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Enhancing Organophosphorus Hydrolase Stability by Immobilization on Chitosan Beads Containing Glutaraldehyde

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ABSTRACT

Organophosphorous Compounds (OPC) are one group of ester and amide or derivatives of toxic thiol-phosphoric compounds widely used to control insects and pests. Such compounds cause the deactivation of acetyl cholinesterase enzyme and create complications in organisms. Wild-type Organophosphorus Hydrolase (OPH) is used for the identification and detoxification of organophosphorus compounds. Immobilization of OPH enzyme on various levels is one of the most effective methods to develop identification and detoxification. As a biopolymer including reactive amine and hydroxyl groups and due to the amine groups as cationic polyelectrolyte, chitosan is particularly important and has many applications, especially in the field of immobilization. In the present study, OPH enzyme was immobilized on chitosan beads and glutaraldehyde was used as cross-linker. Binding the enzyme to chitosan beads was confirmed by Fourier Transform Infrared (FTIR) spectroscopy system and the assessment of its activity was also confirmed. The thermal stability of free and immobilized enzymes was measured in the temperature range of 25-80°C. The pH stability of free and immobilized enzymes was examined at 2-12 pH and the assessment of the reusability of immobilized enzyme was also investigated. The results showed that the thermal stability and pH of the immobilized enzyme was more than that of the free enzyme. Evaluating the reusability of immobilized enzyme indicated that the immobilized enzyme can be applied at least three times without decreasing its activity. The results of this study show the positive role of immobilizing the enzyme in increasing its activity and stability. Immobilization also increases reusability of immobilized enzyme and is economically affordable.

Key words: Organophosphorous compounds, chitosan, organophosphorus hydrolase, glutaraldehyde

INTRODUCTION

Organophosphorous Compounds (OPC) are widely used in various industries, particularly in agriculture and controlling insects and pests as well as in defense industries (Kamelipour *et al*., 2014). These compounds (organophosphates [OPs]) exist in most pesticides in global markets. The use of such compounds as insecticides and pesticides is 25 times more than other compounds in Iran

(Nazarian and Amini, 2008). OPs compounds cause the deactivation of Acetyl Cholinesterase Enzyme (AChE); therefore, they are known as nerve poisons (Kamelipour *et al*., 2014; Nazarian and Amini, 2008; Wu *et al*., 2004; Kazemi *et al*., 2012). These compounds include Paroxon, Parathion, Methyl Parathion, Coumaphos and combat factors of Sarin and Soman (Kamelipour *et al*., 2014; Laothanachareon *et al*., 2008).

Considering the high half-life and stability of OPs compounds in the environment, their dangerous nature and damages caused by their persistence, access to various methods for identifying and disintegration of them is considerable (Gill and Ballesteros, 2000). Development of enzymatic cases for breaking-down of OPs is one of the most important topics for research (El-Boubbou *et al*., 2012). By decomposing the ester bond of these compounds and producing Paranitrophenol (PNP), OPH enzyme is the most important enzyme for decomposition. This homodimeric enzyme was first discovered from *Pseudomonas diminuta* and *Flavobacterium* spp. (Laothanachareon *et al*., 2008; Najavand *et al*., 2012). Due to the high hydrolysis rate of OPs compounds by wild-type OPH enzyme, it is preferably used for identification and detoxification (Liu *et al*., 2004; Raushel, 2002; Gerlt and Raushel, 2003; Efremenko and Sergeeva, 2001; Kumar *et al*., 2006; Ghanem and Raushel, 2005).

The stability of the enzyme is very important for biotechnological applications for future planning and development. An increase in activity, maintenance of quality and sustainability and several use of enzyme are the important challenges may occur through immobilizing the enzyme on proper supports (Laothanachareon *et al*., 2008; Kawaguti *et al*., 2006). Immobilization increases thermo-stability, operational stability, recovery, physical stability, flexibility of reactor design and great operational control (Laothanachareon *et al*., 2008; Cetinus and Oztop, 2003; Zhou *et al*., 2013; Adriano *et al*., 2005). Different types of supports have been used to immobilize an enzyme. Suitable supports should have features such as stability against environmental factors and physical strength, ability to increase the activity of the enzyme and reducer of inhibitors, chemically stability, availability and low cost (Cahyaningrum *et al*., 2014; Krajewska, 2004; Sheldon, 2007; Chen and Chiu, 1999; Singh, 2011).

Chitosan is one of the major supports used in immobilization (Cabuk *et al*., 2014; Chen *et al*., 2013). As a cationic biopolymer including amine and reactive hydroxyl groups, chitosan has many applications in the field of immobilization as gel and bead (Rinaudo, 2006; Altun and Cetinus, 2007; Dincer and Telefoncu, 2007).

Advantages of using chitosan include high affinity to protein, availability of functional groups for direct reaction with enzymes and cross-linker (Chang and Juang, 2005), degradability in environment (Altun and Cetinus, 2007), hydrophilicity, mechanical stability and stiffness, ability to reproduce and easy preparation in various geometrical configurations, nontoxicity, biocompatibility, low cost, availability, anti-tumor and anti-cholesteremic properties, healthy for human and natural environment, etc. (Nasratun *et al*., 2009; Liang *et al*., 2005; Nakorn, 2008; Juang *et al*., 2002; Chang and Juang, 2007).

Several types of enzymes have been immobilized on the chitosan support, including urease (Singh, 2011; Liang *et al*., 2005), acid phosphatase (Juang *et al*., 2002), **β**-glucosidase (Zhou *et al*., 2013), lipase (Nasratun *et al*., 2009), BSA (Nakorn, 2008), pepsin (Altun and Cetinus, 2007), penicillin G-amylase (Adriano *et al*., 2005), catalase (Cetinus and Oztop, 2003), **β**-galactosidase (Chen *et al*., 2013) and plant-esterase enzyme (Dong and Wang, 2009).

Despite some defects, the chitosan beads containing cross-linker have many applications in biochemical engineering (Chang and Juang, 2007). Studies show that stabilization of enzymes on chitosan support causes to increase their pH and thermal stability.

Various methods are applied to immobilize the enzyme including adsorption, covalent binding, affinity immobilization and entrapment (Missau *et al*., 2014).

One of the most common techniques of immobilization is to use bi/multifunctional cross-linkers such as glutaraldehyde, bis-diazobenzidine and hexamethylenediisocyanate and the other one, is to use polymeric supports such as chitosan (Singh, 2011; Lee *et al*., 2006; Hung *et al*., 2003; Datta *et al*., 2013).

Many studies have been conducted to immobilize OPH enzyme on a variety of supports, for example, one of the first cases was immobilization of the enzyme isolated from *Pseudomonas diminuta* on nano composite protein-silicon polymer (NPS). This method proposed by Gill and Ballesteros (2000) caused increasing activity and stability of the enzyme in the field of detoxification in the liquid and gas. This report strengthened the idea of using biopolymers (Gill and Ballesteros, 2000).

In the current study, OPH enzyme was immobilized on chitosan beads containing the glutaraldehyde cross-linker and investigated the increase of thermal and pH stabilities, along with its reusability.

MATERIALS AND METHODS

Preparation of chitosan beads: Six milligram of chitosan powder was added into 6 mL of 0.1 mol acetic acid and vortexed so that it was thoroughly solved and became jelly. Then, it was added to the base solution (NaOH, deionized water, 95% ethanol) drop by drop at a constant time interval. Each drop falling into the base solution immediately forms the beads. The created beads are washed by deionized distilled water so that their pH reaches to neutral pH range. The diameter of beads was about 2 mm and they were used to immobilize the enzyme (Zhou *et al*., 2013; Cahyaningrum *et al*., 2014; Altun and Cetinus, 2007; Chang and Juang, 2007).

Preparation of cross-linking chitosan beads: About 18.86 mL of glutaraldehyde 25% was poured into 40 mL water and thoroughly stirred. Then, 0.16 mg $NaBH_4$ and dinitropyridine were added and thoroughly stirred. In the stirring mode, chitosan beads were slowly and one by one added to the solution containing glutaraldehyde and smoothly stirred for about 8-9 h. Then, glutaraldehyde binds to the amine units (Schiff's base created by produced binding between amino and aldehyde groups) of chitosan and surrounds it. In this reaction, dinitropyridine is as the reaction catalyzer of chitosan amine and glutaraldehyde aldehyde groups and $NaBH_4$ removes Schiff's base and converts it to a firm single bond. If dinitropyridine is not used in this reaction, the time of stirring should be elongated up to 24 h (Altun and Cetinus, 2007; Chang and Juang, 2007). Then, the solution of beads was rinsed so that the absorption of its upper solution in 280 nm reaches to 0.01.

Immobilization of OPH into chitosan beads: Chitosan beads containing glutaraldehyde cross-linker was slowly added to 20 mL enzyme solution one by one and put in very quietly stirred in temperature of 4°C about 24 h. At this stage, dinitropyridine and $NaBH_4$ were used. To demonstrate the binding of enzyme to chitosan beads before and after binding, the protein assay test was done for the enzyme solution (Adriano *et al*., 2005; Krajewska, 2004; Dincer and Telefoncu, 2007). Assessing the protein by Bradford test was performed using bovine serum albumin as a standard (Chang and Juang, 2007; Hung *et al*., 2003; Dwevedi and Kayastha, 2009). In order to prove glutaraldehyde binding to chitosan beads and the enzyme to chitosan bead containing cross-linker, FTIR spectroscopy method was used (Adriano *et al*., 2005; Altun and Cetinus, 2007).

After doing Bradford test, the solution containing the immobilized enzyme was washed so that its absorption in 280 nm reached to less than 0.01.

Chitosan beads containing enzymes can be lyophilized or freeze-dried and be preserved for long periods. The activity of immobilized enzyme was evaluated through measuring PNP by a spectrophotometer (OD = 410 nm, $\varepsilon = 17000\ M^{-1}\ cm^{-1}$) (Laothanachareon *et al*., 2008).

Evaluation of pH stability: The pH stabilities of the free and immobilized enzyme were investigated via measuring the residual activity of the enzyme exposed to pH 2-12 for 5 h. The activity of the enzyme was measured in normal conditions (Cahyaningrum *et al*., 2014; Altun and Cetinus, 2007).

Evaluation of thermal stability: The thermal stabilities of the free and immobilized enzyme were investigated via measuring the residual activity of the enzyme exposed to the temperature of 25-80°C for 5 h. Activities of the samples were performed at optimum conditions.

Evaluation of the reusability: To evaluate the reusability, the enzyme activity was assessed eight times in a way that after assessing, it was rinsed by 50 mmol HCl-tris buffer with pH = 8 and then, it was reused in evaluating another activity.

RESULTS

Preparation of crosslinking chitosan beads: Glutaraldehyde was used as a cross-linker in the study. The aldehyde factor of glutaraldehyde was bound to the amino factor of chitosan via Schiff's base and this binding was confirmed by FTIR (Fig. 1). The resulted peaks in FTIR were as following wavelengths: The peak of the chitosan saccharide structure were 910 and 1153 cm^{-1}, the peak of the N-C bond formation between chitosan and glutaraldehyde was 1574 cm^{-1}, the peak of the methylene groups of glutaraldehyde were 2872 and 2942 cm^{-1}, the peak of the aldehyde H and free carbonyl group of glutaraldehyde were 2750 and 1711 cm^{-1}. These results confirm the binding of glutaraldehyde to chitosan bead (Altun and Cetinus, 2007).

In addition to FTIR spectra, the color change of chitosan is also another reason of binding glutaraldehyde to it. In binding glutaraldehyde to chitosan, the color of beads converted from white to yellow.

Immobilization of OPH into chitosan beads: During this reaction, the enzyme was bond to the aldehyde factor of glutaraldehyde that was confirmed by FTIR (Fig. 1). The resulted peaks in FTIR were as following wavelengths: Peak of the N = C bond formation between glutaraldehyde binding to chitosan and the enzyme was 1600 cm^{-1}, the elimination of peak of the aldehyde H and free carbonyl group of glutaraldehyde a wavelength of 2750 and 1711 cm^{-1}, the spreading the upper region of the wavelength of 3000 cm^{-1} as a result of the presence of enzyme carboxylic acid group that indicate the binding of the enzyme to chitosan bead containing glutaraldehyde cross-linker (Altun and Cetinus, 2007).

Evaluation of pH stability: The pH stability of the free and immobilized enzyme was evaluated in pH of 2-12 (Fig. 2). In pH range of 2-4, the residual activity of free enzyme was less than 20%. The immobilized enzyme in pH range of 2-3 has also residual activity less than 20%. At acidic pH, the decrease in the residual activity of free enzyme was higher than the immobilized enzyme.

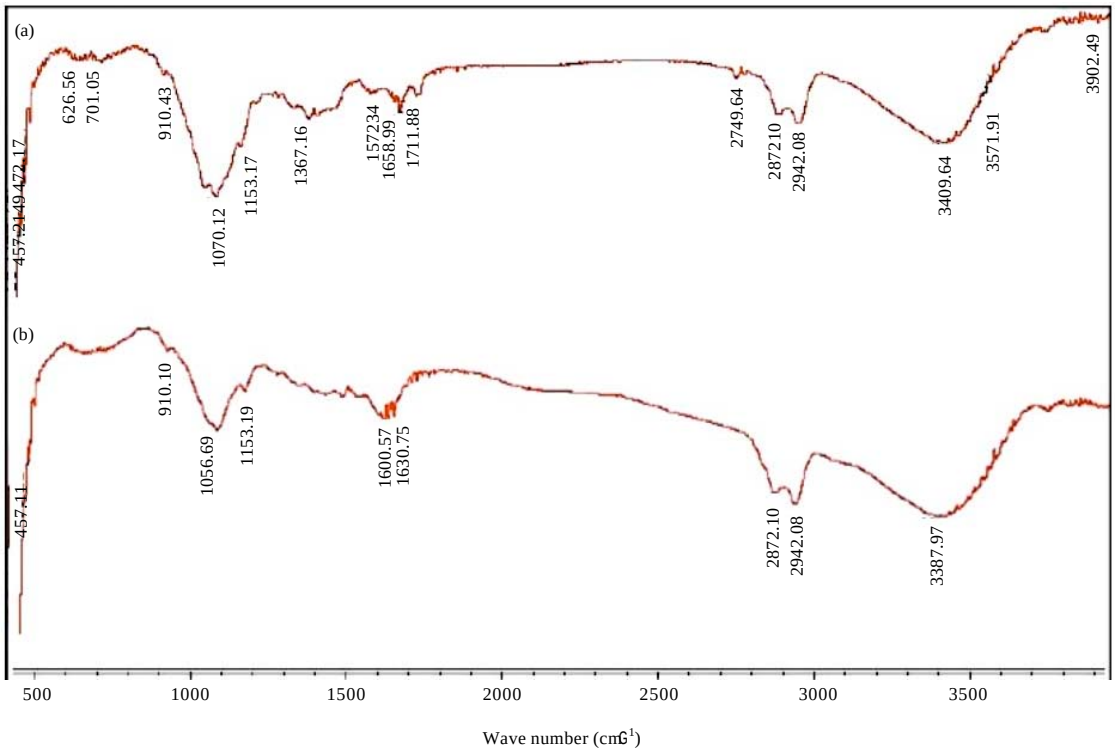

Fig. 1(a-b): IR spectra of chitosan beads, (a) Pretreated glutaraldehyde and (b) OPH immobilized

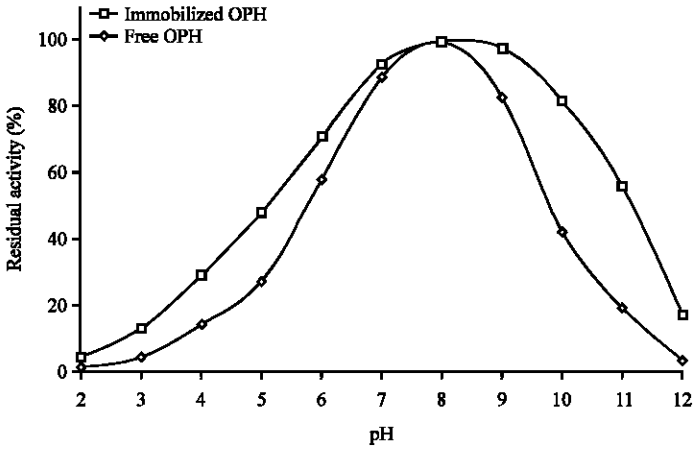

Fig. 2: Evaluation of pH stabilities of free and immobilized OPH

There was also a difference in the residual activity of both enzymes at pH = 6. The free and immobilized enzymes have the highest residual activity in pH = 8. The immobilized enzyme has greater stability in alkaline pH. Its activity at pH = 9 and pH = 10 has no significant reduction and reaches to 80%. At pH = 11, the residual activity is 56% versus 19% of the free enzyme. At pH = 12, the residual activity reduce in both enzymes while the intensity of reduction in the free enzyme is more. Generally, the immobilized enzyme has the pH stability greater than the free enzyme.

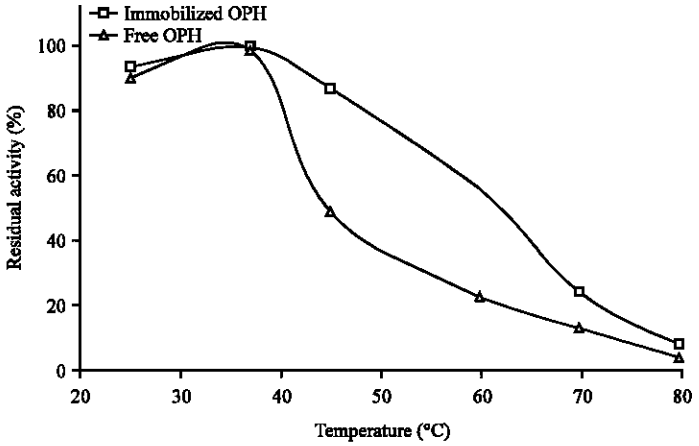

Fig. 3: Evaluation of thermal stabilities of free and immobilized OPH

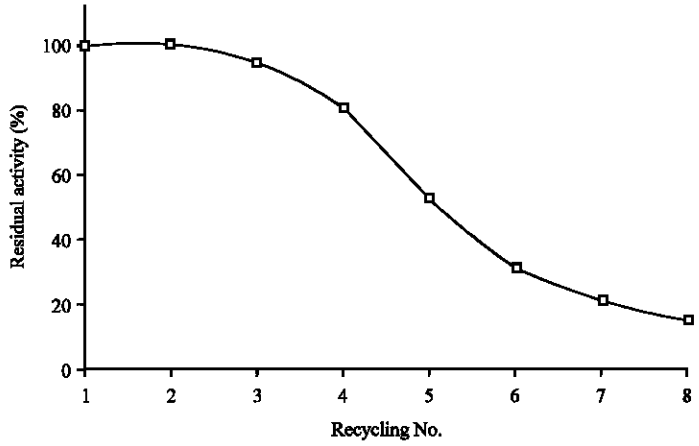

Fig. 4: Evaluation of reusability immobilized OPH

Evaluation of thermal stability: The thermal stability of free and immobilized enzymes was investigated in the temperature range of 25-80 (Fig. 3). As it can be seen, there is no much difference in the residual activity at temperatures of 25 and 37°C. There is a sever reduction in the residual activity of free enzyme at 45°C while the immobilized enzyme has the residual activity of more than 80%. At 60°C, the immobilized enzyme has 56% residual activity compared to 26% of the free enzyme. At the temperature of 70°C, the immobilized enzyme has the residual activity of above 20%. The minimal activity of both enzymes is at the temperature of 80°C.

Evaluation of the reusability: Evaluation of reusability immobilized OPH was performed (Fig. 4). There were no significant changes in the residual activity of the immobilized enzyme in the first three uses. After the third use, the residual activity of the enzyme declined. This reduction is significantly dropped after 5 turns and the residual activity reaches to less than 40% of the initial activity. The free enzyme shows a severe decrease in the residual activity after one use. In general, the reusability of the immobilized enzyme is much better and more than that of free enzyme.

DISCUSSION

The main objective of the present study was immobilization of OPH enzyme on the chitosan beads to increase thermal and pH stability and the enzyme reusability.

In this study, the glutaraldehyde was used as the cross-linker. Glutaraldehyde has two aldehyde groups that are prone to creating chemical bond with amino groups of chitosan on one hand and the organophosphate hydrolase enzyme on the other hand. The major key of this reaction is the creation of Schiff's base bond between the amine group of chitosan and aldehyde group of glutaraldehyde. Since this bond has no much strength, sodium borohydride is used as a reducing agent (Altun and Cetinus, 2007; Liang *et al*., 2005; Dong and Wang, 2009).

As a cross-linker, the utilization of glutaraldehyde is a suitable method to increase activity, stability, sensitivity and fast response to the support. The thermal stability of the immobilized enzyme using glutaraldehyde cross-linker was confirmed in the last decade (Dwevedi and Kayastha, 2009).

Studies conducted by Chang and Lee (2007) and Dong and Wang (2009) showed that the immobilization of enzyme by glutaraldehyde causes to increase the stability and faster response rate versus the decomposition of OPs (Dong and Wang, 2009; Lei *et al*., 2007).

The crystallographic studies by the X-ray diffraction revealed that OPH has six amines in the lysine residual form on each monomer, having the ability to bind to glutaraldehyde cross-linker. Also, since glutaraldehyde cross-linker, leads to restriction by creating the additional links and reduction of the enzyme denaturation, therefore, in the study, we used glutaraldehyde as a cross-linker (Altun and Cetinus, 2007; Liang *et al*., 2005; Dong and Wang, 2009; Dwevedi and Kayastha, 2009).

In this study, the results of assessing pH stability of free and immobilized OPH in the pH range of 2-12 indicated that the immobilized enzyme has a high pH stability compared to the free enzyme. This is agreed with the results of studies on the enzymes of glucose isomerase (Krajewska, 2004), lipase (Lei *et al*., 2007), α and β amylase and glucoamylase (Chang and Juang, 2005). Chang and Juang (2005) demonstrated the pH stability by immobilization of the enzyme. They stated that the presence of amine or amino groups in the structure of chitosan is due to the stability. They believed that these groups may bond to proton in acidic pH and decline the regional concentration of proton nearby the enzyme. LeJeune *et al*. (1999) increased tolerance and stability of the enzyme against pH changes through immobilizing OPH enzyme on the polyurethane. Changes in pH of immobilized enzyme may be due to cationic nature of polymeric support. The cationic support causes the accumulation of OH ions in immobilized enzyme and pH tends to alkaline; therefore, the immobilized enzyme has a more stability at higher pH (Egwim *et al*., 2012).

On the other hand, the immobilization to the charged support often leads to displacements in the immobilized enzyme and the bulk phase leads to the electrostatic reactions with the matrix. So, in general, the immobilized enzyme has more stability in pH changes, especially alkaline pH (Cahyaningrum *et al*., 2014).

In the conducted study, the results of assessing thermal stability of free and immobilized OPH in temperature range of 25-80°C showed that the thermal stability of the immobilized enzyme on a relatively rigid support is greater than that of free enzyme (Altun and Cetinus, 2007).

Our data are consistent with the results of the studies on enzymes of lipase (Hung *et al*., 2003), β-glucosidase (Zhou *et al*., 2013; Chang and Juang, 2007), catalase (Cetinus and Oztop, 2003), α and β amylase and glucoamylase (Chang and Juang, 2005). Present study is also agreed with

the results of the study conducted by Zheng *et al.* (2004) on OPH enzyme and factors affecting the thermal stability during immobilization of the enzyme. Their study demonstrated that an increase in temperature causes intensive kinetic fluctuations in hydrogen bonds between chains. It leads to disintegration and finally, denaturation of the enzyme. In the process of denaturation, the enzyme unfolds, thus, the conformation of correct folding of the enzyme (including alpha helix) is endangered so that it leads to increase the percentage of beta-sheets, beta-turn and incorrect secondary structure. By immobilizing the enzyme on Langmuir, Langmuir-Blodgett film, they observed that the secondary structure of immobilized enzyme was stable and the activity of enzyme did not change at the temperatures up to 60°C (Zheng *et al.*, 2004). Increasing the stability of the immobilized enzyme is due to several reactions, that increase the rigidity of tertiary structure and decrease the denaturation rate of the enzyme in high temperature and effective environmental factors (Laothanachareon *et al.*, 2008; Cetinus and Oztop, 2003; Altun and Cetinus, 2007; Chang and Juang, 2005). The structure change resulted from the immobilization leads to less dependence of the enzyme on the temperature and maintenance of its activity against adverse environmental conditions and high temperature (Zhou *et al.*, 2013; Hung *et al.*, 2003).

Enzyme reusability is one of the important cases for the industrial use of enzyme and since it is economically affordable, it has a significant impotence (Chang and Juang, 2007). In the reusability of free enzyme, there is the possibility of demolishing active site and substrate and reducing the catalytic efficiency of the enzyme (Zhou *et al.*, 2013). These findings indicate that the immobilization of the enzyme may provide its reusability. These results are consistent with the results of studies on enzymes of pepsin (Altun and Cetinus, 2007), β-glucosidase (Adriano *et al.*, 2005; Chang and Juang, 2007), β-galactosidase (Dwevedi and Kayastha, 2009), α and β amylase and glucoamylase (Chang and Juang, 2005). The immobilization of the enzyme causes to prevent the removal or loss of enzyme's nature and to increase the reusability (Altun and Cetinus, 2007). The immobilized enzyme can also be easily detached from the product and be reused (Nasratun *et al.*, 2009). The reusability of the immobilized enzyme can be due to binding the enzyme to chitosan support by a tight link (Zhou *et al.*, 2013; Dwevedi and Kayastha, 2009). This provides "the non-disruption condition" of enzyme's structure in several uses. A study conducted by Efremenko *et al.* (2002) on the immobilization of OPH on poly-cryogel beads (vinyl alcohol) shows 5-fold resistance of the immobilized enzyme to the free enzyme and its 10 times constant use for hydrolysis. In general, the stability of immobilized enzyme is due to the stability of conformation of active site in the enzyme bound to chitosan beads (Laothanachareon *et al.*, 2008).

CONCLUSION

The OPH enzyme was immobilized on chitosan beads by glutaraldehyde cross-linker. The use of cross-linker increases the stability and sensitivity of the enzyme to the substrate. Present study indicated that immobilization, increases pH and thermal stability of the enzyme so that the activity of immobilized enzyme in various ranges of pH and temperature is more and better than that of free enzyme. The reusability of the enzyme increased through immobilization so that the immobilized enzyme can be used 3 times without any loss in the activity. Overall, the immobilization of the enzyme is a suitable method to increase the efficiency of enzyme and it causes enzyme to be affordable and economic. The immobilized OPH enzyme can be used as biosensors in the detection and tools for decomposition of OPs.

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REFERENCES

Adriano, W.S., E.H.C. Filho, J.A. Silva, R.L.C. Giordano and L.R.B. Goncalves, 2005. Stabilization of penicillin G acylase by immobilization on glutaraldehyde-activated chitosan. Braz. J. Chem. Eng., 22: 529-538.

Altun, G.D. and S.A. Cetinus, 2007. Immobilization of pepsin on chitosan beads. Food Chem., 100: 964-971.

Cabuk, B., C. Tari and S.T. Harsa, 2014. β-galactosidase immobilization on chitosan-hydroxyapatite complex: Effects of immobilization conditions. J. Nutr. Health Food Eng., Vol. 1.

Cahyaningrum, S.E., N. Herdyastusi and D.K. Maharani, 2014. Immobilization of glucose isomerase in surface-modified chitosan gel beads. Res. J. Pharm. Biol. Chem. Sci., 5: 104-111.

Cetinus, S.A. and H.N. Oztop, 2003. Immobilization of catalase into chemically crosslinked chitosan beads. Enzyme Microb. Technol., 32: 889-894.

Chang, M.Y. and R.S. Juang, 2005. Activities, stabilities and reaction kinetics of three free and chitosan-clay composite immobilized enzymes. Enzyme Microb. Technol., 36: 75-82.

Chang, M.Y. and R.S. Juang, 2007. Use of chitosan-clay composite as immobilization support for improved activity and stability of β-glucosidase. Biochem. Eng. J., 35: 93-98.

Chang, S.C. and M.S. Lee, 2007. A study on relationship among leadership, organisational culture, the operation of learning organisation and employee, job satisfaction. Learn. Organisation, 14: 155-185.

Chen, J.P. and S.H. Chiu, 1999. Preparation and characterization of urease immobilized onto porous chitosan beads for urea hydrolysis. Bioprocess Eng., 21: 323-330.

Chen, H., Q. Zhang, Y. Dang and G. Shu, 2013. The effect of glutaraldehyde cross-linking on the enzyme activity of immobilized β-galactosidase on chitosan bead. Adv. J. Food Sci. Technol., 5: 932-935.

Datta, S., L.R. Christena and Y.R.S. Rajaram, 2013. Enzyme immobilization: An overview on techniques and support materials. 3 Biotech, 3: 1-9.

Dincer, A. and A. Telefoncu, 2007. Improving the stability of cellulase by immobilization on modified polyvinyl alcohol coated chitosan beads. J. Mol. Catalysis B: Enzymatic, 45: 10-14.

Dong, H. and Q. Wang, 2009. Detection of oraganophosphorus by immobilized wheat esterase in chitosan bead. Proceedings of the 3rd International Conference on Bioinformatics and Biomedical Engineering, June 11-13, 2009, Beijing, China, pp: 1-3.

Dwevedi, A. and A.M. Kayastha, 2009. Optimal immobilization of β-galactosidase from Pea (*Ps* BGAL) onto Sephadex and chitosan beads using response surface methodology and its applications. Bioresour. Technol., 100: 2667-2675.

Efremenko, E.N. and V.S. Sergeeva, 2001. Organophosphate hydrolase-an enzyme catalyzing degradation of phosphorus-containing toxins and pesticides. Russian Chem. Bull., 50: 1826-1832.

Efremenko, E.N., V.I. Lozinsky, V.S. Sergeeva, F.M. Plieva and T.A. Makhlis *et al.*, 2002. Addition of polybrene improves stability of organophosphate hydrolase immobilized in poly (vinyl alcohol) cryogel carrier. J. Biochem. Biophys. Methods, 51: 195-201.

Egwim, E.C., A.A. Adesina, O.A. Oyewole and I.N. Okoliegbe, 2012. Optimization of lipase immobilized on chitosan beads for biodiesel production. Global Res. J. Microbiol., 2: 103-112.

El-Boubbou, K., D.A. Schofield and C.C. Landry, 2012. Enhanced enzymatic thermal stability and activity in functionalized mesoporous silica monitored by ^{31}P NMR. Adv. Healthcare Mater., 1: 183-188.

Gerlt, J.A. and F.M. Raushel, 2003. Evolution of function in $(\beta/\alpha)_8$-barrel enzymes. Curr. Opin. Chem. Biol., 7: 252-264.

Ghanem, E. and M.F. Raushel, 2005. Detoxification of organophosphate nerve agents by bacterial phosphotriesterase. Toxicol. Applied Pharmacol., 207: 459-470.

Gill, I. and A. Ballesteros, 2000. Degradation of organophosphorous nerve agents by enzyme-polymer nanocomposites: Efficient biocatalytic materials for personal protection and large-scale detoxification. Biotechnol. Bioeng., 70: 400-410.

Hung, T.C., R. Giridhar, S.H. Chiou and W.T. Wu, 2003. Binary Immobilization of *Candida rugosa* lipase on chitosan. J. Mol. Catalysis B: Enzymatic, 26: 69-78.

Juang, R.S., F.C. Wu and R.L. Tseng, 2002. Use of chemically modified chitosan beads for sorption and enzyme immobilization. Adv. Environ. Res., 6: 171-177.

Kamelipour, N., A. Mohsenifar, M. Tabatabaei, T. Rahmani-Cherati and K. Khoshnevisan *et al.*, 2014. Fluorometric determination of paraoxon in human serum using a gold nanoparticle-immobilized organophosphorus hydrolase and coumarin 1 as a competitive inhibitor. Microchimica Acta, 181: 239-248.

Kawaguti, H.Y., E. Manrich and H.H. Sato, 2006. Production of isomaltulose using *Erwinina* sp. d12 cells: Culture medium optimization and cell immobilization in alginate. Biochem. Eng. J., 29: 270-277.

Kazemi, M., A.M. Tahmasbi, R. Valizadeh, A.A. Naserian and A. Soni, 2012. Organophosphate pesticides: A general review. Agric. Sci. Res. J., 2: 512-522.

Krajewska, B., 2004. Application of chitin-and chitosan-based materials for enzyme immobilizations: A review. Enzyme Microb. Technol., 35: 126-139.

Kumar, J., S.K. Jha and S.F. D'Souza, 2006. Optical microbial biosensor for detection of methyl parathion pesticide using *Flavobacterium* sp. whole cells adsorbed on glass fiber filters as disposable biocomponent. Biosensors Bioelectron., 21: 2100-2105.

Laothanachareon, T., V. Champreda, P. Sritongkham, M. Somasundrum and W. Surareungchai, 2008. Cross-linked enzyme crystals of organophosphate hydrolase for electrochemical detection of organophosphorus compounds. World J. Microbiol. Biotechnol., 24: 3049-3055.

LeJeune, K.E., J.S. Swers, A.D. Hetro, G.P. Donahey and A.J. Russell, 1999. Increasing the tolerance of organophosphorus hydrolase to bleach. Biotechnol. Bioeng., 64: 250-254.

Lee, D.H., C.H. Park, J.M. Yeo and S.W. Kim, 2006. Lipase immobilization on silica gel using a cross-linking method. J. Ind. Eng. Chem., 12: 777-782.

Lei, C., M.M. Valenta, K.P. Saripalli and E.J. Ackerman, 2007. Biosensing paraoxon in simulated environmental samples by immobilized organophosphorus hydrolase in functionalized mesoporous silica. J. Environ. Qual., 36: 233-238.

Liang, Z.P., Y.Q. Feng, S.X. Meng and Z.Y. Ling, 2005. Preparation and properties of urease immobilized onto glutaraldehyde cross-linked chitosan beads. Chin. Chem. Lett., 16: 135-138.

Liu, Y.H., Y. Liu, Z.S. Chen, J. Lian, X. Huang and Y.C. Chung, 2004. Purification and characterization of a novel organophosphorus pesticide hydrolase from *Penicillium lilacimum* PB303. Enzyme Microb. Technol., 34: 297-303.

Missau, J., A.J. Scheid, E.L. Foletto, S.L. Jahn, M.A. Mazutti and R.C. Kuhn, 2014. Immobilization of commercial inulinase on alginate-chitosan beads. Sustainable Chem. Processes, Vol. 2. 10.1186/2043-7129-2-13

Najavand, S., A.S. Lotfi, K.A. Noghabi, A. Mohsenifar and M.M. Milani *et al.*, 2012. A high potential organophosphorus pesticide-degrading enzyme from a newly characterized, *Pseudomonas aeruginosa* NL01. Afr. J. Microbiol. Res., 6: 4261-4269.

Nakorn, P.N., 2008. Chitin nanowhisker and chitosan nanoparticles in protein immobilization for biosensor applications. J. Met. Mater. Miner., 18: 73-77.

Nasratun, M., H.A. Said, A. Noraziah and A.N. Abdalla, 2009. Immobilization of lipase from *Candida rugosa* on chitosan beads for transesterification reaction. Am. J. Applied Sci., 6: 1653-1657.

Nazarian, A. and B. Amini, 2008. Detection of *Pseudomonas* and *Flavobacterium* species harbouring organophosphorus degrading elements from environment. Iran. J. Basic Med. Sci., 10: 239-244.

Raushel, F.M., 2002. Bacterial detoxification of organophosphate nerve agents. Curr. Opin. Microbiol., 5: 288-295.

Rinaudo, M., 2006. Chitin and chitosan: Properties and applications. Prog. Polym. Sci., 31: 603-632.

Sheldon, R.A., 2007. Enzyme immobilization: The quest for optimum performance. Adv. Synth. Catal., 349: 1289-1307.

Singh, B.D., 2011. Biotechnology Expanding Horizons. 3rd Edn., Ludhiana Kalayani Publishers, India, Pages: 295.

Wu, N., M. Deng, X. Shi, G. Liang, B. Yao and Y. Fan, 2004. Isolation, purification and characterization of a new organphosphorus hydrolase OPHC2. Chin. Sci. Bull., 49: 268-272.

Zheng, J., C.A. Constantine, V.K. Rastogi, T.C. Cheng, J.J. DeFrank and R.M. Leblanc, 2004. Secondary structure of organophosphorus hydrolase in solution and in Langmuir-Blodgett film studied by circular dichroism spectroscopy. J. Phys. Chem. B, 108: 17238-17242.

Zhou, Y., L. Wang, T. Wu, X. Tang and S. Pan, 2013. Optimal immobilization of β-glucosidase into chitosan beads using response surface methodology. Electron. J. Biotechnol., Vol. 16, No. 6. 10.2225/vol16-issue6-fulltext-5