Degradation of Phenol in Waste Water using Alcohol-Free Enzyme Encapsulated Silica Aerogel

Nor Suriani Sani, Lee Siew Ling, Mohd Nazlan Mohd Muhid and **Halimaton Hamdan**

Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia (UTM), 81310 Skudai, Malaysia, hali@kimia.fs.utm.my

ABSTRACT

Tyrosinase encapsulated silica aerogel (TESA) was prepared via an alcohol-free colloidal sol-gel route using organic-silica source at room temperature and neutral pH. The enzymatic activity of TESA; assayed through the reduction of ascorbic acid, was enhanced to higher temperature and a wider pH range. At pH 4 and 9, oxidation of catechol (phenol substitute) by TESA after 60 minutes of reaction was up to 30% with enzymatic activity retained at 80 °C. In contrast, free tyrosinase was totally inactive at these pH values and temperature exceeding 55 °C. The stability of tyrosinase towards extreme temperature as well as acidic and basic conditions is significantly improved with good reusability. 90% of phenol was removed in aqueous solution after three hours of contact time with TESA.

Keywords: silica aerogel, encapsulation, enzyme, tyrosinase, phenol

1 INTRODUCTION

Phenols constitute one of the major classes of pollutants. Due to its toxicity, the concentration of phenol which is greater than 50 ppb is harmful to some aquatic species and the ingestion of 1 g phenol can be fatal in humans. Hence, the removal of phenol in water is important. Conventional methods for removing phenol from industrial waste suffer from serious drawbacks such as high cost, incompleteness of purification, formation of hazardous by-products and applicability to only a limited phenol concentration range.[1-5] Enzymatic treatment has been proposed by many researchers as a convenient method for removing phenol.[6] Enzymes are highly selective and can effectively treat phenol even in dilute wastes.

Tyrosinase was demonstrated to remove phenols and aromatic amines from phenolic industrial effluents; being highly selective and effectively treat phenol even in dilute wastes, operate over a broad aromatic concentration range and require low retention times with respect to other treatment methods. Tyrosinase oxidizes numerous phenols, generates corresponding phenoxy radicals which diffuse from the active centre into solution to react with phenol and form substances that are much less water soluble. These insoluble polymers then precipitate out of the solution and can be separated by simple filtration or flocculation. However, contamination due to the remaining soluble enzyme and non-precipitated products in the aquatic solution after treatment is of concern, especially in drinking water. Furthermore, enzymes are easily denatured, relatively expensive and extremely sensitive to the environment conditions.[7] Free enzymes can be used only once as they are generally soluble in aqueous solutions and not easily recovered.

In order to increase reusability and enhance stability, enzymes are often immobilized on the surface of insoluble supports by physical or chemical means.[8-9] Silica aerogel, made by the sol gel process is a promising platform for the encapsulation of enzymes. Upon encapsulation, the polymeric framework of silica aerogel, grows around the enzymes, creates a cage and protects the enzyme either from aggregation and unfolding or from microbial attack. Therefore, the encapsulated enzymes often retain a sufficient level of activity and functionality presumably because of sufficient retention of their native state conformations. Meanwhile, the matrix pores allow reactant molecules to diffuse through and interact with the encapsulated enzymes. Eventually, encapsulation may improve the activity and storage stability of the enzymes and facilitate its application, recovery and washing.

In most reported applications of silica aerogel, orthosilicate such as tetramethyl orthosilicate (TMOS) or tetraethyl orthosilicate (TEOS) has been used as the silica source. Despite the fact that these chemicals contain relatively pure silica, 70% reduction of enzymatic activity was reported when lipase was encapsulated in TMOS-based silica matrix in 5% volume of methanol. The presence of alcohol is known to be detrimental to the activity of proteins; causing chain unfolding, aggregation, destruction of secondary and tertiary protein structures. Furthermore, such organic silicon precursors are usually too expensive; hence the production in an industrial scale is not economically viable.

In order to overcome these problems, rice husk ash was used as silica precursor in the preparation of sodium silicate required for the synthesis of silica aerogel.[10] This approach completely avoids the generation of alcohol and it

allows the encapsulation to be carried out at neutral pH in order to preserve biological activity of proteins. The organic based silica aerogel is chemically inert and it exhibits higher mechanical strength, enhanced thermal stability and negligible swelling in organic solvents compared to most organic polymers. Silica aerogel can also be tailored as a reservoir for water, thereby enhancing the ability to maintain the biological activity of entrapped enzymes. Eventually, aerogel encapsulation is expected to improve the storage stability of the enzyme and offers a protection for the enzyme against deterioration by the hydrophilic solvent.

2 EXPERIMENTAL

2.1 Synthesis of TESA

Encapsulation of tyrosinase (Fluka) in silica aerogel was carried out via the aqueous sol gel process. Rice husk ash was used as the silica source in producing sodium silicate solution. The silica sol, sulphuric acid and silicate solution were mixed to form a resultant solution with a pH value of ~7. Then, a mixture of tyrosinase and phosphate buffer solution was added to the solution to form a gel. The gel was aged at room temperature before it was washed with phosphate buffers to desorb any excess enzyme molecules. Finally, after drying at 36°C, tyrosinase encapsulated silica aerogel (TESA) was produced.

2.2 Assays of enzymatic activity

The catecholase activity of TESA was determined by monitoring the amount of depletion of ascorbic acid, based on its reaction in the oxidation of catechol to form dehydro-ascorbic acid and *o*-benzoquinone. Buffered substrate solution containing buffer solution (50 mM; pH 7), catechol solution (5.0 mM; pH 7), ascorbic acid (2.1 mM; pH 7) and EDTA acid solution (0.065 mM; pH 7) was incubated at room temperature and stirred gently. The buffered substrate solution was then monitored at 265 nm until the readings were constant. TESA (500 unit/mg) was placed in a beaker containing buffered substrate solution to initiate the oxidation of catechol (substrate). The suspension was filtered and analyzed.

2.3 Enzymatic ability test

The enzymatic ability of TESA and free enzyme at various temperatures and pH ranges were studied. Free tyrosinase and TESA were incubated in buffered substrate solution with pH 7 and temperature range of 5 °C to 70 °C. In order to study the stability in acidic and basic conditions, tyrosinase and TESA were incubated in buffered substrate solution at 25 °C with a pH range of 4 to 9.

2.4 The removal of phenol test

TESA and phosphate buffer (0.1 M, pH7) were put in an artificial phenolic waste water (2.0 mM). The reaction mixture was incubated under aerobic conditions using a stirrer. After the prescribed time, the sample was withdrawn and assayed for phenols by UV-Vis Spectroscopy and the disappearance absorption spectra of reaction solutions were measured.

2.5 Reusability

TESA was recycled for a different number of batches. For each batch, TESA was immersed in artificial phenolic waste water and the phenol degradation for each batch was monitored using UV-Vis Spectrophotometer. Samples were collected at regular intervals for each batch.

2.6 Leaching study

Control studies were designed to confirm that the activity was due to TESA rather than other species present in the reaction vessel. It was undertaken to assess the oxidation of catechol upon exposure to the support alone and to determine if tyrosinase was desorbed from the support and contributed to the oxidation of catechol. Sample from TESA was analysed in the same way using UV-Vis Spectroscopy. The filtrate was re-monitored 48 hours later at 265 nm.

3 RESULTS AND DISCUSSION

3.1 Synthesis of TESA

The UV-Vis spectra of TESA (Fig. 1) show an absorption band at 291 nm while the band of commercial tyrosinase and isolated tyrosinase are at 289 nm and 292 nm, respectively. The absorption bands remained relatively unchanged, suggesting that the enzyme conformation was maintained to a considerable extent after encapsulation. The identical absorbance spectra of free tyrosinase and TESA indicate the success of encapsulation of tyrosinase (95%) into porous silica aerogel via sol-gel process.

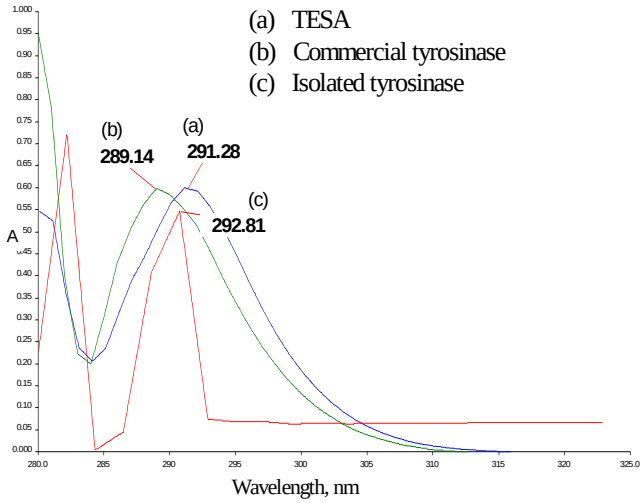

Fig. 1: UV-vis spectra of TESA

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3.2 Assays of enzymatic activity

Fig. 2 shows the enzymatic activity upon oxidation of catechol using free tyrosinase and TESA at pH 7. The amount of tyrosinase in this study was 250 units. It is evident that the free tyrosinase has a higher reduction value compared to the TESA and without the existence of catalyst. This phenomenon can be explained by the diffusion resistance of phenol in the silica aerogel network. Since the diffusion rate of the substrate was sufficiently slow compared to enzymatic catalysis, thus only the enzyme molecules which were encapsulated close to the surface of silica maerogel matrix could oxidise the catechol. Besides, a small amount of the enzymes may have been denatured during the encapsulation process, which led to the lower activity in the encapsulated tyrosinase.

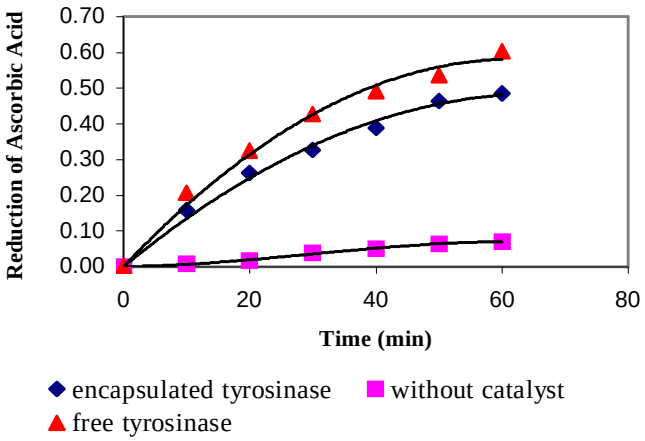

Fig 2: Enzymatic activity of TESA

3.3 Enzymatic ability test

The pH profiles for the enzymatic activity of free tyrosinase and TESA in the pH range of 4 to 9 depicted in Fig. 3 reveals that TESA and free tyrosinase achieved maximum activity at pH 7. Only 50% of activity was detected at pH 8 by using free tyrosinase due to denaturisation of enzymes and polymerization of quinones. During polymerization process, more stable and insoluble intermediates were produced. Accumulation of the intermediates could deactivate tyrosinase, thus, limiting further activity of tyrosinase. Removal of these polymerization products may be the key to improve performance of free tyrosinase at these pH values. Similarly, the enzymatic performance of the free tyrosinase was further decreased at pH 5. Only 5% of activity was detected by using free tyrosinase at pH 5. In contrast, up to 80% activity was achieved when tyrosinase was protected by encapsulation. At pH 4 and pH 9, TESA showed enhanced activity of 20% and 40% respectively, while free tyrosinase was completely inactive at these pH values, which demonstrated that TESA was active at a wider pH range than free tyrosinase and the stability of tyrosinase in acidic and basic conditions is significantly enhanced after encapsulation.

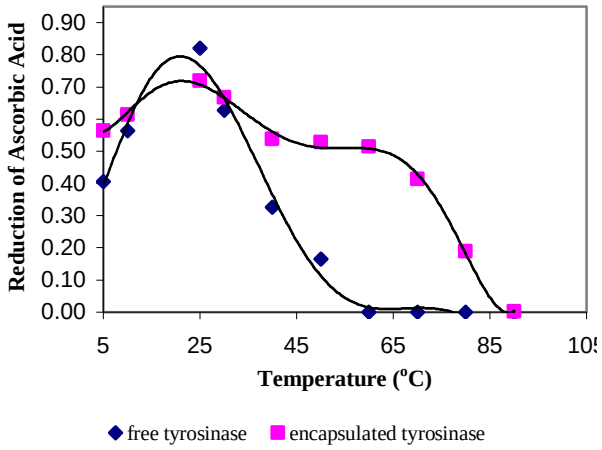

Fig. 3: Relative activity of TESA at different temperature

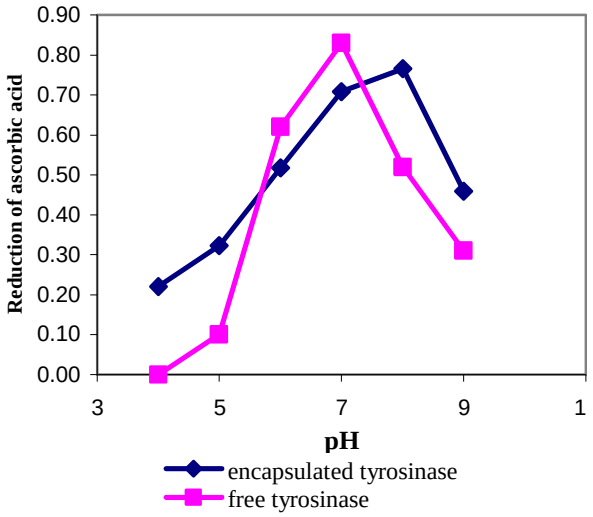

Fig. 4: Enzymatic activity of TESA and free tyrosinase at different temperature

The relative activity of TESA as a function of temperature subjected to 60 minutes of reaction time is presented in Fig. 4. It shows that long-term thermostability of tyrosinase was enhanced after the encapsulation process. The optimum temperature for both free tyrosinase and TESA was 25 °C. TESA was stable up to 70 °C at all contact periods; with loss of activity observed at 80 °C. For free tyrosinase, incubation at temperature above 40 °C was detrimental to enzyme activity and the activity began to decrease sharply when temperature was beyond 35 °C. The enhancement in thermostability of TESA was due to the tight confinement of tyrosinase in the silica aerogel matrix which acted as an insulator for tyrosinase.

3.4 The removal of phenol test

Fig. 5 shows that 70% of phenol was removed after 1 hour of contact with TESA. Evidently, the intraparticle mass transfer resistance affects the percentage of phenol removal. The small percentage of removal of phenol after 80 minutes of contact with TESA might have resulted from either chemical coupling of free radicals or generation of quinones catalyzed by tyrosinase. The occurrence of chemical coupling of free radicals during the reaction could prevent the intermediate complex of the enzyme-substrate for degrading phenol. The excess generation of quinones, which was the product of phenol degradation might have interfered with the activity of free tyrosinase and degrade phenol in the aqueous solution.

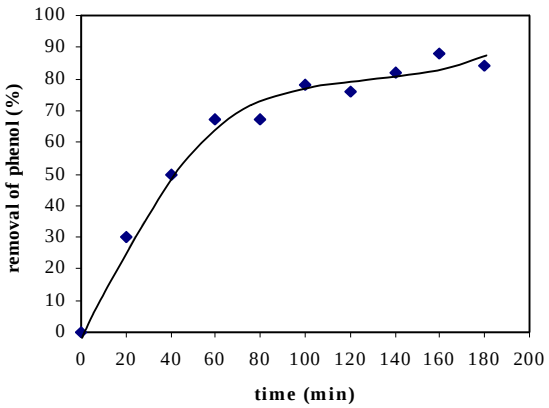

Fig 5: Removal of phenol by TESA

3.5 Reusability

In reusability study, TESA was reused 10 times, in each case; the removal of tyrosinase was studied after 60 minutes of reaction. The reduction over 10 recycles is shown in Fig. 6. The average activity over 10 batches was 60%. It is concluded that TESA is stable since each cycle gave essentially the same activity. It is clear that TESA is initially active which then levelled off due to product inhibition. The accumulation of the product on the support prevents TESA to undergo further reaction. Upon filtration of TESA from the substrate solution of each cycle, brown particles were observed on the recovered TESA suggesting the production of quinones or polyphenolics species which are known to be coloured.

3.6 Leaching study

No activity was observed when silica aerogel alone was immersed in the substrate, confirming that the identified activity was solely due to TESA. In contrast, TESA showed a slight change in the activity of the filtrate due to oxidation of catechol. However it was negligible since the activity was only 0.2% after 60 minutes of reaction. Therefore it suggests that most of the enzyme the molecules are sterically confined in the silica maerogel network. Since the enzyme was added prior to gelation, it is possible that a silica aerogel network was formed around the enzyme, indicating that most of the enzyme was effectively loaded.

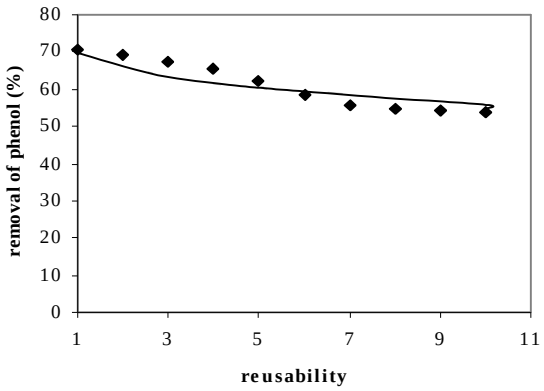

Fig 6: Reusability test of TESA

4 CONCLUSIONS

The optimum condition of enzyme tyrosinase encapsulated in silica aerogel (TESA) is at pH 7 and 35 °C. From the ability test, tyrosinase in silica aerogel is active at a wider pH and temperature range compared to the free tyrosinase. Enhancement of the stability towards acidic and basic conditions was observed when tyrosinase was encapsulated in silica maerogel. TESA removed phenol up to 70% in an hour with high reusability.

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