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Immobilization of α-amylase on Magnetic Fe_3O_4-Nanoparticle

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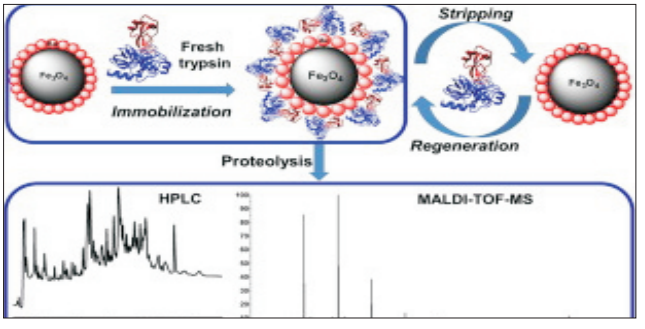
ABSTRACT

The aim of the study is to immobilize α-amylase from Trichoderma harzianum on magnetic Fe_3O_4-nanoparticles. The reusability of the immobilized a-amylase was examined. It was observed that the immobilized enzyme demonstrated 54% of its activity after 6 runs. The free and immobilized a-amylases exhibited pH optima at pH 6.0 and 6.5, respectively. The same temperature optimum for the free and immobilized a-amylases was detected at 50°C. The free and immobilized a-amylases were thermal stable up to 50°C for one h incubation, and retained 15% and 40% of its activity at 80°C, respectively. The Km values of the free and immobilized a-amylases were 3.77 and 2.5 mg starch, respectively. The low Km indicated the high affinity of enzyme toward substrate. Therefore, the results appeared that the affinity of immobilized a-amylase toward starch higher than that of free enzyme. The immobilized a-amylase had high resistance toward all examined metals compared with free enzyme. The EDTA as chelating agent had lower inhibition for the immobilized a-amylase than that for free enzyme. In conclusion, the immobilized a-amylase from T. harzianum could be used for several applications.

KEYWORDS:*Immobilization, α-amylase, magnetic Fe3O4-nanoparticles.*



INTRODUCTION:

α-Amylase is highly demanded in various sectors and is mainly used in food, brew, textile, and paper industries (Sivaramakrishnan *et al.*, 2006; Guilbot and Mercier, 1985). Moreover, the alkaline a-amylase is used as an additive to detergents to degrade residues of starchy foods (Mitidieri *et al.*, 2006). However, the use of free enzymes shows some significant problems, such as thermal instability, susceptibility to attack by proteases, activity inhibition, high sensitivity to several denaturing agents, and the impossibility of separating and reusing free catalysts at the end of the reaction. The enzyme immobilization resolved the most of these problems (O'fagain, 2003; Haring and Schreier, 1999).

Immobilized enzymes have several advantages such as a larger pH range and better thermal stability in addition to easy separation from the solution, making them appropriate as industrial catalysts (Straathof *et al.*, 2002; Batra and Gupta, 1994). Enzyme immobilization has the benefit of providing the practical convenience of a simple regeneration of support by removing the deactivated enzyme and reloading the support with a fresh batch of active catalysts (Brady and Jordaan, 2009). Enzyme immobilization enhanced enzyme activity, modification of substrate selectivity (Wang *et al.*, 2006). The performance of an immobilized enzyme depends greatly on the structure and character of

the carrier materials. Several strategies are currently used to improve carrier-based immobilization include enzyme entrapment achieved using a polymer network, encapsulation, solid support through covalent and non-covalent binding, and self-immobilization (Wang et al., 2006). Functionalized magnetic nanoparticles offer many advantages such as providing large surface areas for immobilization of a significant amount of an enzyme. They are easily separable from the supernatant under magnetic field application, well biocompatible, recyclable, and reusable for several times (Lee *et al*., 2008; Akhond *et al.,* 2016). The aim of this study is to immobilize a-amylase from *Tichoderma harzianum* on magnetic Fe3O4-nanoparticles. The characterization of free and immobilized a-amylases was studied.

MATERIALS AND METHODS

Tichoderma harzianum a-amylase

T. harzianum a-amylase was previously purified and characterized with wide substrate specificity (Mohamed et al., 2011).

a-Amylase assay

a-Amylase activity was determined by measurement of maltose released from starch according to the method of Miller (1959). The reaction mixture was incubated at 37°C for 30 min in tubes containing 5 mg soluble potato starch, 50 mM sodium acetate buffer, pH 6.5, appropriate amount of enzyme solution and distilled water to give a final volume of 0.5 ml. The reaction was stopped by the addition of 0.5 ml dinitrosalicylic acid reagent, followed by incubation in a boiling water bath for 10 min followed by cooling. The absorbance was recorded at 560 nm. The enzymatically liberated reducing sugar was calculated from a standard curve using maltose. One unit of enzyme activity was defined as the amount of enzyme producing 1 µmol reducing sugar as maltose per hour under the standard assay conditions.

Immobilization procedure

Enzyme immobilization was carried out by end over end at 90 rpm on the magnatic Fe3O4-nanoparticles using a solution of *T. harzianum* a-amylase made in 50 mM sodium acetate buffer pH 6.5 at room temperature during overnight. Aliquots of the supernatant were drawn up and the Fe_3O_4-nanoparticles were dried at room temperature to verify the advancement of the immobilization.

Enzyme characterization

Estimates of optimal temperature and pH for free a-amylase and immobilized a-amylase were made by using a temperature ranged from 10 ºC to 80 ºC and a pH ranged from 4.0 to 8.5. The thermal stability was investigated by measuring the residual activity of free a-amylase and immobilized a-amylase after one h of incubation at different temperatures prior to substrate addition. The Km values were determined from Lineweaver-Burk plots by using different concentrations of starch as substrate (1.5 -3.8 mg). The effects of various metal ions on enzyme activity of free and immobilized a-amylases were determined by pre-incubating the enzyme with 5 mM metal ions for 15 min and then assaying the enzyme activity. The activity in absence of metal ions is taken as 100%.

RESULTS AND DISCUSSION

The reusability of the immobilized a-amylase from *T. harzianum* on magnetic Fe_3O_4-nanoparticles was examined (Fig. 1). It was observed that the immobilized enzyme demonstrated 54% of its activity after 6 runs. Similarly, the immobilized a-amylase from porcine pancreatic on magnetite nanoparticles was reusable for 9 times and retaining 68 % of its initial activity (Akhond *et al*., 2016).

The pH profiles of free and immobilized a-amylases were investigated (Fig. 2). The free and immobilized a-amylases exhibited pH optima at pH 6.0 and 6.5, respectively. The greater loss of activity was recorded in alkaline pH for free a-amylase compared with the immobilized enzyme. a-Amylase immobilized on nanoparticles such nano$CaCO_3$, nano-polyethylene film and amino-functionalized magnetite nanoparticles had the same pH optimum at 6.5 (Demir *et al*., 2012; Akhond *et al.*, 2016; Meridor and Gedanken, 2013).

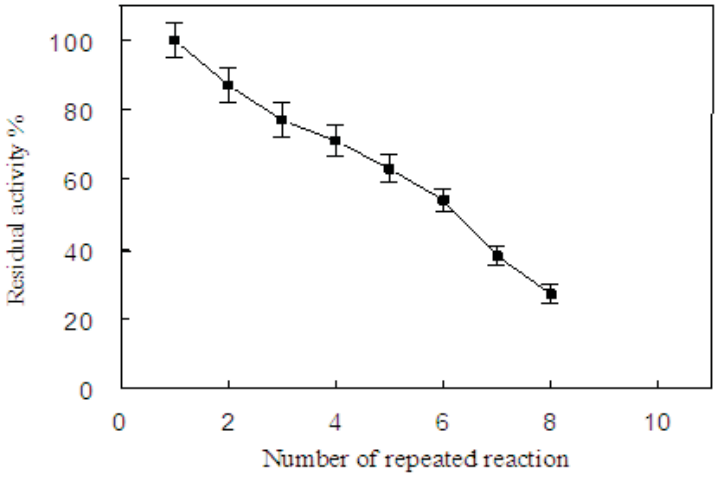


Fig. 1: Reuse of immobilized α-amylase.

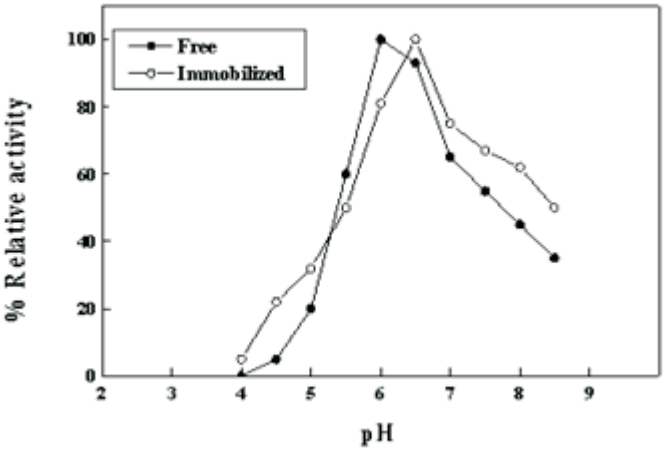


Fig. 2: pH optima of free and immobilized α-amylases.

The effect of temperature on the activity of free and immobilized a-amylases was detected (Fig. 3). The same temperature optimum for the free and immobilized a-amylases was detected at 50°C. On the contrary, the low and the same optimum temperature of free and immobilized a-amylase on nano-polyethylene film was 30°C (Meridor and Gedanken, 2013). The maximum activity was also observed at 40 °C for both free and immobilized a-amylase from porcine pancreatic on magnetite nanoparticles (Akhond *et al.*, 2016). The thermal stability of the free and the immobilized a-amylases was detected (Fig. 4). The results showed that the free and immobilized a-amylases were thermal stable up to 50°C for one h incubation, and retained 15% and 40% of its activity at 80°C, respectively.

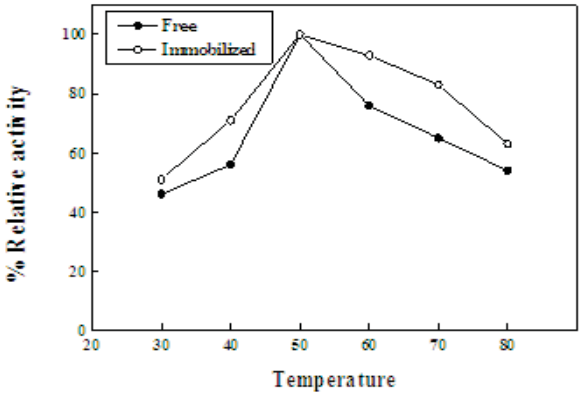


Fig. 3: Temperature optima of free and immobilized α-amylases.

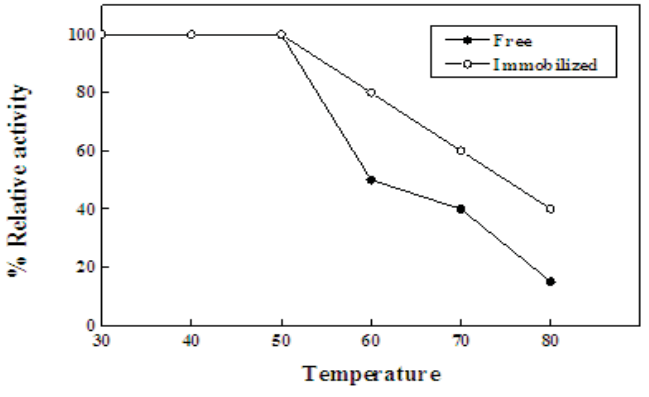


Fig. 4: Thermal stability of free and immobilized α-amylases.

The Km values of the free and immobilized a-amylases were 3.77 and 2.5 mg starch, respectively (Fig. 5). The low Km indicated the high affinity of enzyme toward substrate. Therefore, the results appeared that the affinity of immobilized a-amylase toward starch higher than that of free a-amylase. Similarly, the Km of a-amylase from porcine pancreatic a-amylase immobilized on magnetite nanoparticles and nano-polyethylene film was lower than that of free enzyme (Akhond et al., 2016; Meridor and Gedanken, 2013).

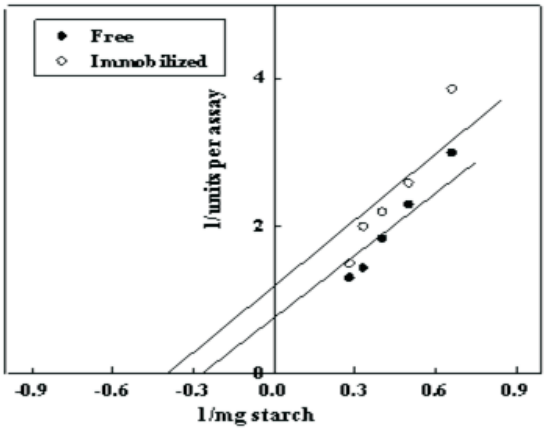


Fig. 5: Km's of free and immobilized α-amylases.

Generally, the resistance of enzymes against inactivation caused by metal ions can be considerably improved by immobilization. The effect of metal ions on the activity of free and immobilized a-amylases was studied (Table 1). The immobilized a-amylase had high resistance toward all examined metals compared with free enzyme. The EDTA as chelating agent had also lower inhibition (42%) for immobilized a-amylase than that for free enzyme (24%). These results are very important considering that inhibiting metal ions are often present in crude materials used in industrial processes. In the same pattern, the activity of a-amylase from porcine pancreatic immobilized on magnetic Fe_2O_3 nanoparticles and amino-functionalized magnetite nanoparticles was less affected in the presence of various metal ions compared with the free enzyme (Akhond *et al*., 2016; Khan *et al*., 2012). In conclusion, the immobilized a-amylase from *T. harzianum* could be used for several applications.

Table 1: Effect of metal ions and EDTA on the free and immobilized a-amylases.

Metal ion	Relative activity (%)	
	Free α-amylase	Immobilized α-amylase
Cu^{2+}	65	80
Ni^{2+}	80	106
Ca^{2+}	60	105
Zn^{2+}	55	70
Co^{2+}	105	115
Pb^{2+}	62	80
Hg^{2+}	22	42
EDTA	24	65

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