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THE CATALYTIC ACTIVITY OF LACCASE IMMOBILIZED ONTO FREE AND SURFACTANT MODIFIED SILICA AEROGELS

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ABSTRACT

The free silica aerogel (FSA) and surfactant (cetyltrimethylammonium bromide) modified silica aerogel (MSA) were synthesized and used as supports for laccase immobilization carried out through adsorption process. The results show that the MSA performed higher laccase adsorption (0.71µmol/g) as compare to FSA (0.22µmol/g). In addition, the enhancement of the catalytic activity of the MSAL was also observed. These results demonstrated that the surface modification of silica aerogel using cationic surfactant (cetyltrimethylammonium bromide) gave higher immobilized mass and catalytic activity of laccase which can be potentially used for degradation of organic micropollutants such dyes, pesticides and antibiotics.

1. INTRODUCTION

Laccase is an extracellular enzyme having molecular weight of approximately 60-70 kDa and acidic isoelectric point of around pH 4 (Baldrian, 2006). It is a monomeric protein, glycoprotein covalently linked to carbohydrate moieties (10 - 45 %) that contributes to its high stability (Duran *et al*., 2002).

Laccase has variety of applications in biotechnology because of its high activity, selectivity and specificity (Mateo *et al*., 2007). It finds applications in oxidizing a wide range of pollutants released from several industries, as biosensors to detect phenolic compound in food industries, as a tool for bioremediation agent to clean up herbicides, pesticides and certain explosive in soil, as a medical diagnostics, used in purification system as a cleaning agent, as a catalyst of anti-cancer drug, and also used in the cosmetics ((Kunamneni *et al*., 2008; Jeon *et al*., 2010).

The industrial use of the laccase is however still limited due to their relative instable under operational conditions such as high temperature. The exposure to other denaturants and organic solvents through the direct contact of laccase with organic solvents affect the catalytically active conformation of the laccase (Markvicheva *et al*., 2005). Therefore, laccase immobilization technology was established to reduce these limitations by increasing the thermostability of the laccase and also it's resistant to the extreme conditions and chemical reagents. Recently, various immobilization methods of laccase have been reported such as covalent bonding, cross-linking, adsorption and entrapment (Edet *et al*., 2013). Among these methods, adsorption is the simplest since it does not require any pre-activation step of the supports which means that the laccase is attached to the matrices of the supports through the non-covalent interactions either by the hydrogen bonding, van der Walls forces, or hydrophobic interactions via the salt linkage (Brena and Viera, 2010; Costa *et al*., 2004). Consequently, many supports have been proposed for immobilization of laccase (Duran *et al*., 2002). These include organic support such as natural polymers or synthetic polymers and inorganic supports such as silica gels (Brena and Viera, 2010)

In this study, the free silica aerogel (FSA) and surfactant modified silica aerogel (MSA) were used as supports for the laccase immobilization. The choice of silica aerogel because it has a large pore size and surface area that can enhance laccase adsorption and stability in wide range of pH and temperature. The modification of synthesized silica aerogel with surfactant (cethyltrimethylammonium bromide, CTAB) (MSA) is to further enhance the surface activity that promote higher laccase adsorption and stability.

2. EXPERIMENTAL

2.1 Materials

Triethoxysilane (TEOS, 98%) was purchased from ACROS Organics (USA). Meanwhile, hydrochloric acid (37%), triethylamine (TEA), 2-propanol, hexane, cetyltrimethylammonium bromide (CTAB), and sodium acetate were purchased from Merck (Germany). 2,2'-Azino-bis(3-ethylbenzoline-6-sulfonic acid) diammonium salt (ABTS), potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Sigma-Aldrich (USA). An industrial grade laccase enzyme of Trametes sp. (30% purity) was purchased from Daiwa Kasei Co. Ltd. (Japan).

2.2 Preparation of silica aerogel (FSA)

Silica aerogel was synthesized using two-step process namely hydrolysis and condensation. Initially, TEOS as a precursor was stirred with the mixture of deionized water and 2-propoanol as a solvent. Then, 0.05 M HCl was dropped and solution was stirred for 12 hours before the TEA was added as a gelatin agent. The aged gel was undergone solvent exchange with hexane:TEOS:2-propanol (3:1:1) at 30±0.5°C for 24 hours in temperature-controlled shaker. Then, the silica aerogel was washed with 2-propanol three times followed by one time with deionized water. The silica aerogel was then dried at 105±0.5°C in oven for two days and kept in desiccator for future use. This silica aerogel sample was denoted as a free silica aerogel (FSA).

2.3 Preparation of CTAB-silica aerogel (MSA)

The CTAB-silica aerogel (MSA) was prepared by mixing 40 mg FSA with 20 mL of 2 mM CTAB solution at 30±0.5°C in the conical flask. The flask was then shaken at 250 rpm for six time intervals within two hours experimental period. The silica aerogel was separated by filtration then washed several times with double-distilled water, dried at 105±0.5°C in oven for a day, and finally kept in the desiccator for future use.

2.4 Laccase immobilization on FSA and MSA

An accurately weight of laccase powder was dissolved in 3mL 0.01 M phosphate buffer solution. The 150 mg FSA or MSA was then added to the solution and stirred for a period of an hour at room temperature (30±0.5°C). The immobilized laccase was separated from the laccase solution by centrifugation (2000 rpm, 10 minutes). The residual laccase in the solution was determined by using UV-Vis spectrophotometer (Perkin Elmer model Lambda 35 (USA) at 280 nm. This allowed calculating the quantity of the laccase adsorbed on the support by using the mass balance. The immobilized laccase (FSAL or MSAL) was freeze dried for 18 hours which was sufficiently long enough to achieved constant mass. After dried, the solid FSAL or MSAL was undergoes the catalytic activity assay.

The immobilization process was investigated at for experimental conditions namely immobilizing pH (3, 4, 5, 7, and 8), initial concentrations of CTAB (0.5. 1, 2, 5, and 10 Mm) and initial concentrations of laccase (0.01, 0.03, 0.05, 0.5, and 0.8 mM).

2.5 Enzyme catalytic activity assay

ABTS is a commonly used substrate to study the reaction kinetics of the multicopper oxidase enzymes including laccase. It was employed as a substrate in the laccase activity assay due to the ability of the laccase to transform the ABTS to radical cation $ABTS^+$ which can be identified by the UV-Vis adsorption peak at 436 nm.

In this study, the catalytic activity was calculated based on Beer-Lambert law where the catalytic activity (U) was defined as the necessary amount laccase for producing 1 µmol of $ABTS^+$ from ABTS per minute which can be calculated using Eq. 1.

$$U = \frac{10^6 \Delta A}{\varepsilon . l \ \Delta t} \qquad (1)$$

where U is the laccase activity (µmol/L.min), ΔA is the increase in absorbance at 436 nm, Δt is the reaction time (min.), ε is the absorptivity (29,300 $M^{-1}.cm^{-1}$) and l is the cuvette path length (cm).

3. Results and Discussion

3.1 Immobilization of laccase

The immobilization capacity of silica aerogels is shown in Fig. 1. When the laccase concentration was 0.05Mm and the pH value of 5.0, the adsorption equilibrium was achieved in less than an hour for both FSA and MSA which was due to the large surface area and surface morphology of silica aerogels (Huang, 2013). In addition, coating silica aerogel with CTAB promoted higher immobilized laccase as observed for the MSA.

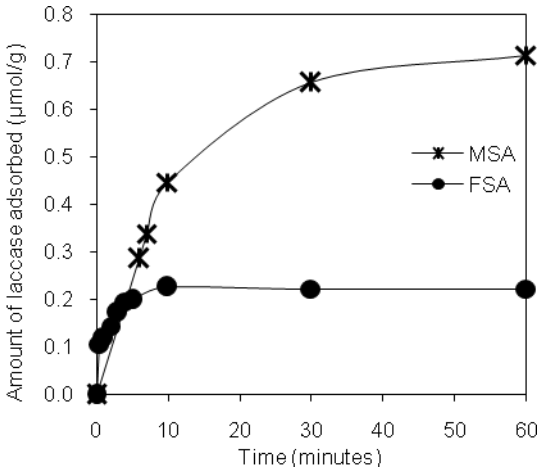
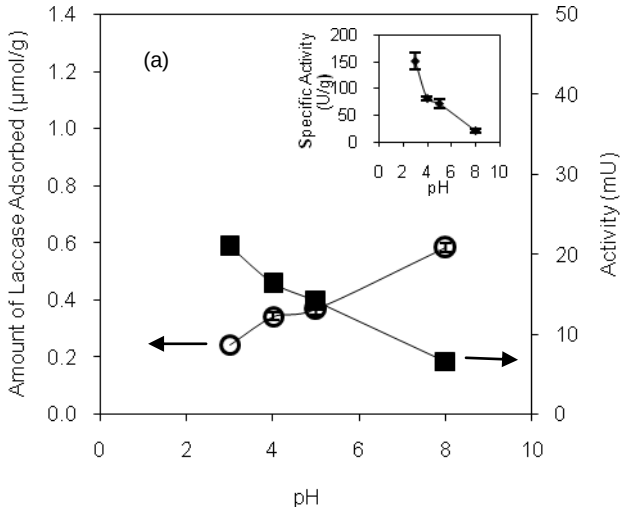


Fig. 1 Effect of contact time on laccase immobilization onto silica aerogel surfaces

3.2 Specific activity of the immobilized laccase

The mass of laccase immobilized by MSA (Fig. 2 (b)) was high as compare to FSA (Fig 2(a)). Fig. 2 indicates that for both FSA and MSA, the increase of solution pH resulted in the decrease of the activity of laccase. It can be seen that the optimum pH for laccase immobilization for both FSA and MSA was in an acidic pH range (Baldrian, 2005). As previously reported by Leonowicz *et al.* (1984), the stability of laccase is generally higher in acidic solution.



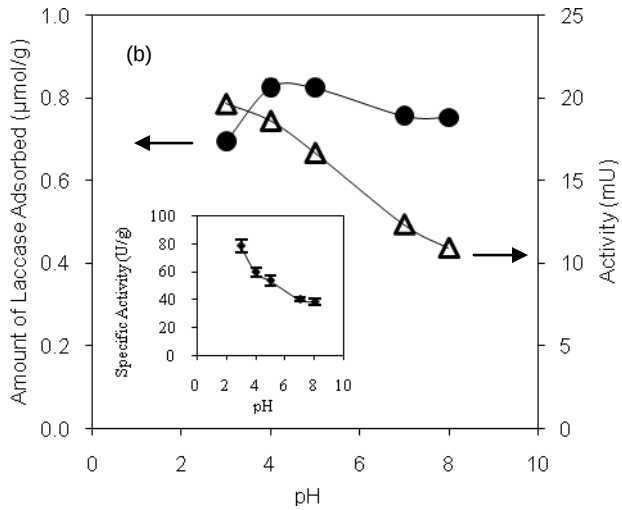


Fig. 2 Effect of pH on laccase immobilization onto (a) FSA and (b) MSA

The effect of CTAB concentration on laccase immobilization is shown in Fig. 3. The mass of immobilized laccase increased with increasing of the CTAB concentration and became almost constant after the CTAB concentration higher than 2mM in which might be due the formation micelles. The critical micelle concentration (CMC) of the CTAB is ~1mM (Li *et al*., 2002). As compared to FSA, MSA shows higher laccase immobilization due to strong positive charge of CTAB which adsorbed on the surface of the silica aerogel attracted to the opposite charge of laccase (Baldrian, 2004). At pH 4 and above, the laccase will have negative charge.

Fig. 3 also shows that the activity of laccase increased with increasing of CTAB concentrations until it becomes almost constant at 5mM CTAB concentration. This is due to large amount of laccase immobilized on the CTAB modified silica surface.

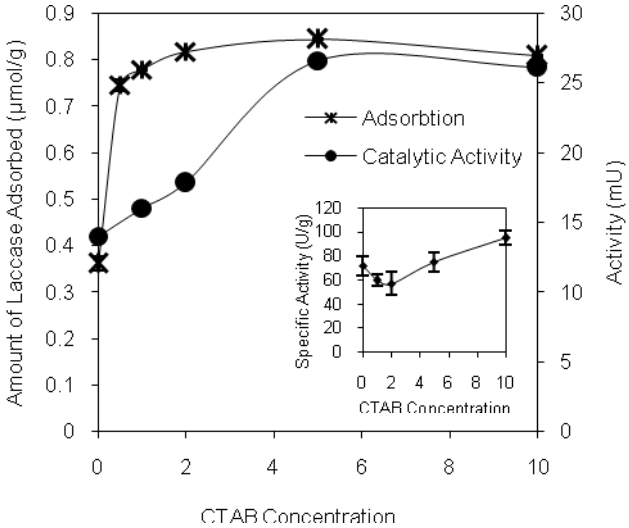


Fig. 3 Effect of CTAB concentrations on laccase immobilization onto silica aerogel

CONCLUSION

Laccase was successful immobilized on free silica aerogel (FSA) and CTAB modified silica aerogel (MSA). The optimum immobilization pH for both FSA and MSA was at pH 5. The mass of laccase immobilized onto MSA was found to be 70% higher than FSA; and thus higher activity of laccase was obtained. The increase of CTAB concentration above 2mM did not give any significant increased in immobilized laccase which might be due to the formation of CTAB micelles. The laccase activity (mU) increased with increasing immobilized laccase mass.

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