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Evaluation of Immobilised Amylase Activity Ondifferent Textile Fabricmaterials

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ABSTRACT

Free enzyme utilization requires large quantity of enzyme which is much expensive economically and reusability is not possible. Hence, with the view to develop the innovative method of an enzyme immobilization, enzyme amylase have been immobilized on different textile fabric materials viz;terecott, cotton, silk and nylon. The stability and reusability of an enzyme was evaluated. The enzyme coated textiles were reacted with starch and enzyme activity has been expressed in terms of maltose released. It was observed that the textile immobilized enzyme can be reused for ten successive reaction set ups, with significant enzyme activity. The maximum activity was seen in case of silk followed by terecott, compact and loose cotton and nylon. The experimental findings enlightened the superiority of textile immobilized enzyme for stability and reusability over free and other enzyme substrate reaction setups.

INTRODUCTION

An enzyme is termed immobilized, if its mobility has been restricted by chemical means. Immobilization of enzymes refers to techniques which represent variety of advantages over free enzyme catalysis including increased stability of enzyme, easy separation of reactant and product [1].Immobilized enzymes have been widely used for many years in different industrial processes [2]. Enzymes can be immobilized to different carriers by entrapment, adsorption, ionic binding and covalent binding. This is beneficial for protecting and stabilizing the enzymes, thereby enhancing their properties and their repetitive utilization. Amylases are starch degrading enzymes. They are widely distributed in microbial, plant and animal kingdom. They are involved in hydrolysis of starch molecules, decomposing them in glucose [3]. The enzymes are high molecular weight proteins that are produced by living organisms [4]. Enzyme amylases are available commercially, and they have almost completely replaced chemical hydrolysis of starch in the starch processing industry [5]. Entrapment is taken as the most preferable method because it prevents excessive loss of enzyme activity after immobilization, increases enzyme stability in microenvironment of matrix, protects enzyme from microbial contamination. It can be reused, involved processes can be operated continuously with better controls, easy separation of the product(s), simpler handling of materials and effective reduction in process cost [6].

Sodium alginate beads are widely used in enzyme immobilization because the gel formation occurs at mild conditions and possesses no risk of harm to humans. Entrapment of amylase in sodium alginate beads has shown to be a relatively easy, rapid and safe technique. Starch molecules are very large, often reaching a molecular weight of 80 million Daltons [7]. It is expected that starch hydrolysis reaction could occur more effectively if enzyme bound to surface. The use of enzymes in a soluble or free form must be considered as wasteful because the enzyme generally cannot be recovered at the end of the reaction. The principal enzymes applied in textile industry are hydrolases (*amylases, cellulases, proteases, pectinases and lipases/esterases*) and *oxidoreductases* (*catalases*) [8].

The use of enzymes in the textile industry is an example of white industrial biotechnology, which allows the development of eco-friendly technologies [9]. Enzyme technology is that concerned with the immobilization of enzymes on insoluble polymers, such as membranes and particles that act as supports or carriers for the enzyme activity [10]. On the other hand the possible use of textile fabric material as carrier material in enzyme immobilization have been reported to be superior in enhancing the surface area followed by turn over in enzymatic reaction, than existing techniques. Hence, in the present study enzyme was used for immobilization with different textile material in comparison with free and sodium alginate encapsulation and further evaluated for its stability and reusability.

MATERIALS AND METHOD

1: Collection of textile fabric materials:

Different textile fabric materials such as cotton, silk, terecott and nylon were purchased from local market Washim, in Vidharbha region of Maharashtra.

2: Activation of carrier textile fabric materials:

Activation of carrier textile fabric material was carried out by adopting method suggested by Rani *et al.*, (2012) [11]. The textile fabric materials were cut into size of 10 × 6 cm. All the pieces of textile fabric were washed 2-3 times using distilled water and allowed to dry at room temperature. Textile fabric materials to clear up the mesh of textile fabric, were chlorinated with NaCl -Cl_2 at the rate (1:3 per cent ratio) solution followed by 3 per cent $NaNO_3$ (w/v) by immersion treatment for 10 minutes and removed to dry at room temperature and finally consider as activated textile fabric (AFMs) and further use for an immobilization.

3: Immobilization of enzyme on activated textile fabric by immersion treatment:

The AFMs then get immersed in sticker solution of 0.1 percent gelatin for 10 minutes. The textile fabric material allowed to drying after laminating with sticker solution. The AFMs were used in its dried form for enzyme immobilization. The immobilization of enzyme was done by adopting immersion treatment. The standard enzyme amylase/diastase solution was made by dissolving 0.5 gm of diastase in 50 ml of sterile buffer solution of pH 6.4. The AFMs were immersed in enzyme solution by hanging on glass rod for 24 hours at room temperature.

4: Drying of enzyme immobilized textile fabric:

After immersion treatment the textile fabric material was removed and allowed to dry aseptically for further 24 hours in a fume disinfected closed vessel.

5: Immobilized textile fabric materials ready to use:
The enzyme treated dried textile fabric was further consider as immobilized textile fabric (ITF) material and use for further study. The ITF further kept for enzyme activation in an incubator at 37 ^{0}C for 30 minutes.

6: Starch hydrolysis by immobilized textile fabric:
Starch solution was prepared by dissolving 0.5 gm of soluble starch in 50 ml of sterile buffer of pH 6.4, and further kept for substrate activation in an incubator at 37 ^{0}C for 30 minutes. The activated ITF material was immersed in 40 ml of activated substrate solution in 50 ml boiling test tube and further incubated for the enzyme-substrate reaction.

7: Estimation of product:
Product estimation was done as per the method recommended by Plummer and Leckenby (1985) [12].The reaction after immersion was allowed to run for 30 min at 37^{0}C.After reaction time course, the (ITF) was removed immediately and transferred to second successive cycle. The reaction was further terminated by adding 1 ml DNSA solution and the reaction mixture was boiled at 100 ^{0}C for 5 minutes. The volume of reaction mixture was made up to 12 ml by adding distilled water. The same procedure was carried out for free enzyme and Gel Bead Immobilized Enzyme. The maltose thus released was further estimated by spectrophotometric analysis at 540 nm and expressed as an enzyme activity.

8: Preliminary Viability analysis Per cent immobilization analysis:
The ITF was further used for successive cycle and the Enzyme-Substrate (ES) reaction was carried out up to 10 repeated cycles with an interval of 30 minutes. By this way viability of immobilized textile fabric was carried out. The viability analysis was carried out by comparing the maltose released per cycle and maltose release in standard free and encapsulated enzyme amylase respectively, adopting the method suggested by Bernfeld (1955) with slight modifications [13].

RESULT AND DISCUSSION
The viability analysis was carried out by comparing the maltose released per cycle. The observations were recorded in terms of optical density at 540 nm, and graphically represented in (Figure 1). The enzyme substrate reaction was repeated up to 10 cycles; further the maltose released was estimated for each cycle so as to check retention viability of an immobilized enzyme. There after the pooled mean was expressed so as to compare the enzyme activity of immobilized and standard enzyme. The observations were recorded in terms of maltose released as mg/ml, and graphically represented in (Figure 2). The result revealed that, initially the enzyme activity record was very low up to 2 cycles in all the immobilized setups except silk, however the initial rise in enzyme activity was recorded in case of free and gel bead immobilized enzyme.

Incase of enzyme immobilisation in tericott ITF, the initial 1st and 2nd cycle of reaction the maltose released was recorded tobe 0.23 mg/ml and further it was declined to 0.15 mg/ml upto 4th cycle of reaction. There after the enzme activity showed dramatic enhancement in releasing the matose in 4th and 5th cycle, at 5th cycle the maximum maltose i.e 0.7mg/ml was recoerded. Further it was also observed that the enzme activity was found tobe significantly stable up to 9th cycle of reaction. The maltose concentration recorded was minimum i.e. only 0.1 mg/ml in 10th cycle of reaction.

Incase of enzyme Amylase activity as maltose release in loose woven cotton ITF, initially in 1st, 2nd and 3rd cycle of reaction the maltose released was high and recorded to be 0.47 mg/ml andwas at par with each other. Further it was declined to 0.39 mg/ml in 4th cycle of reaction. There after the enzme activity showed dcreseased in releasing the matose, the maltose concentration recorded was 0.31 mg/ml in 5th, 6th and 7th cycle and was at par with each other in releasing the maltose. At 8th and 9th cycle the maltose concentration was found to be decrease upto 0.15 mg/ml respectively. Further it was also observed that the enzyme activity was increased in 10th cycle of reaction at which the maltose concentration recorded was 0.39 mg/ml. The maximum activity was recorded in initial cycle of reaction in loose woven cotton ITF and decreased there after.

Incase of enzyme Amylase activity as maltose release in nylon ITF, it was observed that initially from 1st to 8th cycle of reaction the maltose released was significantly low and recorded to be only 0.15 mg/ml. Further it was inclined to 0.7mg/ml and was maximum in 9th cycle of reaction. There after the enzme activity showed decreased in releasing the matose. The maltose concentration recorded was minimum i.e. only 0.1 mg/ml the results indicates the unsutability of nylon as the carrier matterial for enzme immobilization.

Incase of enzyme Amylase activity as maltose release in compact woven cotton ITF, it was observed that initiallyfrom 1st to 6th cycle of reaction the maltose released was significantly low and recorded to be 0.15 mg/ml. Further it was inclined to 0.7 mg/ml in 7th cycle and was maximum. There after the enzme activity in 8th and 9th cyclewas at par in releasing the matose. There after in 10th cycle the enzme activity showed again decreased in releasing the matose. The maltose concentration recorded was minimum i.e. only 0.1 mg/ml.

Incase of enzyme Amylase activity as maltose releases in silk ITF, it was observed that initiallyin 1st and 2nd cycle of reaction the maltose released was maximum and coparetively high among all the materials studied, the maltose recorded was 0.63 mg/ml. In 3rd 0.55 and in 4th 0.63 maltose mg/ml was recorded. Further it was declined and at par from 5th to 9th cycle and it was 0.47 mg/ml respectively. There after in 10th cycle the enzyme activity showed again decreased in releasing the matose. The maltose concentration recorded was minimum i.e. only 0.3 mg/ml.

In case of enzyme Amylase activity as maltose release in standard free enzyme, initially in 1st cycle of reaction the maltose released was high and recorded to be 0.87 mg/ml. Further it was declined to 0.71 mg/ml in 2th cycle of reaction. There after the enzme activity showed decreased in releasing the matose and the maltose concentration recorded was 0.63 and 0.55 mg/mlin 3rd and 4th cycle respectively. In 5th and 6th cycle maltose concentration was found to be 0.63 and was at par with each other in releasing the maltose. In 7th cycle maltose concentration was found to be 0.55 mg/ml. In 8th cycle the maltose concentration was found to be increase upto 0.63 mg/ml. Further in 9th and 10th cycle it was also observed that the enzme activity was decreased and the maltose concentration recorded was 0.55 and 0.47 mg/ml respectively.

In case of enzyme Amylase activity as Gel bead immobilized enzyme, initially in 1st and 2nd cycle of reaction the maltose released was recorded to be 0.97 mg/ml and was at par with each other in releasing the maltose. Further it was inclined to 1.40 mg/ml in 3th and 4th cycle of reaction and was at par with each other in releasing the maltose which was maximum as compared to all the enzyme setups in the study. Thereafter the enzme activity showed decreased in releasing the matose and the maltose concentration recorded was 1.39 mg/ml in 5th, 1.27 mg/ml in 6th and 7th cycle. The maltose concentration was found to be again decreased upto 0.99 and 0.95 mg/ml in 8th and 9th cycle respectively. In 10th cycle maltose concentration was found to be increase upto 1.0 mg/ml.

The mean maltose released up to all the cycles was calculated and graphically represented in (Figure 3). From the results it was observed that, the enzyme activity was superior incase of encapsulation method in gel bead 1.16 mg/ml, followed by free enzyme 0.59 mg/ml, whereas in case of silk ITF maltose released was recorded to be 0.50 mg/ml. In tericott,compact woven cotton and loose woven cotton maltose released was recorded to be comparatively low i.e. 0.42, 0.32, and 0.34 mg/ml respectively. The nylon (TFM), showed minimum enzyme activity i.e. the maltose released recorded was 0.2 mg/ml. The study enlights that only silk, terecott, compact woven cotton and loose woven cotton may be the possible carrier material for immobilization of enzyme, it may be due to the significant ion exchange potential for this textile. However, this textile can further be studied for the research and development to develop the functional fabrics.

CONCLUSIONS

The experimental findings on the present studies entitled "Studies on amylase immobilization using different textile fabric materials as carriers and evaluation of its activity", the conclusions drawn indicated that, the textile fabric material can be positively used as the carrier material in enzyme immobilization, which can be the lucrative alternative to avoid the technical constraint in enzyme recovery for its subsequent use. The enzyme amylase activity is superior after the two successive cyclic runs which may be due to the standardization period required by fabric material in substrate solution. In case of silk and terecott the enzyme amylase activity was superior from the initiation of the reaction, which indicates that the standardization period is not required by fabric material in substrate solution. The fabric material of silk and terecott may be the choice of carrier material for immobilization of amylase enzyme.

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Figure1: Optical densities of ITF materials, free enzyme & gel beads at 540 nm for 10 cycles.

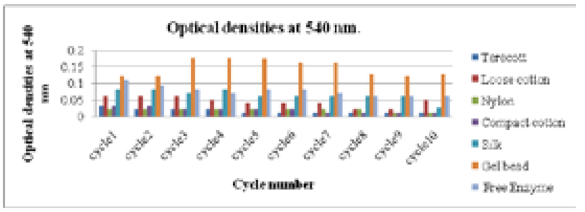


Figure2: Amylase activity in terms of maltose released (mg/ml) in all cycles by free and immobilized setups.

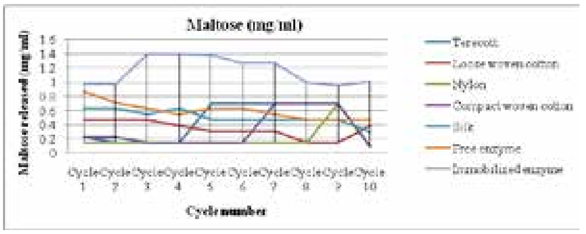
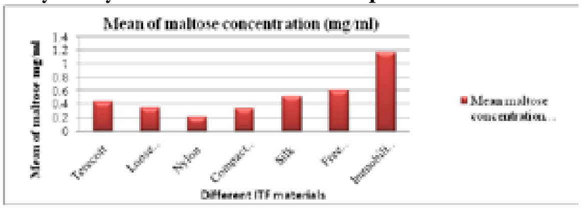


Figure 3: Mean maltose concentration (mg/ml) produced in all cycles by free and immobilized setups.



REFERENCE

1 Raviyan P., J. Tang and B. A. Rasco (2003). "Thermal Stability of Alpha-Amylase from Aspergillus orhyzaeEntrapped in Polyacrylamide Gel". J. Argic. Food Chem., 51(18):5462-5466. | 2 Swaisgood H. E. (1985). "Immobilization of Enzymes and Some Applications in the Food Industry", in Enzymes and Immobilized Cells in Biotechnology, A I Laskin (Ed.). The Benjamin/Cummings Publishing Company, Inc., London, 1:1-24. | 3 Paquet V., C. Croux, G. Goma and Soucaille (1991). "Purification and Characterization of the Extracellular Alpha Amylase from Clostridium acetobutylicumATCC 824".Applied Environmental Microbiology, 57:212-218. | 4 Jenkins R. O. (2003). In:Textile Processing with Enzymes, Edited by Cavaco-Paulo A. and Gübitz GM, Woodhead publishing Ltd.,CRC Press, Boca Raton, ISBN 18557366101. | 5 Gupta R, Gigras P, Mohapatra H, Goswami V. K., Chauhan B(2003) 'Microbial -amylases: A biotechnological perspective'. Process Biochem, 38:1599-1616. | 6 Kennedy J.F. (1979) In: Immobilized microbial cells. ASC Symposium Series 106, Venkatsubramanian K. (ed.), Washington D. C., American Chemical Society, 119. | 7 Wolfgang Gerhartz (1990). 'Enzymes in Industry'.Weinheim, FRD. | 8 Lenting H.B. M. (2007). Enzymes in textile production. In: Aehle, W., editor. Enzymes in industry, production and applications, 3rdedn.Weinheim: Wiley-VCH VerlagGmb H and Co.: 218-230. | 9 O'Neill C., F.R. Hawkes, D.L. Hawkes, N.D. Lourenco, H. M. Pinheiro and W. Delee, (1999).Color in textile effluents sources, measurement, discharge consents and simulation: a review. J ChemTechnolBiotechnol,74(11):1009-1018. | 10 Smith E. J. (1998) Biotechnology: Studies in Biology (3rd ed), Cambridge Univ., Cambridge: 83-91. | 11 Rani K. (2011). "Immobilization of AzadirachtaindicaAlkaline Phosphatase onto Polyethylenimine-Treated Chlorinated Woven BombyxmoriiSilk Fabric".Int. J. Biochem Biotech, 1(1):1-4. | 12 Plummer J. T. and J. D. Leckenby (1985).Copy research foundation and futures.Incopy testing. New York: The Advertising Research Foundation. : 24-34. | 13 Bernfeld P. (1955), "Amylases α and β". Methods in Enzymology, clockwick S. P. Kalpan N. O. (Eds.) Academic Press New York.1:149-158. |