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Comparative Study of the Properties of the α-Amylase Enzymes Isolated from Different Sources and Immobilized in Different Materials

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ABSTRACT: α-amylase enzyme was immobilized in 4% sodium alginate and the immobilization efficiency was 70%, the immobilized enzyme was more active in the 3% substrate than the freeα-amylase. The amount of α-amylase enzyme immobilized by covalent binding in phytalol chloride and functionalized glass beads was 25.2 ± 3.1 mg / g. The covalently bound α-amylase enzyme activity was retained 98% after 6 cycles, 81.4% after 25 cycles. The enzymes immobilized in the presence of Ca^{2+} ions retained 90.7 and 80.0% of their original activities even after 30 days for ECH-activated P(HEMA) and P(St-HEMA) systems, respectively. When amylase was immobilized in dialdehydecellulose, the optimum temperature was 90-95 ° C and the enzyme activity was 44%.

KEYWORDS: α-amylase, immobilization, sodium alginate, covalent binding, enzyme, phytalol chloride, dialdehyde cellulose, temperature.

I. INTRODUCTION

The α-amylase enzyme produced in the world is one of the main enzymes used in the manufacturing industry and is one of the most promising sources of products such as glucose-fructose syrups, polysaccharides and oligosaccharide-dextrins as a result of enzymatic processing of starch-containing wastes (carbohydrates - glucose, maltose, isomaltose). The α-amylase enzyme, which is synthesized by microorganisms, is widely used not only in the food industry and medicine, but also in the production of alcohol, beer, textiles and confectionery, and in the pharmaceutical industry. Microbial amylases have been shown to be very successful in replacing the processes of chemical hydrolysis of starch in starch processing, pharmaceutical industry, as well as in clinical medicine and analytical chemistry [1, 18].

Today, due to the growing demand for amylase enzymes in the world, one of the current problems of the enzyme industry is to ensure their stability, immobilization and study of the properties of various materials, to increase the efficiency of the enzyme. Currently, although several methods have been developed to immobilize enzymes, the problem of immobilization of some enzymes in different materials, studying their properties, and ensuring their stability remains unresolved.

Accordingly, immobilization of enzymes in a solid carrier for industrial utilization has a number of advantages, such as reuse of the enzyme, the ease of separation of the product, increasing the stability of the enzyme. Materials used as matrices may affect the stability of the enzyme and the effectiveness of the enzyme immobilization, but it is difficult to predict which materials will be used as the most suitable matrix for a particular enzyme. The matrix must be water-insoluble, have high strength to bind the enzyme, be mechanically stable, and not adversely affect enzyme activity [12].

Immobilized enzymes have been widely used in various fields for many years. The nature of the matrix chosen for immobilization is very important in this process, as it affects the activity and other properties of the enzyme. The α-amylase enzyme isolated from various sources was also immobilized on different matrices, and in the process its properties were observed to be improved relative to the free enzyme [3]. A number of studies have been conducted in this area, and the results obtained when using different matrices differ to some extent.

Therefore, despite the fact that the immobilization of enzymes in various materials, the study of their properties, the development of theoretical and practical problems, as well as the ever-increasing range of types of

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immobilized enzymes and the expansion of their use, there are many unresolved issues in the field of immobilization evidence of relevance.

The main purpose of the work is to compare the properties of the enzyme α-amylase obtained from different sources and immobilized in different materials. In the some references, there is information about different methods of immobilization of the α-amylase enzyme. However, the lack of sufficient material for a comparative study of articles published to date indicates that the problem of immobilization of this enzyme has not yet been fully resolved. This research also aims to increase the efficiency of the amylase enzyme immobilization process, which uses different carriers for immobilization and provides practical guidelines for the use of immobilized amylase.

Details of study. The enzyme α-amylase was immobilized on Na alginate at different concentrations, and the efficiency of immobilization in 4% Na alginate solution was reported to be the highest (70%). The optimal substrate concentration for the immobilized enzyme was also higher than free enzyme, when the 3% substrate was used in the reaction, the activity of the immobilized enzyme was the highest. The pH stability of the immobilized enzyme was also higher than that of the free enzyme, 7.5 and 7.0, respectively [13].

When the α-amylase enzyme was immobilized by covalent binding to phytalol chloride and functionalized glass beads [2], the amount of covalently bound α-amylase in glass beads was 25.2 ± 3.1 mg / g. The optimum pH for the free amylase enzyme was 6.5, while for the immobilized enzyme was 7.5. Immobilized α-amylase exhibited better thermostability than the free enzyme. While the free enzyme lost all activity within 15 days, covalently bound amylase remained stable for up to 5 days and lost only 20% activity within 25 days. The covalently bound enzyme showed 98% activity after 6 reuse and 81.4% more activity after 25 reuse [2].

The α-amylase enzyme isolated from Bacilllus subtilis was immobilized to Na alginateby covalent binding, chitin, cephadex by physical adsorption, and dionexby ionic bonding [4]. The pH of the immobilized α-amylase was 8.0 and that of the free amylase was 7.0. While free amylase showed optimal activity at 40 °C, the immobilized enzyme showed optimal activity at 50 °C. The optimal reaction temperature of free amylase ranged from 50 °C to 70 °C for the enzyme immobilized on dionex and Ca alginate, and 80 °C for the enzyme immobilized in cephadex and chitin. The minimum level of free and immobilized α-amylase activity was 90 °C [3]. The thermal stability of free and immobilized α-amylase was fully stable at 60 °C for 10 min. The free enzyme lost 50% of its relative activity after 15 minutes, retaining 60% of its activity when immobilized using cephadex and dionex; maintained 80% activity after 30 minutes when chitin and Ca-alginate were used for immobilization. After 60 minutes, the free and immobilized enzyme lost more than 50% of its activity [3,14].

α-amylase from Bacillus amyloliquefaciens was immobilized by a covalent binding to a solution of Ca^{2+} salts and an aqueous mixture of Na alginate. Concentrations of alginate and $CaCl_2$ salts were 2% and 5%, respectively. The immobilization efficiency was 89%. The immobilized enzyme retained 51% activity after 6 re-uses and 38% activity after 7 re-uses [4].

α-amylase from *B. Subtilis* KIBGE-HAR *was* partially purified and then immobilized by entrapment in calcium-alginate beads. The catalytic properties of the immobilized α -amylase were compared with that of the free enzyme. The optimum pH of the free enzyme was 7.0 while that of immobilized enzyme was pH 7.5. The optimum temperature for free and immobilized enzyme was 60 °C and 70°C respectively. The activity yield of the immobilized enzyme was 65 %. A substrate maximum for immobilized enzyme was changed from 2 % to 3%. Incubation time for enzyme-substrate reaction was remained same i.e. 5 minutes for the free and immobilized α –amylase[5,17].

α-amylase had been immobilized on dialdehyde cellulose also. The aldehyde groups of the dialdehyde cellulose were able to react with amino groups of a thermostable alphα-amylase to form covalent bonds and resulted in a dialdehyde cellulose immobilized enzyme. The optimum pH of this immobilized enzyme was pH 7-9 while that of the free enzyme was pH 7.0. The optimum temperature for free and immobilized enzymes was 90 °C and 95 °C, respectively. The activity yield of the immobilized enzyme was 44%[6] .

α-amylase from Bacillus subtilis was immobilized on insoluble chitosan and its amino acid (L-glutamic acid and 4-aminobutyric acid) condensation adductswith the direct covalent attachment method and with glutaricdialdehyde (GDA) as a crosslinking agent. For the assays carried out via the crosslinking method at 25 C and pH 6.9, the retained activities were found to be 68.59, 97.36, and 79.50% for chitosan, chitosan– L-glutamic acid, and chitosan–4-aminobutyric acid crosslinked with 1% GDA, respectively. The immobilized α-amylase had better stability and higher

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retained activities with respect to the pH, temperature, and storage stability than the free a-amylase. In the repeated-use experiments, the α-amylase immobilized with chitosan– GDA (1%) retained about 46.45% of its original activity after 25 uses. In contrast, the activities of α-amylase immobilized on chitosan–L-glutamic acid–GDA (1%) and chitosan– 4-aminobutyric acid–GDA (1%) did not change after 11 and 8 uses, respectively. The retained activities after 25 uses were 79 and 71% with respect to the original activity for the aforementioned carriers[7].

α-amylase from mung beans (*Vignaradiata*) was immobilized on two different matrices, Amberlite MB 150 and chitosan beads. Maximum immobilization obtained was 72% and 69% in case of Amberlite and chitosan beads, respectively. The pH optima of soluble α-amylase were 5.6, whereas that for immobilized amylase on chitosan and Amberlite was 7.0. Soluble amylase and Amberlite immobilized amylase showed maximum activity at 65 ◦C, whereas chitosan immobilized amylase showed maximum activity at 75 ◦C. The Amberlite-amylase and chitosan-amylase showed a residual activity of 43% and 27%, respectively, after 10 uses[8].

The produced α-amylase by Bacillus licheniformis was immobilized on various carriers by different methods and the properties of the enzyme were compared before and after immobilization. Compared to the free enzyme, the optimum pH after immobilization enzyme changed to acidic range and the optimum reaction temperature was shifted slightly to 70 - 80 °C. The thermal stability of the immobilized enzyme was found to be higher than that of the free one. Thermal stability of free and immobilized α–amylasewas completely stable at 60°C for 10 min. Thefree enzyme lost 24% of the relative activity after 15min. while the immobilized ones retained 96% and 97%in case of utilization of sephadex and dowex; to 87% and91% of the activity after 30 minutes when chitin and Ca alginatewere used for immobilization respectively. After 60 min., the free enzyme lost more than 90% of its activity[9].

An isolated strain (from rice), Bacillus acidocaldarius was able to produce extracellular α -amylase. Immobilized α -amylase on glass beads (covalent binding) and cation exchange resin (ionic binding) had the highest immobilization yield (85.6 and 84.3%), respectively. It was further observed that, thermal and pH stabilities of immobilized enzymes were higher compared to free enzyme. Immobilized enzyme on glass beads showed the highest operational stability for up to 8 reuses with 70% residual activity. On the other hand, α -amylase immobilized on cation exchange resin retained 66.2% of its original activity after 8 cycles[10].

α -amylase (1,4-a-d-glucan-glucanohydrolase; EC 3.2.1.1, Type VI-B from porcine pancreas, extra pure 29 units mg/1) was covalently immobilized on poly (2-hydroxyethyl methacrylate), p(HEMA), and poly (styrene- 2-hydroxyethyl methacrylate), p(St-HEMA) microspheres, which were activated by using epichlorohydrin (ECH). For the assays carried out at 25°C and pH 6.9, the relative activities were found to be 61.7 and 67.0% for ECH-activated P(HEMA) and P(St-HEMA) bound enzymes, respectively. The maximum activities were obtained at lower pH values and higher temperatures upon immobilization compared to free enzyme. Immobilization, storage stability and repeated use capability experiments that were carried out in the presence of Ca^{2+} ions demonstrated higher stability. The enzymes immobilized in the presence of Ca^{2+} ions retained 90.7 and 80.0% of their original activities even after 30 days for ECH-activated P(HEMA) and P(St-HEMA) systems, respectively[11].

The purified and thermostable α-amylase from soybean seeds was immobilized by entrapment on agarose and agar matrices and their catalytic properties were compared. The optimum pH of α -amylase immobilized on both matrices was 7.0. The 1% of agarose (w/v) and 4% of agar (w/v) yielded an optimum immobilization of about 75 and 77% respectively. The reusability of agarose and agar immobilized enzyme was found to be upto 5 cycles[15].

The thermal stability increase of α-amylase obtained from locale bacteria isolate *Bacillus subtilis* ITBCCB148 was achieved by immobilization process using an ionic exchange matrix of DEAE-Cellulose.The result showed that the immobilized enzyme has an optimum temperature of 60°C, the thermal stability storage temperature of 60°C, pH 9.0 and 60 minutesdemonstrated the immobilized enzyme has residual activity of 28.1%. Although the immobilized enzyme's thermal stability was only increased 1.5 times, athigher temperatures, it was much more stable than the native enzyme[16].

II. CONCLUSION

The results showed that when the α-amylase enzyme was immobilized in 4% sodium alginate and the immobilization efficiency was 70%, the immobilized enzyme was more active in the 3% substrate than the free α-amylase. The amount of α-amylase enzyme immobilized by covalent binding in phytalol chloride and amine group functionalized glass

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REFERENCES

1. Mobini-Dehkordi,M; Javan, F.A. *Application of alphα-amylase in biotechnology.* Journal of Biology and today's world. 2012, 1:39-50.
2. M. VezirKahraman, G.Bayramoglu, et.all. α-amylase immobilization on functionalized glass beadsby covalent attachment, Food Chemistry.. 2007. 104:1385-1392.
3. T E. El-Banna, A. A.Abd-Aziz, M. I. Abou-Dobara and R. I. Ibrahim. Production and Immobilization of α-amylase from *Bacillus subtilis,* Pakistan Journal of Biological Sciences.2007.12:2039-2047.
4. E.Demirkan, S.Dincbas, N.Sevinc and F.Ertan.Immobilization of B. amyloliquefaciens α-amylase and comparison of some of its enzymatic properties with the free form.Romanian Biotechnological Letters. 2011. 6:6690-6701.
5. A.Riaz, S.A.UlQader,et all. Immobilization of a Thermostable á-amylase on Calcium Alginate Beads from Bacillus Subtilis KIBGE-HAR, Australian Journal of Basic and Applied Sciences. 2009. 3(3): 2883-2887.
6. S.Varavinita, N.Chaokasema and S.Shobsngob. Immobilization of a thermostable alpha-amylase,ScienceAsia. 2002. 28: 247-251.
7. M. A. Abd El-Ghaffar, M. S. Hashem. Immobilization of α-amylase onto Chitosan and Its Amino Acid Condensation Adducts,Wiley Periodicals, Inc. J ApplPolymSci. 2009. 112: 805–814.
8. P.Tripathi, A.Kumari, P.Rath, A.M.Kayastha. Immobilization of á -amylase from mung beans (Vignaradiata) onAmberlite MB 150 and chitosan beads: A comparative study, Journal of Molecular Catalysis B: Enzymatic. 2007. 49:69–74.
9. T.E. El-Banna, A.A. Abd-Aziz, M.I. Abou-Dobara and R.I.Ibrahim.Optimization and Immobilization of α-amylase from Bacillus licheniformis.CATRINA, 2008. 3 (1): 45 -53.
10. P.Singh, P. Gupta, R.Singh and R. Sharma.Activity and stability of immobilized alphα-amylase produced byBacillus acidocaldarius. Int. J. of Pharm. & Life Sci. (IJPLS), 2012. 3(12): 2247-2253.
11. Hayrettin,SerpilAksoy, NesrinHasõrcõ. Covalent immobilization of α-amylase onto poly(2-hydroxyethyl methacrylate) and poly(styrene -2-hydroxyethyl methacrylate) microspheres and the effect of Ca^{2+} ions on the enzyme activity. Food Chemistry. 2000. 68:259±266
12. Sumitra Datta L. Rene Christena Yamuna Rani SriramuluRajaram. Enzyme immobilization: an overview on techniques and support materials. Biotech. 2013. 3:1–9.
13. Sankaran, K. and Ravikumar. S. Enhanced production and immobilization of alpha amylase using recombinant Bacillus subtilis (MTCC 2423). International Journal of Current Research.2011. P.176-181.
14. Borgio,J.F. Immobilization of Microbial(Wild and mutant strains) Amylase on coconut fiber and alginate matrix for enhanced activity. American Journal for Biochemistry and Molecular Biology. 2011. 1(3), 255-264.
15. Prakash.O. and Jaiswal.N.Immobilization of a Thermostable α -amylase on Agarose and Agar Matrices and its Application in Starch Stain Removal. World Applied Sciences Journal. 2013. 13 (3): 572-577.
16. Yandri, Suhartati,T. and Hadi,S. Immobilization of α-amylase from locale bacteria isolate Bacillus subtilis ITBCCB148 with diethylaminoethyl cellulose (DEAE-Cellulose). *Material Science Research India,* 2010. 7(1), 123-128.
17. Prabakaran,G. and Pugalvendhan,R.Production and immobilization of alpha amylase by using Bacillus subtilis.Recent Research in Science and Technology, 2009.1(4): 189–194.
18. Monteiro de Souza,P., Pérola de Oliveira e Magalhães.Application of microbial α -amylase in industry – a review, Brazilian Journal of Microbiology. 2010. 41: 850-861.

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