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Research Article

Determination of Optimum Immobilization Conditions of *Trametes versicolor* Laccase with Sodium Alginate Beads

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Abstract

Laccase (*EC* 1.10.3.2) is a multi copper enzyme that catalyzes the oxidation of various environmental pollutants such as phenolic compounds. The efficiency of the enzyme for environmental and industrial applications can be increased by immobilizing the enzyme on a carrier. In the present study, laccase obtained from *Trametes versicolor* (fungi) was immobilized on sodium alginate beads and kappa-carrageenan and the effect of contact time, pH, temperature, the amount of carrier and enzyme concentration were investigated to determine optimum conditions of laccase immobilization. Sodium alginate beads were chosen as the most efficient carrier due to their high immobilization yield. Maximum laccase immobilization was determined as 30 min of contact time, pH 4.5, 30 °C of temperature, 200 mg of sodium alginate amount.

Keywords: Laccase, sodium alginate, kappa-carrageenan, immobilization.
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Aljinat Boncukları ile *Trametes versicolor* Lakkazının Optimum İmmobilizasyon Koşullarının Belirlenmesi

Özet

Lakkaz (*EC* 1.10.3.2), fenolik bileşikler gibi çeşitli çevresel kirleticilerin oksidasyonunu katalizleyen çoklu bakır enzimidir. Çevresel ve endüstriyel uygulamalar için bir taşıyıcı üzerinde enzimin immobilize edilmesiyle etkinliği arttırılabilir. Bu çalışmada, *Trametes versicolor'dan* elde edilen lakkaz sodium aljinat ve kappa-karagen üzerinde immobilize edilmiş, temas süresi, pH, sıcaklık, taşıyıcı miktarı, enzim konsantrasyonun etkisi değerlendirilmiştir. Sodyum aljinat ve kappa-karragenanın immobilizasyon etkinliği araştırılmıştır. Sonuç olarak, sodyum aljinat boncukları yüksek immobilizasyon veriminden dolayı en iyi taşıyıcı olarak seçilmiştir. Maksimum lakkaz immobilizasyonu, 30 dakika temas süresi, pH 4.5, 30 °C, 200 mg sodyum aljinat koşullarında elde edilmiştir.

Anahtar Kelimeler: Lakkaz, sodyum aljinat, kappa-karragenan, immobilizasyon

Introduction

Trametes versicolor and *Phanerochaete chrysosporium* are fungi that produce lignolytic enzymes such as lignin peroxidase, Mn-dependent peroxidase and laccase (Thurston 1994; Orth and Tien 1995). Laccases (*EC* 1.10.3.2), are multi copper enzymes cata- lyzing the oxidation of different phenolic compounds. Furthermore, laccases have been stimulated by their potential use in detoxification of environmental pollutants, paper processing, enzymatic conversion of chemical intermediates, and the production of useful chemicals from lignin (Duran and Esposito 2000; Jia Li and Yi Zheng 2004).

Various complex aromatic compounds and some xenobiotics may be degraded by a few organisms especially white-rot fungi. The interest for extracellular enzymes obtained from white-rot fungi has increased due to their potential to degrade both highly toxic phenolic compounds and lignin (Mansour et al. 1998; Gianfreda and Rao 2004).

The practical use of an enzyme in bioremediation applications can be increased by immobilizing the enzyme on a solid carrier and/or entrapment. This process can lead to the possi- bility of their reuse via operational stability and durability of the enzyme. Immobilization of laccase on appropriate polymeric supports may principally provide advantages (Brandi et al. 2006) such as the easy separation of reaction products from the reaction mixture. Indeed, immobilization can protect laccase from denaturation by organic co-solvents, thereby extending its half-life (Brandi et al. 2006; D'Annibale et al. 2000; Palmieri et al. 1994). Furthermore, some advantages have been reported for several immobilized laccase preparations (Brandi et al. 2006; Duran et al. 2012).

In the present study, laccase obtained from *Trametes versicolor* was immobilized on two different carriers, sodium alginate beads and kappa-carrageenan. In order to determine the optimum conditions of laccase immobilization, the effect of contact time, pH, temperature, the amount of sodium alginate and enzyme concentration were systematically investigated.

Materials and methods

Culture conditions and laccase production
Trametes versicolor ATCC *T. versicolor* ATCC200801 used in laccase enzyme production studies was obtained from Amrican Type Culture Collection of USA. Stock cultures of organism were maintained by growing the fungus on malt agar slants according to the procedure suggested previously (Ünal et al. 2011). Mycelial suspension of stock culture of T.versicolor prepared by suspending the stock culture of the organism in 5 ml of steril deionized water was used as inoculation source during the experiments. 250 mL conical flasks containing 100 mL of relavant culture medium were inoculated with 1 mL of mycelia suspension and allowed to incubate at 30 °C in an incubator shaker agitating at 150 rpm for 12 days . Culture media, Mycologic Liquid Medium (MLM), Stock Basal Medium (SBM), and Modified Vogel's Medium (MVM) used in experiments were prepared according to the description given by various researchers (Paice and Jurasek 1989; Forney and Reddy 1979; Aktaş 1999).

Culture filtrates obtained from *T.versicolor* cultures by using the procedure described by Ünal et al. 2011 was used as enzyme source in the experiments.

Enzyme immobilization

The methods described by Rozie et al. (1988) for alginate beads immobilization and by Eikmeier et al. (1984) for kappa-carrageenan beads immobilization were employed. Alginate and kappa-carragenan beads were maintained in 0.03 M CaCl2 and 0.9 % KCl, respectively, at +4 °C until use (Ünal et al. 2011).

Laccase assay

As was described in our previous study 0.1 ml of enzyme source, 4.9 ml of 0.1 M acetate buffer (pH 4.5) and 1 mM guaiacol (Ünal et al. 2011) as substrate were utilized for enzyme ac- tivity measurements. The reaction mixture pre- pared was incubated at 37 °C for 5 min. Blanks contained inactive enzyme boiling enzyme source (Ünal et al. 2011). Enzyme activity in the tubes was colorimetrically measured by a spectrophotometer (Jenway 6105 UV/VIS)

at 465 nm wavelength. One unit of activity was defined as enzyme activity that elicited an increase in A465 of 0.1 absorbance unit per minute (Ünal et al. 2011).

Results

In this study, *Trametes versicolor* has been found to have maximum laccase production (6.1 U/mL) capacity when it was grown in M.V.M as com- pared with the other culture media examined (Fig.1). Therefore M.V.M was chosen and used as appropriate growth medium to produce lac- case from *Trametes versicolor* for the laccase assays. Na-alginate beads prepared for laccase immo- bilazition had much more stabile and homoge- nic morphology than of the cappa-carrageenan beads (Fig.2). On the other hand, immobilization efficiency of sodium alginate was superior to kappa-carrageenan (Fig.3). For these reasons sodium alginate beads were chosen as carrier in further studies dealing with immobilization. Earlier investigations have reported different carriers of enzyme immobilization such as alginate, magnetite, chitin, polyurethane (Datta et al. 2013). Between many different carriers as a supporting material for the immobilization of laccase, sodium alginate was found the most effective matrix (Kirkpatrick et al. 1990; Wada et al. 1992; Wada et al. 1993; Sun and Payne 1996; Duran and Esposito 2000). In the present study, it is also found that sodium algi- nate may be a useful carrier of immobilization for laccase, due to observed low activity lost (18.4%) both during immobilization procedure and storage in 0.03 M CaCl2. Also, immobi- lized laccase was found to be very stable over a long period. The same findings were reported by some earlier researchers (Leonowicz et al. 1988; Cho et al. 2008). Accordingly sodium alginate was chosen as immobilization material in the present study for *Trametes versicolor* laccase.

According to the results of the experiment to find out the effect of contact time, it was observed that there was a nearly linear increase in relative enzyme activity and % immobilization up to 30 min of period (Fig.4) and in following periods activity and % immobilization did not significantly change with contact time.

The effect of pH on the immobilization of laccase is presented in Fig.5. Similar trends were observed with % immobilization and rela- tive enzyme activity between pH 3 -10. Relative enzyme activity was increased with increasing pH from 3.0 to 4.5. Also, enzyme activity and

% immobilization were decreased with increas- ing pH from 5.0 to 10. Maximum enzyme activ- ity was observed at pH 4.5.

The effect of the temperature on the immobilization process was investigated at a constant value at pH 4.5 and 30 min contact time. The variation of the % immobilization and enzyme activity with temperature is presented in Fig. 6. As it is seen from this figure the immobilization of laccase appears to be temperature dependent in the temperature range studied. When the temperature was increased from 4 to 50 percentage of immobilization increased from 2,24 % to 59,93 %. However, enzyme activity was highly affected with temperature after 30 °C.

It has been determined that significant increase of relative enzyme activity was observed in 200 mg sodium alginate and that the relative enzyme activity of immobilized laccase did not significantly change up to 800 mg sodium alginate (Fig.7). While the amount of % immobilization increased with the increasing of sodium alginate concentration as expected, there was no change in relative enzyme activity

The effect of enzyme concentration was investigated in the range of 3.54 to 22.52 mg ml-

1. The experiments were performed under the determined optimum contact time, pH values, temperature and amount of sodium alginate. The results are presented in Fig.8. With increase in the laccase concentration from 3.54 mg ml-1 to

22.67 mg ml^{-1} the percentage immobilization was increased from 33.93% to 81.18% which is the maximum value obtained as the amount of enzyme are increased. Further increase up to

22.52 mg ml^{-1} did not change the maximum immobilization capacity of the sodium alginate beads which stayed almost constant.

Discussion

Since it has broad subtrate specifity and large biotechnological applications, the pro- duction of laccase from appropriate sources, optimization of conditions for production of the enzyme and enhancing its stability immobilising by appropriate matrix have been subject of various investigations. Although laccase is found in a wide range of higher plants and fun- gi (Leontievsk et al. 1997 and Kiiskinen et al. 2004), some of white-rot fungal strains seems to be more potent laccase producer than the other organisms examined in this respect (Bourbonnais 1995; Leontievsk et al. 1997). In this study, a member of white-rot fungi, *Trametes versicolor,* which has been reported as a good laccase producer (Arcand and Archibald 1991; Limura et al. 1996; Taşpinar and Kolankaya 1998; Novotny 2004) was selected as laccase source for the production of the enzyme needed to use in immobilization studies. The data that we have obtained during the studies shows that produc- tion yield of laccase activity seems to be associ- ated with culture conditions such as the chem- ical composition of culture medium. However, higher laccase activity was yielded when we have grown *T.versicolor* in the culture medium,

M.V.M comparing with others examined. This means that culture conditions such as chemical composition of culture medium is affecting the level of enzyme activity. Similar data were also obtained in other studies aiming to find out the effect of culture conditions on laccase produc- tion by some white-rot fungi (Dong et al.2005; Couto and Toca-Herrera 2007).

This is the first time that the conditions of immobilization for the laccase obtained from *Trametes versicolor* grown in very productive culture medium, M.V.M., is studied. There are number of studies reporting that Na-alginate is most effective matrix for immobilization of laccase enzyme (Kirkpatrick et al. 1990; Wada et al. 1992; Wada et al. 1993; Sun and Payne 1996; Duran and Esposito 2000). The results of our present study indicate that there is very low activity lost both during immobilization procedure and storage period of immobilized laccase when Na-alginate beads were used as supporting material. Similar findings dealing with low activity lost of laccase due to its immobilization by Na-alginate have been announced by some investigators (Leonowicz et al. 1988; Cho et al. 2008). Taking the results of our study into consideration, we can offer Na-alginate as an appropriate supporting material for the immobilization of laccase enzyme.

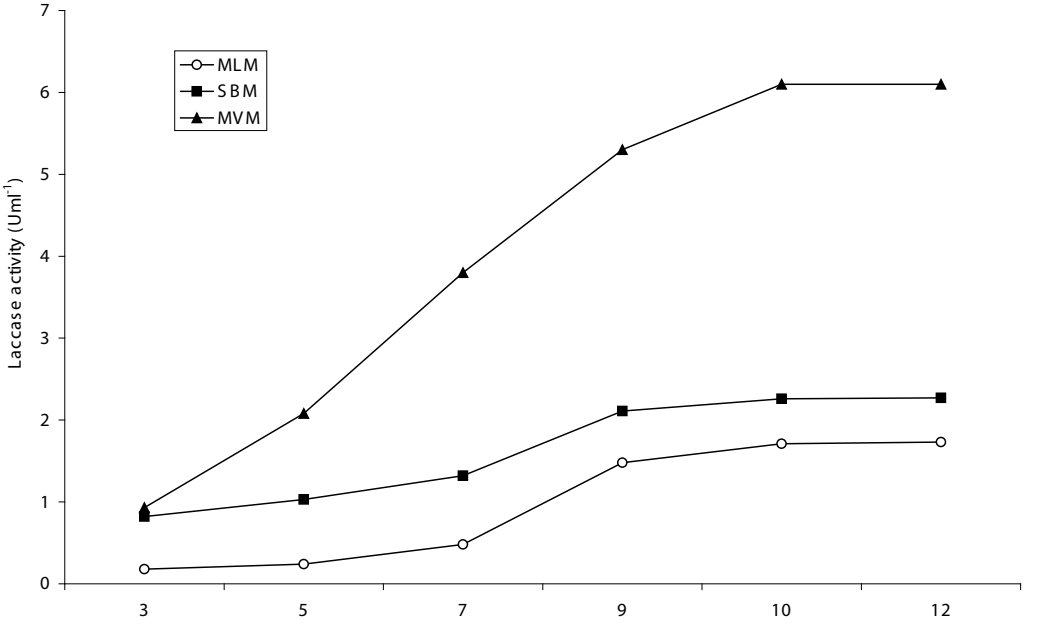

Figure 1. Laccase enzyme activity of *T. versicolor* in three different culture media: M.L.M., S.B.M. and M.V.M.

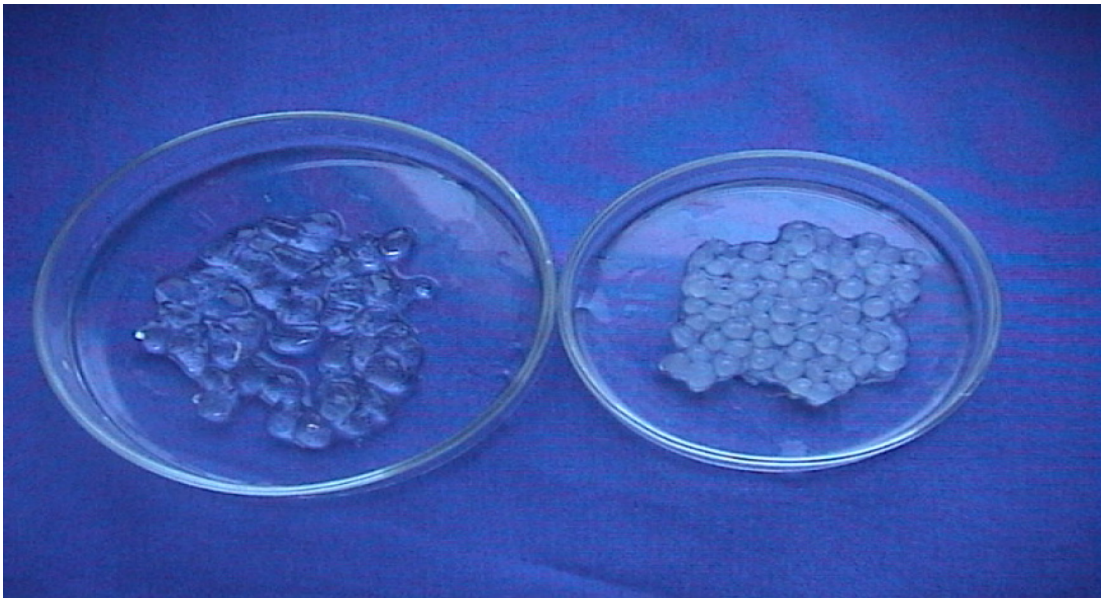

Figure 2. Typical morphology of kappa-carragenan (a) and sodium alginate beads (b).

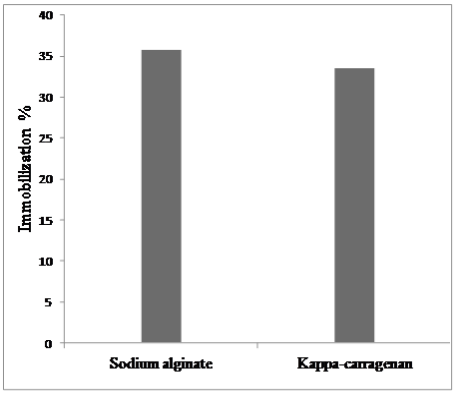

Figure 3. Immobilization efficiency of sodium alginate beads and kappa-carrageenan beads.

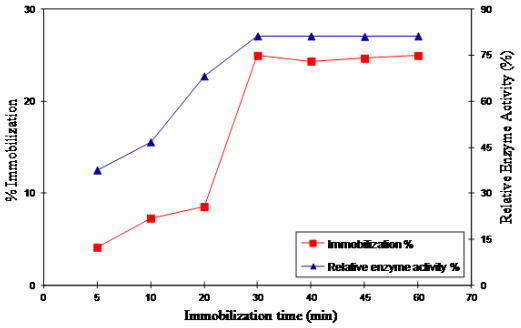

Figure 4. The effect of immobilization yield and relative enzyme activity of immobilization time.

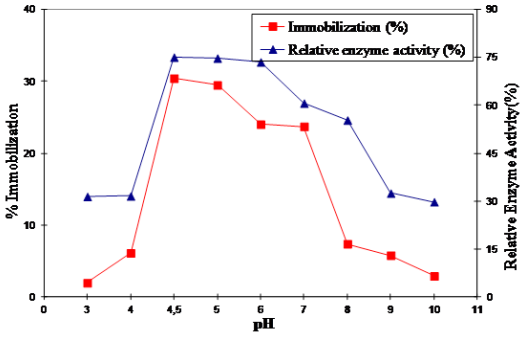

Figure 5. The effect of pH.on immobilization yield and relative enzyme activity.

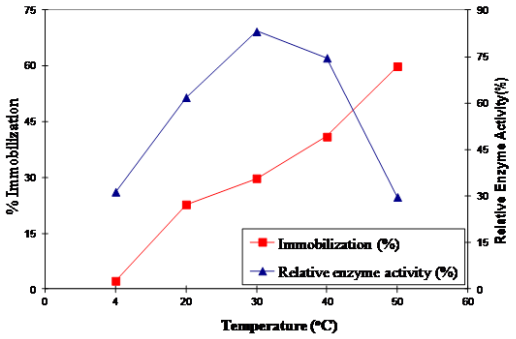

Figure 6. The effect of temperature on the immobilization yield and relative enzyme activity.

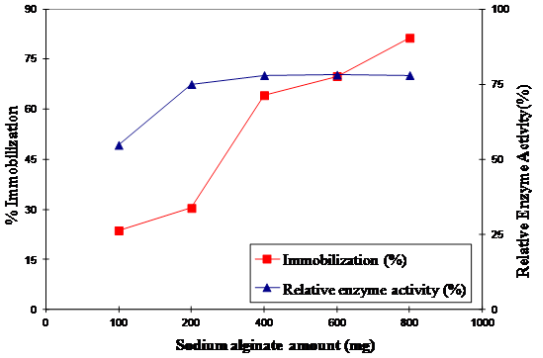

Figure 7. The effect of sodium alginate beads amount on immobilization yield and relative enzyme activity.

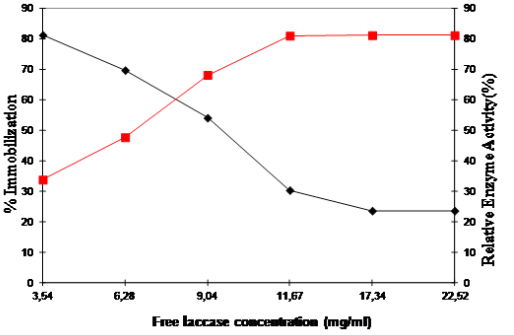

Figure 8. The effect of immobilization yield and relative enzyme activity of free laccase enzyme concentration.

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