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Preparation new immobilized phytase from *Bacillus sp* for improve its thermal stability

Ohood D. Mufwez[1], Enas N. Danial[1,2]* and Hanan S. Alnahdi[1]

[1]Biochemistry Department, Faculty of Science- Al Faisaliah King Abdulaziz University, Jeddah, Saudi Arabia.
[2]Chemistry of Natural and Microbial Products, National Research Center, Cairo, Egypt.

Abstract : The aim of this study is to evaluate the biological importance of free and immobilized phytase enzyme. The support material used for phytase immobilization was alginate with concentration 2%. Mostly, the immobilization of phytase in gel alginate exhibit more thermal stability than the free enzyme. The effect of metal ions Mg2+, Fe3+, Hg2+, Cu2+, Na+ andCa2+at a concentration 0.2 M used in the study showed inhibitory effect on free more than the immobilized phytase enzyme. These results highlighted the biochemical and technical advantage benefit of immobilized phytase over the free enzyme.
Key words : phytase; kinetic studies; thermal stability; inhibitor and immobilized.

Introduction

Phytase enzyme (EC 3.1.3.8), is one of phosphatase family, its chemical name is Myo-inositol hexakisphosphate 3- phosphohydrolase[1]. These enzymes able to liberate phosphate from phytic acid [2]. Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisdihydrogenphosphate) is considered as the master form of phosphours stored in plant tissues. Monogastric animals cannot utilize phytic acid, because of the absence of phytase in their digestive system, [1]. The reaction of phytate with proteins and essential dietary minerals (Ca, Mg, Zn, Fe, ect.) are the most factors limiting the nutritional values of legumes and cereals in animals and man, so the effect of the antinutritional factor was appear. On the other hand reducing the phytate in plant materials increases their nutritional values. Phytases are the primary enzymes responsible for the hydrolysis of phytic acid. Hence, phytases have major value in enhancing the nutritional characteristic of phytate-rich food [3, 4]. Phytases are an eco-friendly product due to its effect on the level of phosphorus in the environment and fixation of nutrient factors from soil[5].These enzymes were produced by microorganisms such as bacteria, *Lactobacillus sanfranciscensis* CB1 [1], *Lactobacillus pentosus*[6]and fungi, *Aspergillus fiucuum*[7].

Immobilization is characterized as impediment of development of biocatalysts as per substance or physical treatment[8]Elnashar, 2010).Immobilization of enzyme has shuffling them highly applicable to range of improve biotechnologies. [9].

The present work deals with the immobilized phytase enzyme from Bacillus sp. And characterization with respect to kinetic parameters (optimum pH, optimum T°, thermal stability, substrate concentration, and the effect of inhibitor) on both free and immobilized phytase enzymes.

Materials and Methods

Phytase producing bacteria

Among different bacterial strains isolated from soil samples collected from different sites from north Jeddah-Saudi Arabia, a potent strain which gave a high yield of phytase was chosen for further study. The isolated strain was fully identified using morphological, biochemical and as *Bacillus sp.*The bacteria was routinely grown on phytase solid medium (PSM) at 50°C for 2-3 days then preserved at 4°C in nutrient agar medium.

1) Phytase activity assay

The activity of Phytase was determined by estimating the amount of inorganic escaped phosphate. 0.2 ml of enzyme solution with 0.9 ml of (0.5% w/v) Na-phytate in acetate buffer 0.2 M (pH 5.5) was carried out at 50°C for 30min then the reaction was stopped by 15% trichloroacetic acid adding with equal volume. One unit of enzyme activity was defined as to liberate 1µmol of phosphate per min under the assay condition [10].

Preparation of immobilized phytase enzyme

Phytase enzyme was mixed with 2% Sodium alginate, then the mixture was Dripping into 2%,$CaCl_2$ solution (w/v) from a constant [8].

Effect of different pH on phytase enzyme

The free and immobilized enzymes were incubated for 30 min at various pHs (4, 4.5, 5, 5.5, 6,6.5,7,7.5,8), phytase activity was determined.

Optimum temperature of phytase enzyme

The free and immobilized enzymes were incubated for 30 min at various temperatures (30, 40, 50, 60℃),phytase activity was determined.

Thermal stability of phytase enzyme

The effect of thermal stability on phytase activity was monitored by incubating the enzyme in different time period (0, 15,30,45,60,75,90 and 105 min) at different incubation temperature (30,40,50 and 60℃).Phytase activity was measured.

Effect of different substrate concentration on phytase enzyme

The effect of substrate concentration on phytase activity, 0.1 ml of enzyme was added to 0.9 ml of acetate buffer containing various concentrations of sodium phytate (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and 0.1), phytase activity was measured.

Effect of inhibitor on phytase enzyme

Ions and cations with concentration of 0.2 M were added to acetate buffer to study the effect of these ions and cations on free and immobilized phytase.

Results and Discussion

Optimum pH

The ideal pH esteems for the free and the immobilized enzyme were close as they were at pH 6.5., the immobilized enzyme demonstrated higher relative activities98% when contrasted with 100% for the free enzyme as appeared in Figure 1. The move to more fundamental ideal pH upon immobilized could be clarified because of the diffusional limitation of the help holding a higher centralization of enzyme product, phosphate, in the closeness of the pore space of support that adsorbed enzyme exhibit [11]. Comparable perceptions upon the immobilization of phytase and different enzyme have been accounted for by Arica et al. (2001)[12].

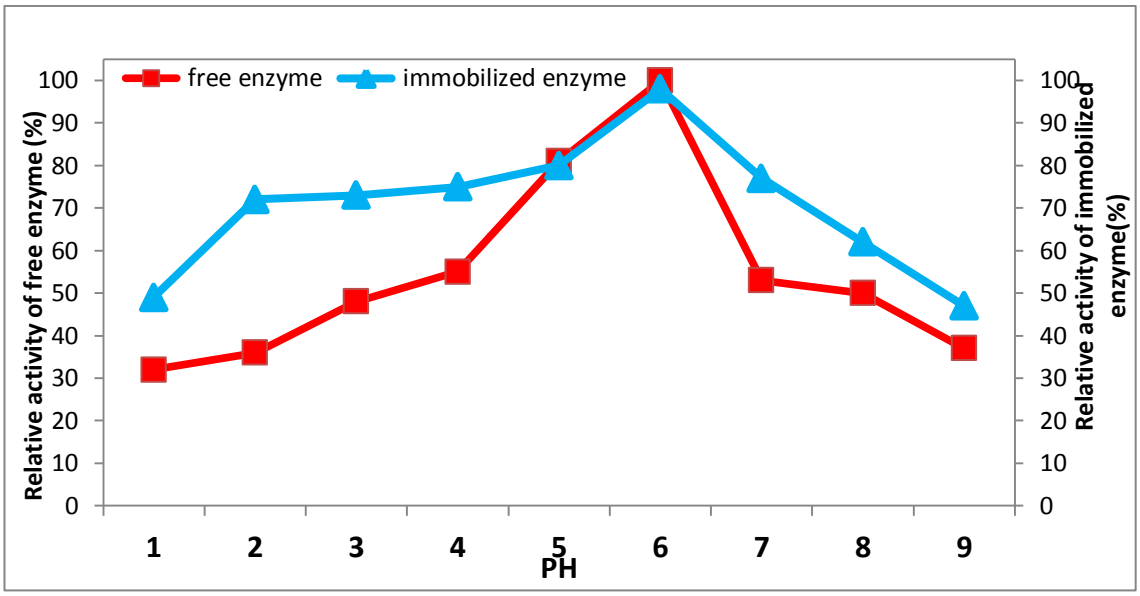

Figure 1 - pH profile of free and immobilized phytase.

Optimum temperature

The ideal temperature of the free enzyme was 50°C; however the immobilized enzyme was still at the ideal states movement from 50 to 60°C (Fig. 2). This was bolstered by Danial (2010) who announced that exclusive polyamines generously enhanced hydrogels warm solidness. This activity of the enzyme ideal temperature after immobilization could be because of the conceivable assurance of immobilized enzyme from the clot temperature through the development of a molecules chest around the enzyme protein.

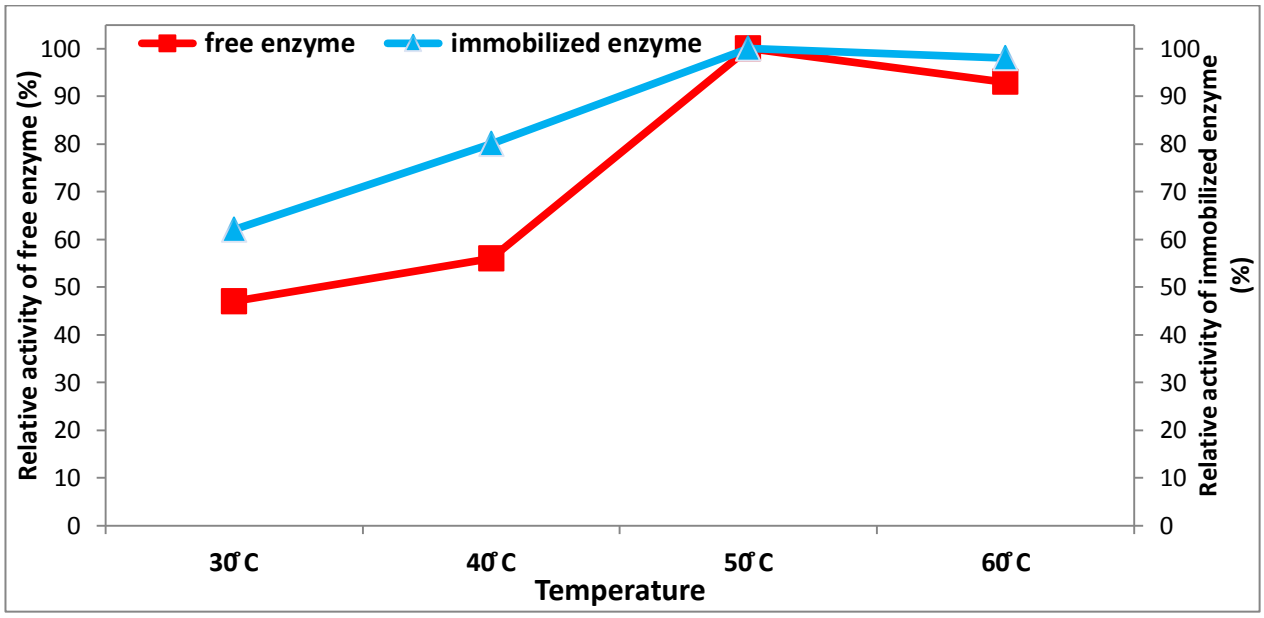

Figure 2 - Optimum temperature profile of free and immobilized phytase.

Thermal Stability of phytase enzyme

One of the primary objectives of this examination was too efficient the enzyme thermal stability to be reasonable for applicable utilize. The immobilized enzyme expose a higher thermal strength over the free protein as appeared in Table (1), which demonstrated the profile of stability for free and immobilized phytase. Obviously the relative activities of the immobilized phytase were 78% while the free phytase39% at 50ºC after 30 min for a similar incubation period. The relative activities of the immobilized and free compounds were

keeping at 82 and 62% at 60°C for 30 min, respectively. The thermal stability of immobilized phytase expanded significantly because of immobilization in alginate gel. These outcomes could be clarified by the presence of the immobilized proteins, which was less harming than free proteins in solution. These outcomes were as per Bailey and Ollis (1986)

Table (1): temperature-stability for free and immobilized phytase

Time (min)	Relative activity of enzyme %							
	Temperature							
	30 °C		40 °C		50 °C		60 °C	
	Free	immobilized	Free	immobilized	Free	immobilized	Free	immobilized
0	100	95.501	100	95.501	100	95.501	100	95.501
15	97.508	94.53	93.139	93.995	75.199	85.908	62.186	82.357
30	80.758	84.086	84.848	94.608	39.3	78.062	35.415	75.329
45	58.672	56.069	44.915	50.120	19.445	41.364	25.878	62.465
60	38.222	48.391	30.935	23.666	6.3952	23.27	15.058	34.839
75	20.022	26.473	19.037	9.8345	2.2308	2.8072	0.4833	1.6917
90	1.4314	7.0831	6.5067	0.8923	0.3943	1.3532	0	0.6506
105	0	0	0	0	0	0	0	0

Effect of sodium phytate concentration on phytase activity

Phytase activity was measured at different concentration of substrate from 0.01 mM to 0.1mM. The result (Figure3) showed that for rising substrate concentration from 0.01 to 0.09mM, there was a corresponding increase in the rate of reaction with the increase in the substrate concentration from 0.01 to 0.09mM.This results were agreement with [13]who reported the increasing of substrate value indicated that the enzyme has high affinity and specificityto the sodium phytate.

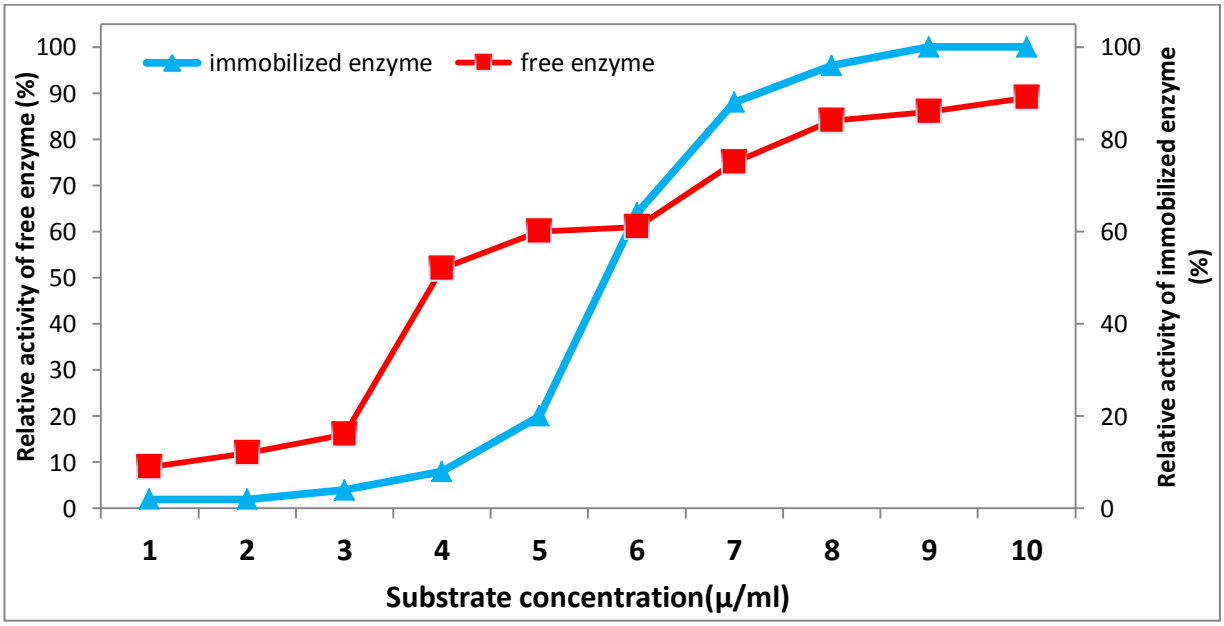

Figure 3 –Substrate concentration of free and immobilized phytase.

Effect of inhibitoron phytase activity

The metal ions used(Mg2+, Fe3+, Hg2+, Cu2+, Na+ andCa2+)in the study had an inhibitor effects when used at a concentration 0.2 M. The reduced phytase activity in the presence of these metal ionsare

attributed to a lower phytate concentration in the enzyme assay because of the appearance of a phytate precipitate[14].

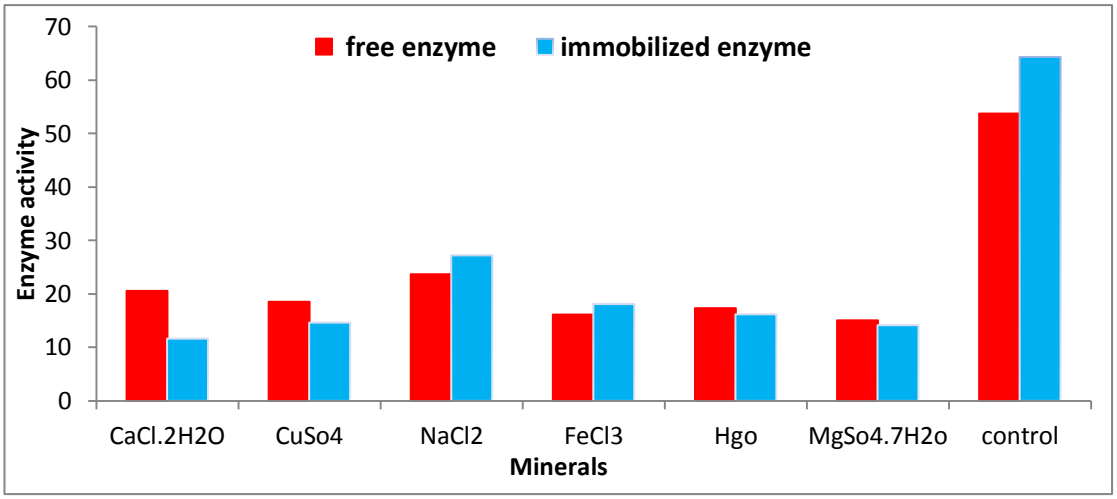

Figure 4 –metal ions profile of free and immobilized phytase.

Conclusion

In view of the outcomes, it could be reasoned that the immobilized phytase demonstrated many advantages over the free enzyme, which could make its utilization and application in ventures more significant. This was appearing through the expansion thermal stability and pH level of the immobilized enzyme over the free enzyme.

References

1. Mullaney, E.J., C.B. Daly, and A.H. Ullah, *Advances in phytase research.* Advances in applied microbiology, 2000. 47: p. 157-199.
2. Liu, B.-L., et al., *The induction and characterization of phytase and beyond.* Enzyme and Microbial Technology, 1998. 22(5): p. 415-424.
3. Grases, F., et al., *Urinary phytate in calcium oxalate stone formers and healthy people: dietary effects on phytate excretion.* Scandinavian Journal of Urology and Nephrology, 2000. 34(3): p. 162-164.
4. Onomi, S., Y. Okazaki, and T. Katayama, *Effect of dietary level of phytic acid on hepatic and serum lipid status in rats fed a high-sucrose diet.* Bioscience, biotechnology, and biochemistry, 2004. 68(6): p. 1379-1381.
5. Awad, G.E., et al., *Optimization of phytase production by Penicillium purpurogenum GE1 under solid state fermentation by using Box–Behnken design.* Saudi journal of biological sciences, 2014. 21(1): p. 81-88.
6. Hanafi, E.M., et al., *A novel phytase enzyme for Poultry feed.* World Applied Sciences Journal, 2013. 26(2): p. 194-199.
7. Singh, B. and T. Satyanarayana, *Improved phytase production by a thermophilic mould Sporotrichum thermophile in submerged fermentation due to statistical optimization.* Bioresource technology, 2008. 99(4): p. 824-830.
8. Danial, E.N., M.M. Elnashar, and G.E. Awad, *Immobilized inulinase on grafted alginate beads prepared by the one-step and the two-steps methods.* Industrial & Engineering Chemistry Research, 2010. 49(7): p. 3120-3125.
9. Barabino, R.C., D.N. Gray, and M.H. Keyes, *Coupled reactions of immobilized enzymes and immobilized substrates: clinical application as exemplified by amylase assay.* Clinical chemistry, 1978. 24(8): p. 1393-1398.
10. Arpana, M., et al., *Isolation and biochemical characterization of acido-thermophilic extracellular phytase producing bacterial strain for potential application in poultry feed.* Jundishapur Journal of Microbiology, 2017. 2011(4, Autumn): p. 0-0.

11. Elnashar, M., *Chapter in a book entitled "Low-cost foods and drugs using immobilized enzymes on biopolymers".* Book entitled Biopolymers, published by www. sciyo. com, 2010.
12. Arica, M.Y., et al., *Reversible immobilization of lipase on phenylalanine containing hydrogel membranes.* Process Biochemistry, 2001. 36(8): p. 847-854.
13. Surchandra, T., et al., *Partial purification and biochemical characterization of acid phosphatase from germinated mung bean (Vigna radiata) seeds.* African Journal of Biotechnology, 2012. 11(103): p. 16777-16782.
14. Konietzny, U. and R. Greiner, *Molecular and catalytic properties of phytates degrading enzymes (phytases).* International journal of food science & technology, 2002. 37(7): p. 791-812.

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