the immobilized enzyme free enzyme

Entrapped laccase into (poly (vinyl alcohol)-Ca alginate) beads was found to have better temperature, pH and strorage stability when compared to the free enzyme. The immobilized enzymes were protected 75% of its original activities. These properties of immobilized laccase enzyme can be used for various biotechnological and industrial applications, such as in waste water treatment.

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REFERENCES

[1]. Laurent N., Haddoub R., Flitsch SL., Enzyme catalysis on solid surfaces, Trends Biotechnol., 26 (2008) 328–337.

[2]. Tischer W. and Kasche V., Immobilized enzymes: crystals or carriers, Trends Biotechnol., 17 (1999) 326–335.

[3]. Pang R., Li M., Zhang C., Degradation of phenolic compounds by laccase immobilized on carbon nanomaterials: Diffusional limitation investigation, Talanta., 131(2015) 38-45.

[4]. D'annibale A., Stazi S.R., Vinciguerra V., Mattia E.D., Sermanni G.G., Characterization of immobilized laccase from Lentinula edodes and its use in olive-mill wastewater treatment, Process Biochem., 34 (1999) 697–706.

[5]. D'annibale A., Stazi S.R., Vinciguerra V., Sermanni G., Oxirane-immobilized Lentinula edodes laccase: stability and phenolics removal efficiency in olive mill wastewater, J Biotechnol., **77** (2000) 265–273.

[6]. Jiang D.S., Long S.Y., Huang J., Xıao H.Y., Zhou J.Y., Immobilization of Pycnoporus sanguineus laccase on magnetic chitosan microspheres, Biochem. Eng. J., 25 (2005) 15–23.

[7]. Durán N., Rosa M.A., D'annibale A., Gianfreda L., Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: a review, Enzyme Microb. Tec., 31(2002) 907–931.

[8]. Jolivalta C., Brenon S., Caminade E., Mougin C., Pontié M., Immobilization of laccase from Trametes versicolor on a modified PVDF microfiltration membrane: characterization of the grafted support and application in removing a phenylurea pesticide in wastewater, J. Membrane Sci., 180(2000) 103–113.

[9]. Wan Y., Lu R., Xiao L., Du Y., Miyakoshi T., Chen C., Knill C., Kennedy J., Effects of organic solvents on the activity of free and immobilised laccase from Rhus vernicifera, Int. J. Biol. Macromol., 47 (2010) 488–495.

[10]. Rotkova J., Sulakova R., Korecka L., Zdrazilova P., Jandova M., Lenfeld J., Horak D., Bilkova Z., Laccase immobilized on magnetic carriers for biotechnology applications, J. Magn. Mater., 321 (2009) 1335–1340.

[11]. Curulli A., Cusma A., Kaciulis S., Padeletti G., Pandolfi L., Valentini F., Vitocelli M., Immobilization of GOD and HRP enzyme on nanostructured substrates, Surf. Interface Anal., 38 (2006) 478–481.

[12]. Gokgoz M. and Altinok H., Immobilization of laccase on polyacrylamide and polyacrylamide - κ - carragennan-based semi-interpenetrating polymer networks, Artificial Cells, Blood Substitutes, and Biotechnology, 40 (2012) 326–330.

[13]. Yamak O., Kalkan N.A., Aksoy S., Altinok H., Hasirci N., Semi-interpenetrating polymer networks (semi-IPNs) for entrapment of laccase and their use in Acid Orange 52 decolorization, Process Biochemistry, 44 (2009) 440-445.

[14]. Koklukaya S.Z., Sezer S., Aksoy S., Hasirci N., Polyacrylamide-based semi-interpenetrating networks for entrapment of laccase and their use in azo dye decolorization, Biotechnology and Applied Biochemistry, 63(5) (2016) 699-707.

[15]. Makas Y.G. , Kalkan N.A., Aksoy S., Altinok H., Hasirci N., Immobilization of laccase in -carrageenan based semi-interpenetrating polymer Networks, Journal of Biotechnology, 148 (2010) 216–220.

[16]. Leonowicz A. and Grzywnowicz K., Quantitative estimation of laccase forms in some white rot fungi using syringaldazine as a substrate, Enzyme Microb. Tech., 3 (1981) 55–58.

[17]. Lante A., Crapisi A., Krastanov A., Spettoli P., Biodegradation of phenols by laccase immobilised in a membrane reactor, Process Biochem., 36 (2000) 51–58.

[18]. Al-Adhami A.J.H., Bryjak J., Markiewicz B.G., Chozch W.P., Immobilization of woodrotting fungi laccases on modified

cellulose and acrylic carriers, Process Biochem., 37 (2002)1387–1394.

[19]. Dodor D.E., Hwang H., Ekunwe S., Oxidation of anthracene and benzo[a]pyrene by immobilized laccase from Trametes versicolor, Enzyme Microb. Tech., 35 (2004) 210–217.

[20]. Fang H., Huang J., Ding L., Li M., Chen Z., Preparation of magnetic Chitosan nanoparticles and immobilization of Laccase, Journal of Wuhan University of Technology-Mater. Sci. Ed., 24 (2009) 42-47.

[21]. Yang W.Y., Min D.Y., Xiao S.W., Jin L., Rong L., Tetsuo M., Bo C., Immobilization and characterization of laccase from Chinese Rhus vernicifera on modified chitosan, Process Biochem., 41 (2006) 1378-1382.

[22]. Lineweaver H. and Burk D.J., The determination of enzyme dissociation constant, Am. Chem. Soc., 56 (1934) 658–666.

[23]. Xiao H., Huang J., Liu C., Jiang D., Immobilization of Laccase on amine-terminated magnetic nano-composite by glutaraldehyde crosslinking method, T. Nonferr. Metal Soc., 16 (2006) 414-418.

[24]. Zamora P. P., Pereira M. C., Tiburtius R. L., Rosa M.A., Minussi C.R., Duran N., Decolarization of reactive dyes by immobilized Laccase, Appl. Catal B-Environ ., 42 (2003) 131-144.