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RESEARCH ARTICLE

Stability and immobilization of D-hydantoinase from *Bacillus theorgensis* on chitosan

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Abstract

D-hydantoinase (EC 3.5.2.2) was purified from *Bacillus theorgensis* with specific activity of 201.7 Umg^{-1} and 16.5-fold. The enzyme was successfully immobilized on chitosan bead. The best substrate for the enzyme was D-hydantoin followed by 5-hydroxy hydantoin, 5-phenyle hydantoin and 5-benzyle hydantoin. The optimal pH values were 8.0 and 9.0 for the free and immobilized enzyme, respectively. The optimal temperatures were 40 and 50 $^{\circ}C$ for. V_{max} values were 66.67 and 47.17 units mg^{-1} protein and K_m values were 1.25 and 0.55 mM. The immobilized enzyme exhibited higher heat stability at 70 $^{\circ}C$, higher storage stability at 4 and 25 $^{\circ}C$ than the free enzyme. The immobilized enzyme kept 80% of its activity after 7 cycles. Thus, D-hydantoinase represents a candidate to be used in the industry purpose.

INTRODUCTION

Enzymes are the focal point of biotechnological processes since enzymes are involved in all aspects of biochemical conversion (Ebbs, 2011). Enzymes have a critical importance for processes of pharmaceutical, industrial and biotechnological application (Sanchez and Demain, 2011). The application of enzymes processes for chemical industry is dependent on cost competitiveness (Tufvesson *et al.*, 2011).

The microbial enzymes are preferred over the plant sources because of their economic production and optimization (Pozo *et al.*, 2002). Furthermore, microbial enzymes provide a greater diversity of catalytic activities (Adrio and Demain, 2014).

Hydantoinase (EC 3.5.2.2) is a metalloenzyme which was first described in the 1940s (Eadie *et al*., 1949). Hydantoinase is characterized by its steorespecificity and substrate promiscuity. These characteristics are of considerable value for industrial application (Clemente-Jiménez *et al.*, 2008).

The stabilization of the enzymes is an important concern especially during thermal processing. The stabilization of enzymes in soluble form is an important desirable goal to achieve in the purification processes of enzymatic proteins and in the storage of purified enzymes (Aehle, 2009).

The soluble forms of enzymes are unstable to fulfill economical requirements for an industrial purpose. Thus, the immobilized enzymes are used in industrial bioprocesses especially in nutritional, food and pharmaceutical technologies (Sheldon, 2007). Immobilized D-hydantoinase is used to produce D-amino acids (Arcuri *et al*., 2002).

D-hydantoinase plays particular role in industry in the preparation of optically active compounds (Scheomaker *et al.*, 2003).

Thus, this work aimed to: 1) purify and immobilize D-hydantoinase from *B. theorgensis* and 2) investigate its kinetic properties and stability.

Materials and Methods

Growth *medium*

The growth medium of *B. theorgensis* contained 10 g/l sucrose, 10 ml corn steep liquor, 0.1 g yeast extract, 3 g NaCl, 2 g KH_2PO_4, and 0.25 g $MgSO_4$ at pH 8.0. The effects were studied separately using D-hydantoin as substrate and examining for production of *N*-carbamoyl-glycine. All media were sterilized at 121°C for 15 min.

Preparation of the crude extract

Bacterial cells were harvested by centrifugation at 10,000 rpm for 20 min then washed by 150 mM potassium phosphate buffer (pH 8.0). The cells were then resuspended in the same buffer. The resuspended cells were disrupted by ultra-sonication to give the crude enzyme extract.

Purification of the D-hydantoinase

The purification of D-hydantoinase was carried out at 4°C. A solid ammonium sulfate was added continuously to the supernatant until reaching 55% saturation. The precipitate was removed by centrifugation at 6,000 rpm, for 20 min at 4°C and added solid ammonium sulfate to 75% saturation and stirred at 4°C for 24h. After centrifugation, the obtained pellet was dissolved in 20 ml 100 mM Tris-HCl buffer, pH 8.0 and the mixture was dialyzed against the same buffer for 12h.

After dialysis the enzyme solution was applied to a Phenyl-Sepharose column (1.6x10 cm) pre-equilibrated with 100 mM Tris–HCl buffer, pH 8.0. The column was washed with the 100 mM Tris-HCl buffer, pH 8.0 and the enzyme was eluted with the same buffer at a flow rate of 1 ml/min.

The elution peaks was pooled together for the next step. The obtained enzyme solution from Sepharose column was loaded onto a Sephacryl S-200 column (1.6x100 cm) after being pre-equilibrated with 100 mM Tris-HCl buffer, pH 8.0. The enzyme was then eluted at a flow rate of 0.5 ml/min with the same buffer and the active fractions were pooled then concentrated by dialysis. The purified enzyme was kept at 4°C.

Assay of D-hydantoinase

The enzyme assay was carried out according to Niu *et al.* (2007) with some modifications. D-hydantoinase activity was estimated by adding 2.0ml of D-hydantoinase solution to 1.0 ml of D-hydantoin 2.0 g/l in 150 mM potassium phosphate buffer (pH 8.0). The mixture was incubated at 40°C with constant shaking for 10 min. The reaction was then terminated by adding 0.5 ml of 5% $ZnSO_4$ solution followed by centrifugation at 5,000 rpm and the supernatant was used for the *N*-carbamoyl-D-amino acid assay according to Morin *et al*. (1987). The assay included 1 ml of the reaction mixture supernatant, 0.5 ml Ehrlich's reagent (dissolve 250 g *p*-dimethylaminobenzaldehyde in 250 ml 6 N HCl and 2.5 ml distilled water).

The concentration of the *N*-carbamoyl-amino acid produced can be calculated using a standard curve of *N*-carbamoyl-amino acid subjected to the Ehrlich's assay.

Immobilization of D-hydantoinase on chitosan beads

1-Preparation of chitosan beads

Chitosan powder (3 g) was suspended in 99 ml of distilled water followed by stirring for 20 min followed by adding one ml of glacial acetic acid and stirring was allowed for 4h at room temperature. The solution was filtered and dried. Drops of 2% (w/v) NaOH were added to neutralize the acetic acid in the chitosan film. The films were washed twice with distilled water and finally dried again.

2-Reinforcement of chitosan beads with glutaraldehyde

The cross-linking was carried out by adding the dried chitosan to 0.1% (w/v) glutaraldehyde solution in 150 mM cold potassium phosphate buffer (pH 8.0) for 2h. The brownish reinforced beads were washed three times by 150 mM cold potassium phosphate buffer (pH 8.0) to remove the excess of glutaraldehyde.

3-Immobilization of D-hydantoinase on chitosan beads

The prepared chitosan beads were mixed with 2 mg/ml D-hydantoinase solution in 150 mM potassium phosphate buffer (pH 8.0) for 3h with slight stirring and washed at 4°C. Then, the beads were dried at room temperature and stored at 4°C. The activity of immobilized enzyme was determined by subtracting the recorded activity in the supernatant after immobilization from the activity added to chitosan bead.

Assay of immobilized D-hydantoinase

Approximately 0.1 g of the immobilized enzyme was used for enzyme assay as described earlier for the free enzyme.

Determination of immobilization yield for D-hydantoinase

The immobilization yield of the enzyme was defined as the yield of the enzyme immobilized on chitosan and expressed as:
Immobilization yield (%) = (Activity of immobilized enzyme/ Activity of the added enzyme) x 100

Determination of K_m and V_{max}

The Michaelis–Menten equation can be written as: $V = V_{max}\, S/(K_m + S)$. Where V is the reaction rate and S is the substrate concentration). The equation was used to determine the reaction kinetic parameters. Plotting 1/V against 1/S (Lineweaver–Burk plot) gives a linear fit, which is used to determine V_{max} (the maximum reaction rate of the enzymatic reaction) and K_m (the apparent Michaelis–Menten constant) (Engel *et al*., 2012).

Effect of pH on free and immobilized D-hydantoinase activity

The optimal pH for the enzyme activity was determined at a range of pH from 4 to 10. The following buffers were used: sodium acetate/acetic (pH 4.0 – 5.0), potassium phosphate buffer (6.0 – 7.0), Tris-HCl buffer (8.0-9.0) and sodium bicarbonate buffer (10.0).

Effect of temperature on D-hydantoinase activity

The reaction mixture of free and immobilized D-hydantoinase was incubated at different temperatures 10, 20, 30, 40, 50 and 60°C. The optimal temperature was determined from the graph of enzyme activity against temperature.

Heat stability of D-hydantoinase

The heat stability of the free and immobilized D-hydantoinase was determined by measuring enzyme activity at 70°C through different time intervals (20, 40, 60, 80, 100 and 120 min). The relation between the enzyme activity and the time of incubation was plotted.

Storage stability of free and immobilized D-hydantoinase

Free and immobilized enzyme extracts were stored at 4°C or 25°C for 24 days. The enzyme activities of both forms were determined every 4 days.

Statistical analysis

All the data in the present study are expressed as mean ± SE obtained from three measurements.

Results and Discussion

One of the principles for the purifying of an enzyme is finding a source of large quantities of the enzyme in a soluble form. Many procedures reported for the purification of hydantoinases (Pietzsch *et al.,* 2000). Hydantoinases have been purified from a number of native species including *Bacillus* sp. (Luksa *et al.,* 1997), *Blastobacter* sp. (Soong *et al.,* 1999), *Agrobacterium* sp. (Runser and Meyer, 1993) and *Arthrobacter* sp. (Siemann *et al*., 1999).

The present work showed that D-hydantoinase was purified with specific activity of 201.7 units mg^{-1} protein and 16.5-fold (Table 1). Specific activity obtained in the present work is higher than 48.5 units mg^{-1} protein reported for the enzyme from a recombinant *E. coli* (Shi *et al*., 2006). The fold of purification for the enzyme from *B. theorgensis* was 16.5-fold which is higher than 3.4-fold recorded for the enzyme from a recombinant *E. coli.*

The substrate specificity of hydantoinase enzymes is fundamental for this industrial application because it defines the types of reaction products. The present results showed that hydantoin was the best substrate compared with 5-hydroxy hydantoin, 5-benzyle hydantoin, and 5-phenyle hydantoin (Fig. 1). These are in agreement with those of Kikugawa *et al.* (1994). However, Shi *et al.* (2006) found that the best substrate was dihydrouracil for the enzyme from *E. coli.* This indicates that the best substrate for the enzyme depends on the source of the enzyme.

D-hydantoinase was immobilized on chitosan bead and the immobilization yield ranged between 76-91 % (Table 2). The effect of glutaraldehyde concentration on the immobilization yield was studied. Glutaraldehyde was tested at 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 (%v/v). The results in Fig. 2 show that there was corresponding increase in the immobilization yield by increasing glutaraldehyde concentration up to 1%. In this case, it is likely that glutaraldehyde forms intramolecular bonds that result in a more stable enzyme preparation (Taravati *et al*., 2007).

After 1% glutaraldehyde, the immobilization yield decreased at the higher concentrations. Such decrease is possibly due to the reaction of aldehyde groups with the amine groups of D-hydantoinase which promotes cross-linking of the protein chains blocking the active site of the enzyme and results in enzyme inactivation during the stabilization process. Similar results were reported of catalase stabilization (Costa *et al.,* 2002) using glutaraldehyde.

K_m and V_{max} values for the free D-hydantoinase from *B. theorgensis* were 1.25 mM and 66.67 units mg^{-1} protein (Fig. 3). A lower K_m value (0.2 mM) was reported for D-hydantoinase from a recombinant *E. coli* (Shi *et al.,* 2006). However, K_m and V_{max} of the immobilized enzyme in the present work were 0.55 mM and 47.17 units mg^{-1} protein, respectively.

The pH of the free D-hydantoinase was 8.0 whereas that of the immobilized one was 9.0 (Fig. 4). The optimal pH of both free and immobilized D-hydantoinase from *E. coli* was 9 (Chen *et al.* 2014). A shift in the pH optimum for the immobilized enzymes on chitosan was reported by Palmieri *et al*. (1994).

At extreme pH the tertiary structure of protein may be disrupted and the protein is denaturated. At moderate pH values where the tertiary structure is not disrupted the enzyme activity may depend on the degree of ionization of certain amino acid chains and pH profiles of an enzyme may suggest the identity of those residues (Palmer, 1985). The inhibitory effect of higher acidic and basic pH on D-hydantoinase activity may suggest the change on enzyme

ionization state, dissociation of subunits and modifying its surface change, thus disrupting the enzyme-substrate intermediate.

The optimal temperature of the free D-hydantoinase from *B. theorgensis* was 40°C; however it increased up to 50°C for the immobilized enzyme (Fig. 5). Chen *et al.* (2014) reported that optimal temperature of 40°C and 60°C for free and immobilized enzyme from *E. coli*. The increase in the optimal temperature of immobilized D-hydantoinase may be caused by the changing enzyme conformational structure upon immobilization (Srinivasa Rao *et al.,* 1994).

The increase in the temperature of the enzyme reaction causes an increase in the inherent energy of the system and more molecules obtain the necessary activation energy for the reaction to take place. However, there comes a point where the increase of the reaction due to the effect of temperature on the activation of molecules is equal to decrease of reaction rate due to the destruction of tertiary structure (Palmer, 1985). At this point the activity is a maximum and this temperature is often known as the optimum temperature (Lizott *et al*., 1990).

The immobilization of D-hydantoinase on chitosan improved heat stability of D-hydantoinase at 70°C compared to the free D-hydantoinase (Fig 6). The support of the immobilization generally has a protecting effect at high temperatures at which deactivation occurs. The conformational flexibility of the enzyme is affected by immobilization. The immobilization of enzyme causes an increase in enzyme rigidity, which is commonly reflected by an increase in stability toward denaturation by raising the temperature (Chang and Juang, 2004).

It should be noted that the biocompatibility of the support could also play an important role on stabilization of enzyme confirmation. The thermostability depends on the microenvironment of the enzyme and its subunit reorganization. Also, large repulsions contributes to the confirmation and stability of the proteins, this repulsion between charged groups present in the protein is the main driving force for protein to be stabilized in open conformation (Daniel *et al*., 1996).

The storage stability of free and immobilized D-hydantoinase was investigated at 4°C (Fig. 7) and 25°C (Fig. 8) for different time intervals (4, 8, 12, 16, 20 and 24 days). The immobilized enzyme expressed higher stability than the free enzyme at both 4°C and 25°C. This might be due to protein-protein interaction (autolysis of D-hydantoinase) as in case of other enzyme expressed higher stability which is probably a result of the prevention of autolysis by immobilization.

Enzyme reuse is known to be one advantage of immobilized enzymes. Therefore, the reusability of the chitosan-immobilized D-hydantoinase in repeated biocatalytic reactions was assessed in order to further evaluate the stability of the enzyme. The results in Fig. 9 indicate that D-hydantoinase immobilized on chitosan retained 80% of its activity after 7 cycles. These results have a significant importance for industrial use of D-hydantoinase.

The soluble enzyme cannot be used to catalyze more reactions because the free enzyme cannot be recovered from the reaction mixture but the process of immobilization can make it feasible. However, the loss of some activity of the immobilized D-hydantoinase in repeated use is a common phenomenon (Arasaratnam *et al.,* 2000). The decrease in the enzyme activity throughout the various cycles might be attributed to protein deactivation, protein damage or physical loss of the chitosan-bound protein.

In conclusion, the present investigation offers a successful method for purification and immobilization of D-hydantoinase from *B. theorgensis.* The immobilized enzyme exhibited higher thermostability than the free one which is important for industrial purposes.

Table 1: Purification of D-hydantoinase from *B. theorgensis.*

Purification steps	Total protein (mg)	Total activity (Units)	Activity recovery (%)	Specific activity (Units/mg)	Purification (-fold)
Crude extract	79.3	970.0	100.0	12.2	1.0
$(NH_4)_2SO_4$	63.4	844.5	87.1	13.3	1.1

(55-75%)					
Phenyl Sepharose	11.2	633.2	65.3	56.5	4.6
Sephacryl S-200	2.6	524.4	54.1	201.7	16.5

Table 2: Summary of immobilization of D-hydantoinase purified from *B. theorgensis* on chitosan beads.

No. of experiment	Added activity (U/mg protein)	Immobilized activity (U/mg protein)	Immobilized yield (%)
1	29±0.9	22±0.4	76
2	34±0.6	27±0.5	79
3	39±0.7	32±0.9	82
4	45±0.5	41±0.6	91

References

Adrio, J. L. and Demain, A. L. (2014). Microbial Enzymes: Tools for Biotechnological Processes., 117-139; doi:10.3390/biom4010117.

Aehle, W.C. (2009). Enzyme in industry: Production and applications. Vch Verlagsgesellschaft Mbh.pp. 81 – 120.

Arasaratnam, V., Galaey, I. Y. and Matiasson, B. (2000). Reversibly soluble biocatalyst: optimization of trypsin coupling to eudargit S-100 and biocatalyst activity in soluble and precipitated forms. Enz. Microb. Technol. 27: 254-263.

Arcuri, M. B., Sabino, S. J., Antunes, O. A. C. and Oestreicher, E. G. (2002). Kinetic study and production of *N*-carbamoyl-D-phenylglycine by immobilized D-hydantoinase from *Vigna angularis*. Catal. Lett. 79: 17–19.

Chang, M. Y. and Juang, R. S. (2004). Stability and catalytic kinetics of acid phosphatase immobilized on composite beads of chitosan and activated clay. Proc. Biochem. 39: 1087-1091.

Chen, S. Y., Chien, Y. W and Chao, Y. P. (2014). In vivo immobilization of D-hydantoinase in *Escherichia coli*. J. Biosci. Bioeng., http://dx.doi.org/10.1016/j.jbiosc.2013.12.020.

Clemente-Jiménez, J. M., Martínez-Rodríguez, S., Rodríguez-Vico, F. and Las Heras-Vázquez, F. J. (2008). Optically pure α-amino acids production by the "Hydantoinase Process". Recent Patents on Biotechnology 2: 35-46.

Costa, A. S., Tzanov, T., Carneiro, A. F., Paar, A., Gubitz, M. G. and Cavaco-Paulo, A. (2002). Studies of stabilization of native catalase using additives. Enz. Microb. Technol. 30: 387-391.

Daniel, B. A., Dines, M. and Petach, H. H. (1996). The denaturation and degradation of stable enzymes at high temperatures. Biochem. J. 317: 1-11.

Eadie, G., Bernheim, F. and Bernheim, M. L. C. (1949). Pyrimidine metabolism in germinating seedlings. J. Biol. Chem 181: 449–458.

Ebbs, S. (2011). Biological degredation of cyanide compound, Curr. Opin. Biotechnol., 15: 231-236.

Engel, U., Syldatk, C. and Rudat, J. (2012). Stereoselective hydrolysis of aryl-substituted dihydropyrimidines by hydantoinases, Appl. Microbiol. Biotechnol. 94: 1221–1231.

Kikugawa, M., Kaneko, M., Fujimoto-Sakata, S., Maeda, M., Kawasaki, K., Tak-agi, T. and Tamaki, N. (1994): Purification, characterization and inhibition of dihydropyrimidinase from rat liver. Eur. J. Biochem. 219: 393–399.

Lizott, P. A., Henson, C. A. and Duke, S. H. (1990). Purification and characterization of pea epicotyl α-amylase. Plant Physiology. 92: 615-621.

Luksa, V., Starkuviene, V., Starkuviene, B. and Dagys, R. (1997). Purification and characterization of the D-hydantoinase from *Bacillus circulans*. Appl. Biochem. Biotechnol. 62: 219-231

Morin, A., Hummel, W. and Kula, M. R. (1987). Enrichment and selection of hydantoinase-producing microorganisms. J. Gen. Microbiol. 133: 1201–1207.

Niu, L. X., Zhang, X. Y., Shi, Y. W. and Yuan, J. M. (2007). Subunit dissociation and stability alteration of D-hydantoinase deleted at the terminal amino acid residue. Biotechnol Lett 29: 303-308.

Palmer, T. (1985). Understanding Enzymes 2nd Ed., Ellis Horwood Publisher.

Palmieri., F. (1994). Mitochondrial carrier proteins. FEBS. Lett. 346: 48-54.
Pietzsch, M., Wiese, A., Ragnitz, K., Wilms, B., Altenbuchner, J., Mattes, R. and Syldatk, C. (2000). Purification of recombinant hydantoinase and L-N-carbamoylase from *Arthrobacter aurescens* expressed in *Escherichia coli*: comparison of wild-type and genetically modified proteins. J. Chromatography B 737: 179-186.
Pozo, C., Rodelas, B., de la Escalera, S. and Gonza,´lez-Lo´pez. J. (2002). D, L-Hydantoinase activity of an *Ochrobactrum anthropic* strain. J. Appl. Microbiol. 92: 1028–1034.
Runser, S. M. and Meyer, P. C. (1993). Purification and biochemical characterization of the hydantoin hydrolyzing enzyme from *Agrobacterium sp.*I-671: a hydantoinase with no 5,6-dihydropyrimidine amidohydrolase activity. Eur. J. Biochem. 213: 1315–1324.
Sanchez, S. and Demain, A. L. (2011). Enzymes and bioconversions of industrial, pharmaceutical and biotechnological significance. Org. Process Res. Dev., 15: 224–230.
Schoemaker, H. E., Mink, D and Wubbolts, M. G. (2003). Dispelling the myths--biocatalysis in industrial synthesis. Science 299: 1694–1697.
Sheldon, R.A. (2007). Enzyme immobilization: The quest for optimum performance. Adv. Synthesis Catalysis, 349: 1289-1307.
Shi, Y., Niu, L., Feng, X. and Yuan, J. (2006). Purification, enzymatic properties of a recombinant D-hydantoinase and its dissociation by zinc ion. World Journal of Microbiology & Biotechnology. 22: 675–680.
Siemann, M., Alvarado-Marin, A., Pietzsch, M. and Syldatk, C. (1999). A D-specific hydantoin amidohydrolase: properties of the metalloenzyme purified from *Arthrobacter crystallopoietes*. J. Mol. Catal. B 6: 387-397.
Soong, C. L., Ogawa, J., Honda, M. and Shimizu, S. (1999). Cyclic-imide-hydrolysing activity of D-hydantoinase from *Blastobacter* sp. Strain A17p-4. Appl. Environ. Microbiol. 65: 1459-1462
Srinivasa Rao, M., Chellapandian, M. and Krishnan, M. R.V.(1994). Immobilization of urease on gelatin – poly(HEMA) copolymer preparation and characterization. J. Bios. Engin. 13: 211-214.
Taravati, M., Shokrzadeh, M., Ebadi, A. G., Valipour, P., Hassan, A. T. and Farrokki, F. (2007). Various effects of sugar and polyols on the protein structure and function: Role as osmolytes on protein stability. World Appl. Sci. J. 2: 353-362.
Tufvesson, P., Lima-Ramos, J., Nordblad, M. and Woodley, J. M. (2011). Guidelines and cost analysis for catalyst production in biocatalytic processes. Org. Process Res. Dev. 15: 266–274.

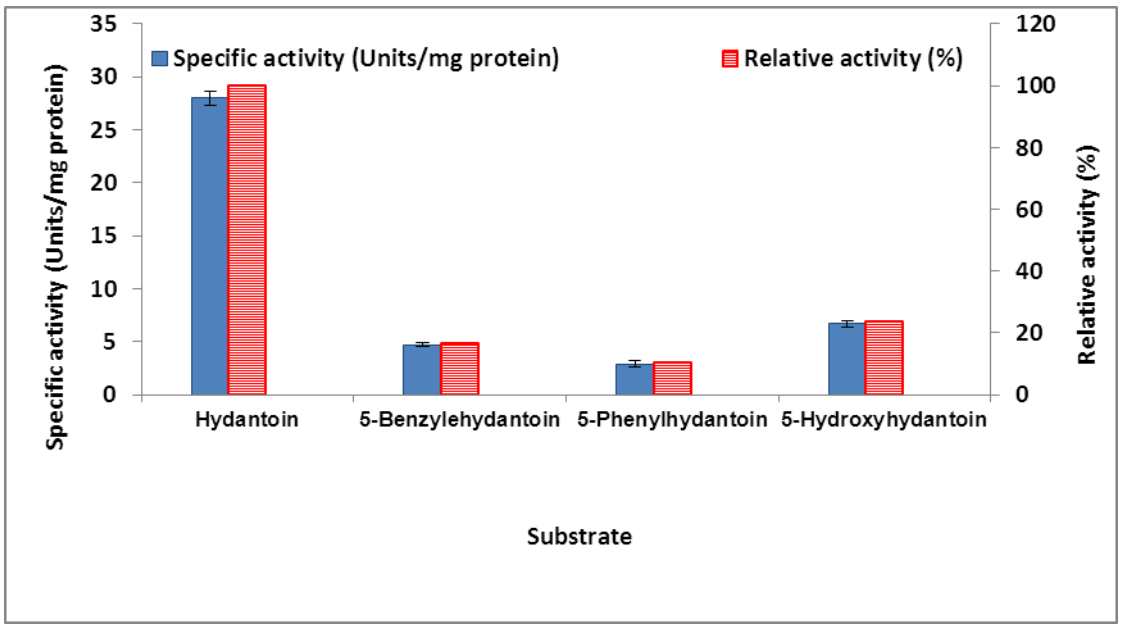

Fig. 1: Substrate specificity of D-hydantoinase from *B. theorgensis.*

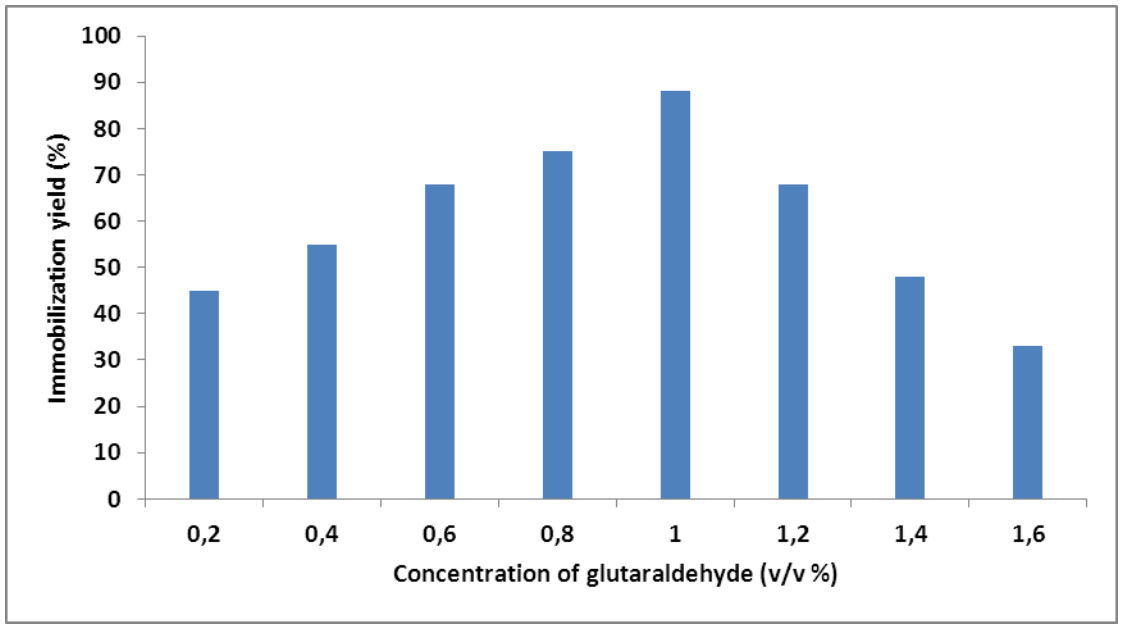

Fig. 2: Effect of glutaraldehyde concentration on the immobilization yield of D-hydantoinase from *B. theorgensis.*

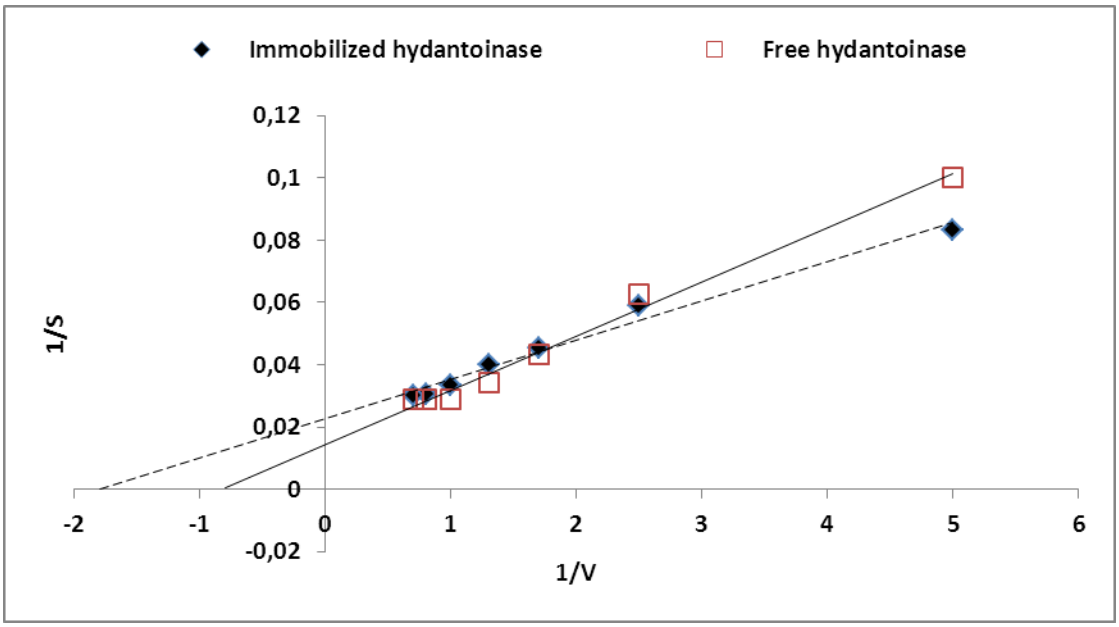

Fig. 3: Lineweaver-Burk plot for free and immobilized D-hydantoinase from *B. theorgensis.*

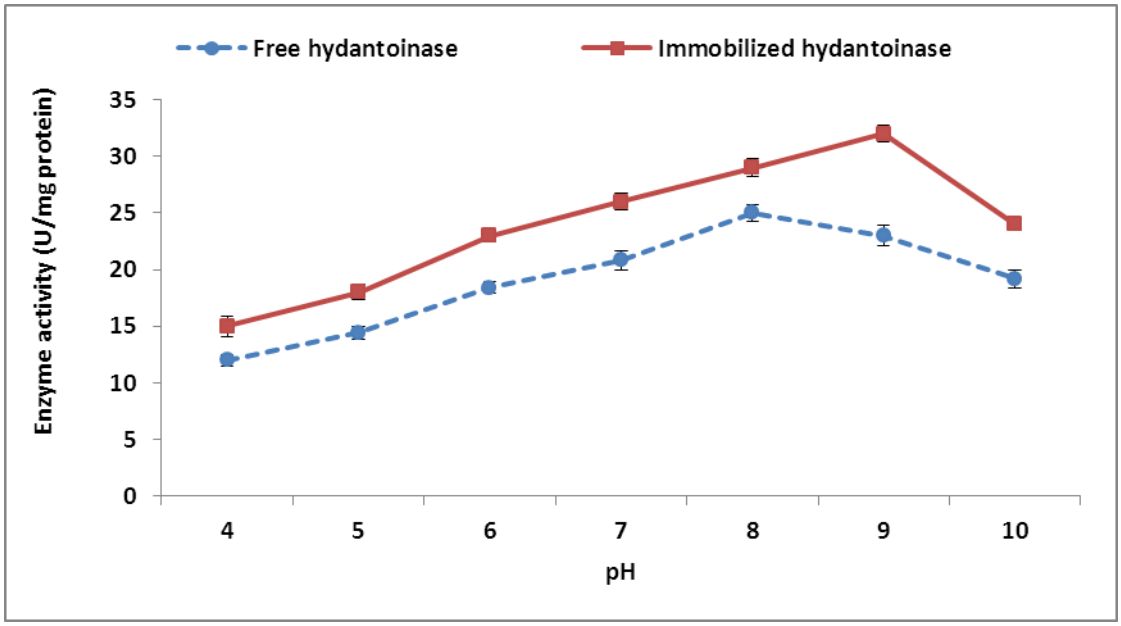

Fig. 4: Effect of pH on free and immobilized D-hydantoinase activity from *B. theorgensis.*

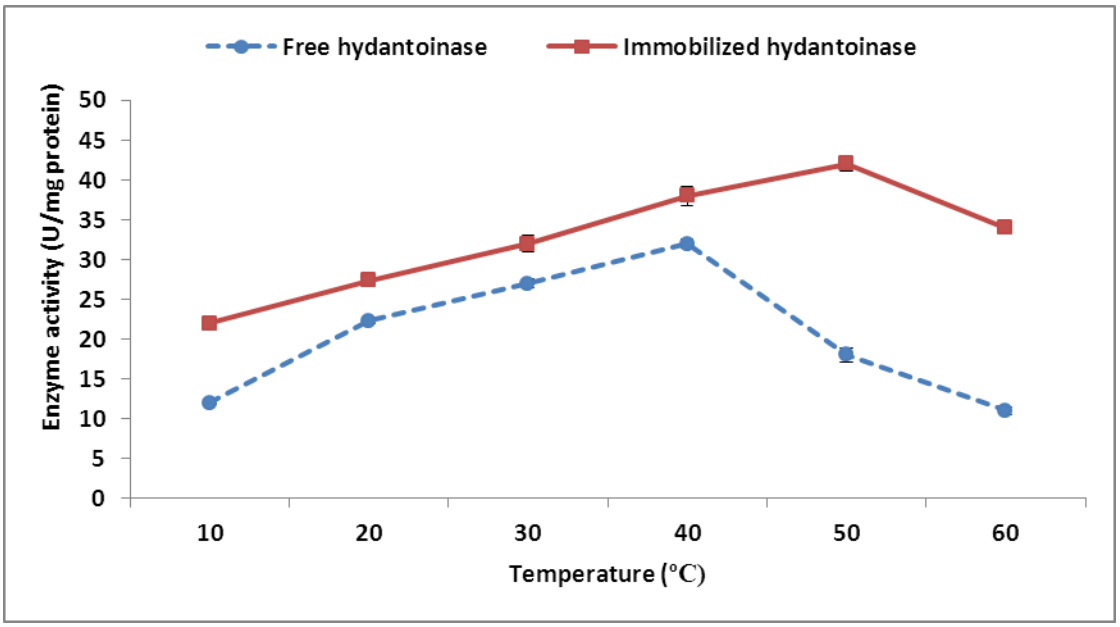

Fig. 5: Effect of temperature on free and immobilized D-hydantoinase activity from *B. theorgensis.*

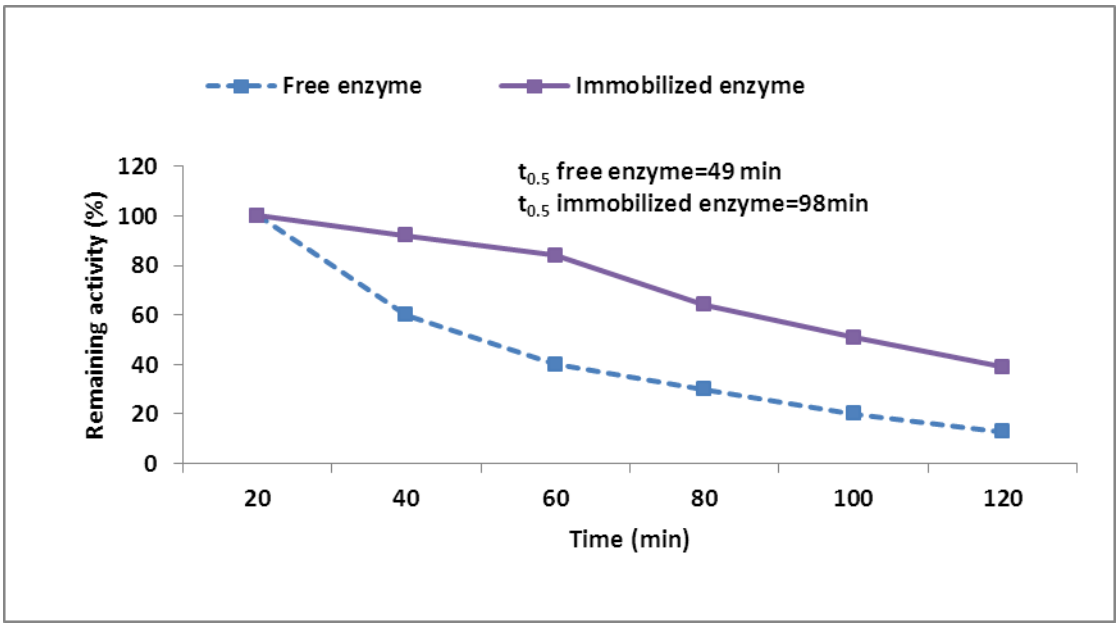

Fig. 6: Heat stability of free and immobilized D-hydantoinase *B. theorgensis* at 70°C.

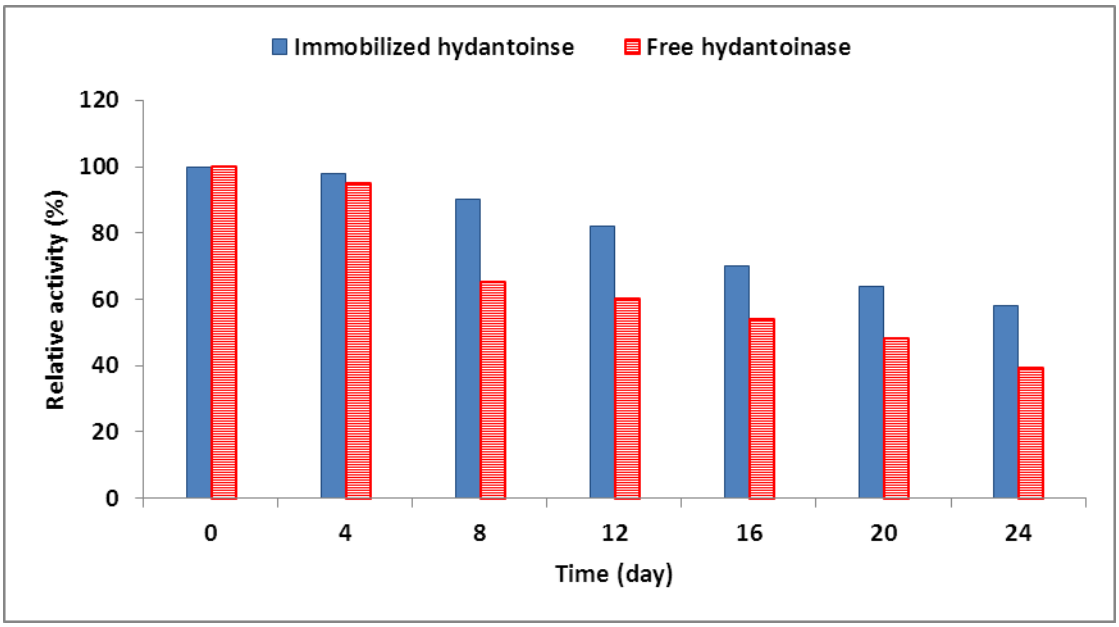

Fig. 7: Storage stability of free and immobilized D-hydantoinase from *B. theorgensis* at 4°C.

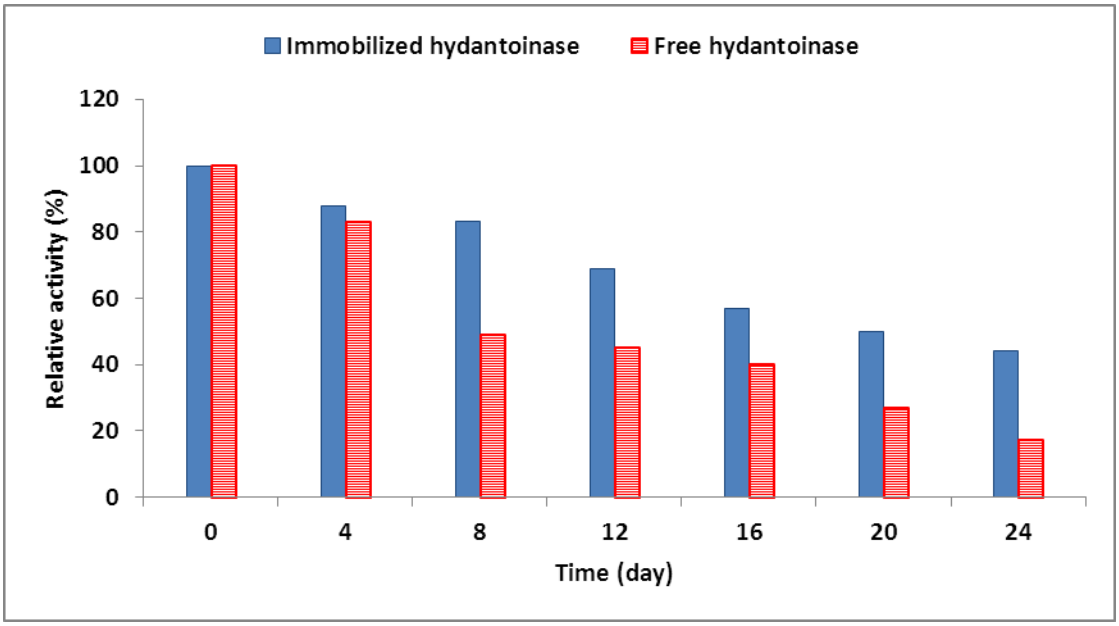

Fig. 8: Storage stability of free and immobilized D-hydantoinase from *B. theorgensis* at 25°C.

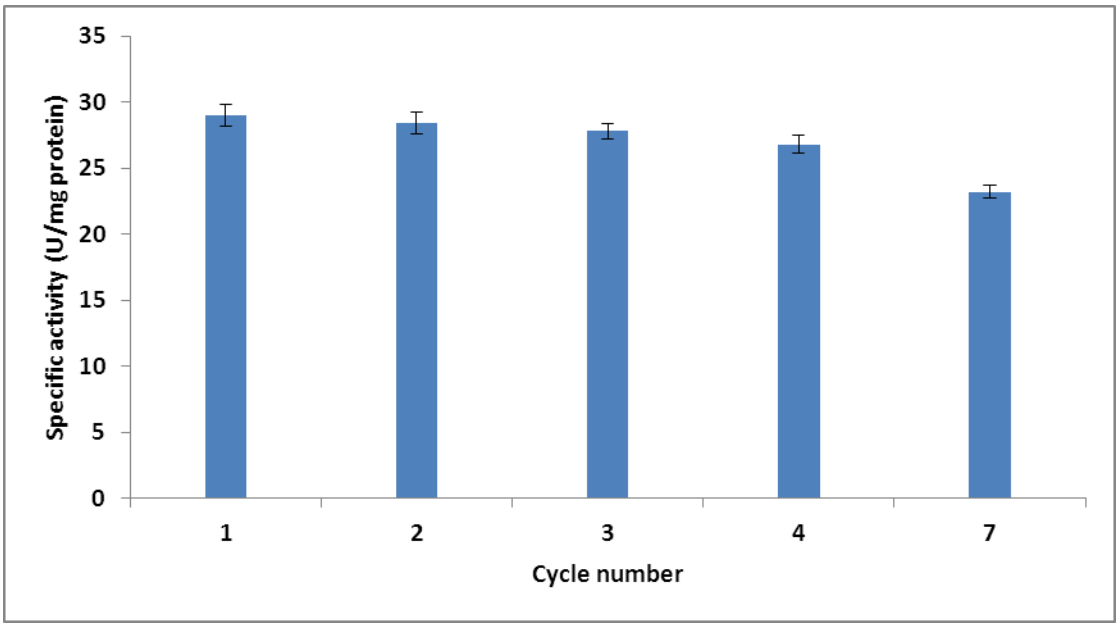

Fig. 9: Reusability of immobilized D-hydantoinase from *B. theorgensis.*