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Immobilization of protease on modified glass beads by salinization: its use in commercial activity

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ABSTRACT:-

In the present work immobilization of Protease enzyme on glass beads by covalent coupling was done. Glass beads were bought from scientific stationary were used. Protease was immobilized by covalent coupling. After immobilization process for further study, various parameters were optimized and carried out the comparison studies by using protease loaded glass beads. Furthermore, FTIR and SEM analysis were done to prove that the enzyme was immobilized on the support bead. In application protease of it was applied to purified the fruit juice i.e. Pineapple juice, watermelon juice and sugarcane juice. In addition, this study introduces a new achievement of the eco-friendly effect of immobilized enzyme, due to reuse full function of immobilized enzyme.

Keywords: Protease, Glass beads, Enzyme Immobilization, FTIR & SEM study of alcohol protease.

Introduction:-

Enzyme have high specificity and gives catalytic activity under mild conditions. Therefore, they have larger space of applications in commercial field. They have efficient and eco-friendly nature. Industrial applications of free enzymes are till poor because, their separation is difficult and reuse as well as low stability upon exposure to pH values, organic solvents and heat. On other hand, Immobilized enzymes provides many advantages as compared to free enzymes. They are reusability, stability and easy separation from the reaction solutions.

Now a days, many techniques are developed like physical adsorption, ion exchange, matrix entrapment, covalent bonding, micro capsulation have been largely applied in enzyme immobilization process on the bases of property of various support materials, like synthesized and also which are found from nature. Synthesized support like, hydrogels, HEMA, carrageenan, glass beads, agar, poly vinyl alcohol (PVA). Most important criteria for the application in industrial application of biocatalyst like immobilized enzymes, different kind of immobilized microbial cells, and its operational stability.

In present research work investigation of different study of immobilized protease on glass beads, also worked on the stability and activity. Mainly focused on reusability and activity of protease as its advantages in commercial level. Beneficial results were achieved by this research work as used more reaction cycles rather than one reaction cycle.

2. Experimental

Materials

Glass beads which are normally used in chemistry lab, NaOH from organic lab, H_2O_2 from medical store, Concentrate H_2SO_4 which is used in chemistry lab, Protease **(200U/mg)** & 3-aminopropyl-trirthoxysilane(3-APTES) from sisco research laboratories Pvt. Ltd.

Characterization

FTIR Characterization was recorded on FT-IR Spectrometer. At CHARUSET UNIVERSITY Changa, Gujarat. SEM electron microscopic analysis was one by Scanning electron microscope at CHARUSET UNIVERSITY Changa.

Surface cleaning and activation process of glass beads:

For cleaning and surface activation the of surface of glass beads were added in solution of 30% V/V NaOH and 6% V/V H_2O_2. After, then heating at 60˚- 80˚ C for 15 minutes. On completing this step beads were filtered out from the solution, washed with distilled water, and dri ed. In next step beads were added in to the solution of 30% V/V H_2SO_4 , 6% V/V again heated for 60 minutes at 90˚ C. This is the pre-conditioning method provides suitable surface with enough amount of silanol group for silan derivation to form siloxane covalent bonds. Formation of Si-O-Si bonds.

Procedure for synthesized organ functionalized glass beads:

21 gm of glass beads from activated glass beads were taken, 150 ml of dry toluene and 5 ml of 3 -aminopropyl-triethoxysilan were added into reaction under nitrogen atmosphere. Then the was stirred under reflux for 15 h. afterwards, amino functionalized glass beads were separate out from the solution and kept in to the oven at 110˚ C for 2 h. for achieved complete

Immobilization of protease on glass beads:

After done all the cleaning and surface activation process glass beads were slowly stirred in reaction flask which contains 20% V/V glutaraldehyde solution. Stirring process was done for 2 h. at room temperature approx. 23 - 25˚ C. The glass beads were than immersed in protease solution which was diluted with sodium acetate buffer ($_p$H=4) with ratio of 1:4. (6 ml enzyme solution in 24 ml of buffer solution) at 4˚ C. Then beads were used for further measurements and application.

$$\text{Activity yield} = \frac{\text{Activity of immobilized protease}}{\text{Specific activity of free enzyme}} \times 100$$

Preparation of juice.

Freshly picked up pineapples were taken and kept at normal room temperature. Pineapple was washed properly and outer skin was removed from it, then fresh juice was made by normal mixture without adding any extra things. After complete filter process, 150 ml of juice was separate out in a beaker to perform assay method.

Treatment of immobilized Protease to check reusability

5 ml of juice from was taken in test vial; 0.5 ml of starch solution was added. After adding beads of immobilized enzyme contains 0.05 g of enzyme and phosphate buffer solution ($_p$H=6.9) was incubated at room temperature for 10 minutes. 2 ml of 3, 5 -dinitrosalicylic acid was added and vail was incubated in boiling water bath for 10 minutes reducing sugar was determine by spectrophotometer at 420 nm.

pH activity profile

As enzymes consist of protein, the catalytic activity is markedly affected by environmental conditions, especially the *pH* of the aqueous medium. Thus, information on changes in *pH*-activity behavior caused by the immobilization of enzymes is useful for an understanding of the structure-function relationship of enzyme protein. Hence, the activity of the free and immobilized protease has been measured by incubating free and immobilized enzymes at 27 °C for 30 min in the 50 mm phosphate buffers of different pH ranging from 5 to 9 and using ethanol as a substrate. The absorbance of the reaction mixture was measured at 420 nm and correlated to the concentration of the enzyme. From the calibration, plot activity of the enzyme was determined.

Thermal stability

Because of the immobilization of enzyme the heat stability is enhanced, it is advantageous for the industrial application of immobilized enzymes and is thus important in determining the feasibility of immobilized enzymes for a particular application. Therefore, the thermal stability of free and immobilized enzymes was investigated. Free and immobilized enzymes were placed in the optimum pH buffer and incubated at different temperatures (30 to 70 °C) for different time intervals, activity of the enzyme was then determined as described earlier Thermal deactivation constant (Kd) was calculated by using the following equation :

Ln At = In Ao - Kd (t)

Where ‘Ao’ is the initial activity and ‘At’ is the activity after heat-treat for minutes**.**

3. Result and Discussion

pH Outline

Every enzyme has an optimum pH at which it shows optimum activity. **Figure 1**shows the pH activity of free and bounded. We have observed that free and entrapped enzyme was showing a maximum of 5 to 8 pH. During immobilization, results shows that there is no conformational change.

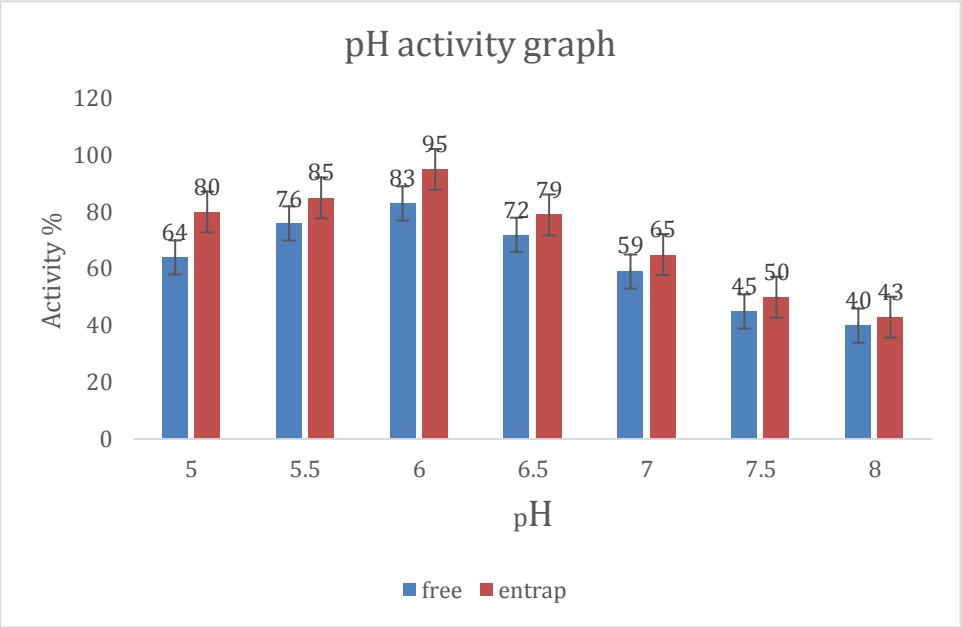

Fig-1 pH activity graph of immobilized protease on glass beads

Thermal Stability:

Enzymes is temperature-dependent. When the temperature increases enzyme reactivity increases and beyond a definite limit the enzyme, gets deactivated. **Figure 2**shows that entrapped enzyme show better thermal stability compared to free enzyme. The entrapped enzyme showed better thermal stability as they are encapsulated within the beads.

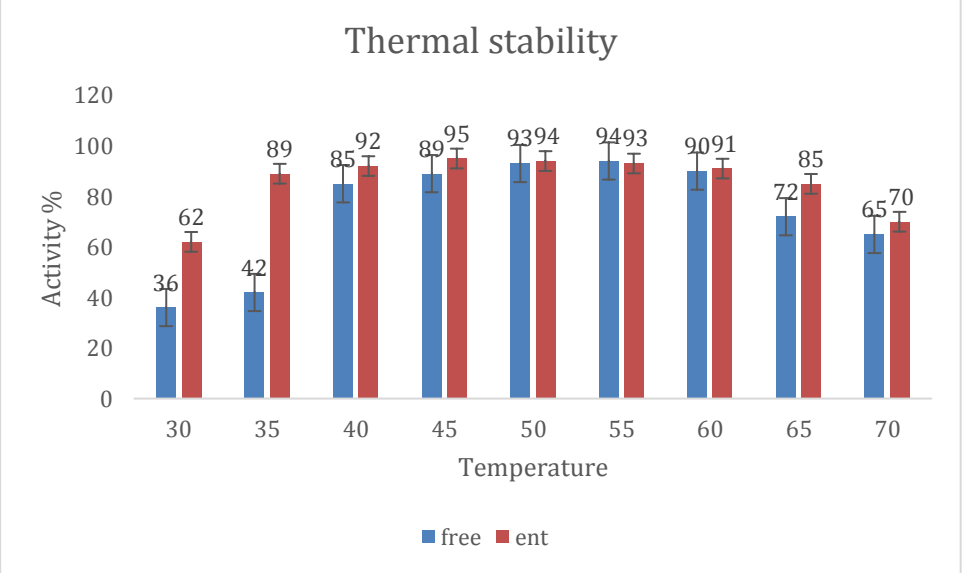

Fig-2 Thermal stability graph of immobilized protease on glass beads

FTIR analysis

FTIR analysis of bead was done. FTIR analysis shows the chemical presence of specific chemical groups of enzyme. Spectra were recorded in the spectral range of 4000 – 500 cm-1.α-amylase have amide group in its structure. Pease peak pears at 1651.58 m^{-1} shows stretching of -C=O and 1026.07 cm-1 show the bond starching vibration of amide. 3395.48 cm-1 shows –OH starching vibration.

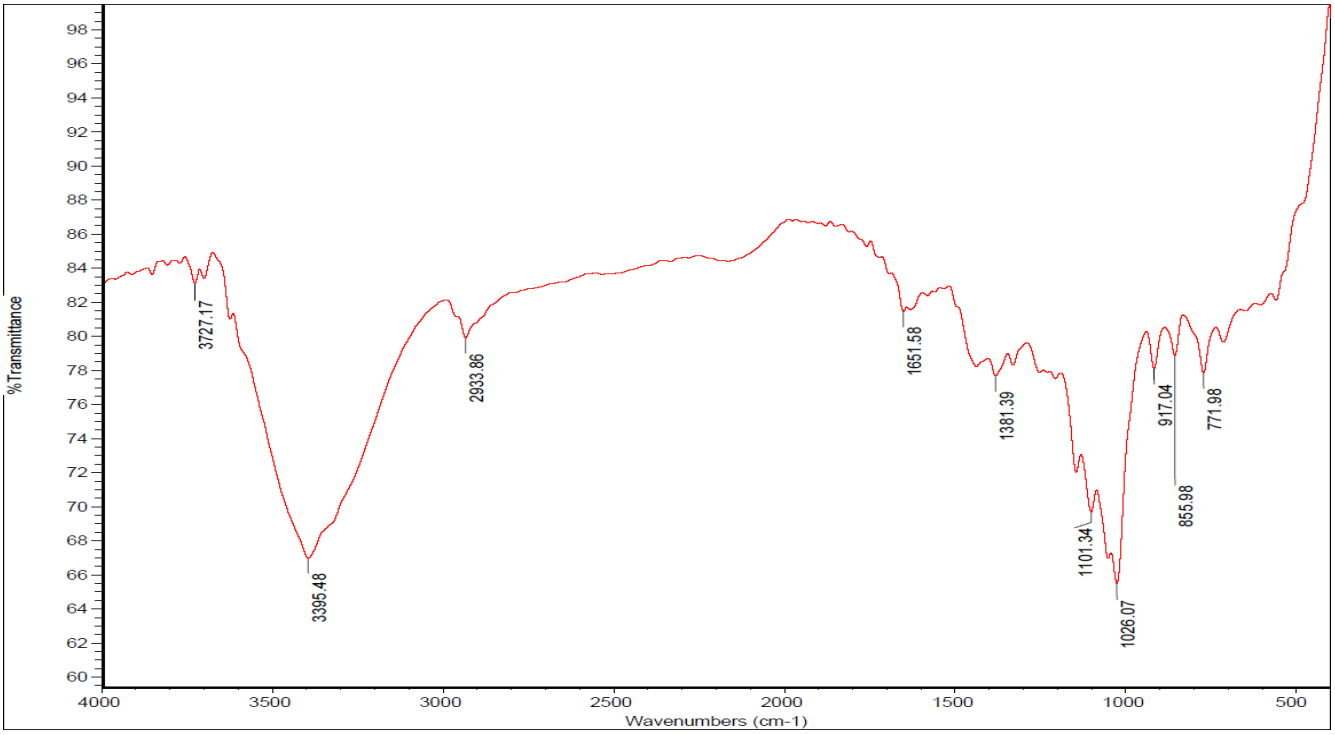

Fig-3 FT-IR of immobilized protease on glass bead.

SEM analysis (Scanning electron microscopy)

SEM analysis of bead in which have enzyme was entrapped, purpose of this study was to obtain a topographical Characterization of the support beads. SEM photographs were taken using a scanning electron microscope, at required magnification at room temperature. Distance of working 9.5 mm was maintained, and the acceleration voltage used was 20.00 KV

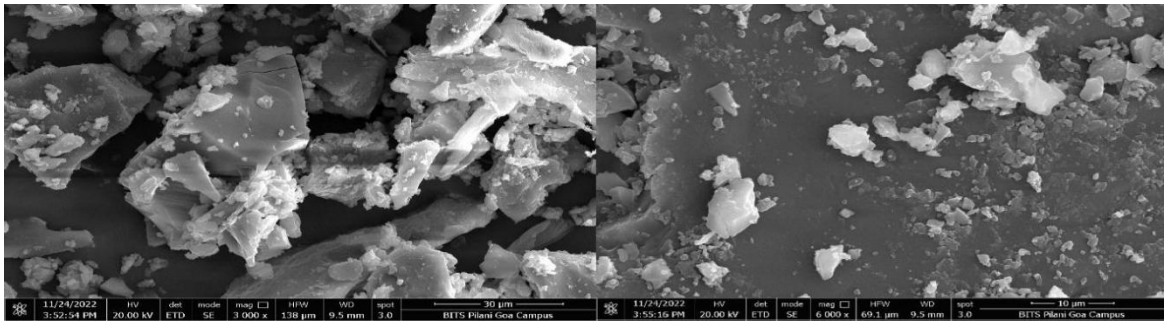

Fig-4 SEM image of immobilized protease on glass beads

Storage stability

For study of residual activities of the free and immobilized enzymes stored at room temperature (30 °C) were determined and the activities were expressed as percentage retention of their residual activities at different times. Immobilized beads were also kept at 5 °C for 45 days and residual activity was examine after every 7 days.

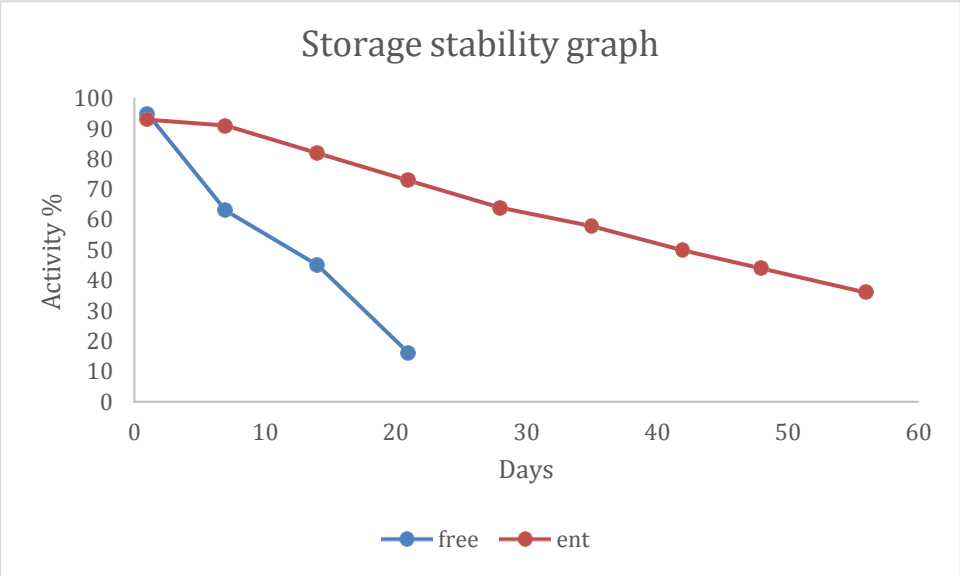

Fig-5 Thermal stability graph of immobilized protease on glass beads

Use of immobilized Protease in Juice purification.

Fresh pineapple juice, watermelon juice, sugarcane juice weaken. For this 5ml of juice was taken and added to the vial. Then entrapped enzymes beads were added in it after this in next step 5 ml of buffer solution was added in the test vial, and 1 ml of casein solution was added. Then vial was incubated in a shaker incubator at 35 ˚C for 30 min. after incubation 5 ml of carbonate and 1 ml of folin's reagent were added. And again kept the test vial for 30 min at 35 ˚C. Practical was performed with a free enzyme and with entrapped enzyme. OD (optical density) was carried out for further calculation at 420 nm.

Reusability of bead:

In industries and bio-chemical reaction, reusability of entrapped enzyme has great importance. It was checked by using beads in the assay method in place of free enzyme solution. The reusability of entrapped enzyme beads was checked and it was founded as shown in. **Figure-5** bounded enzyme employed 75% of its enzyme reactivity after 5 or 6 rotations and 50-40 % activity after 8 cycles showing the advantage of immobilized enzyme and which increases its applicability.

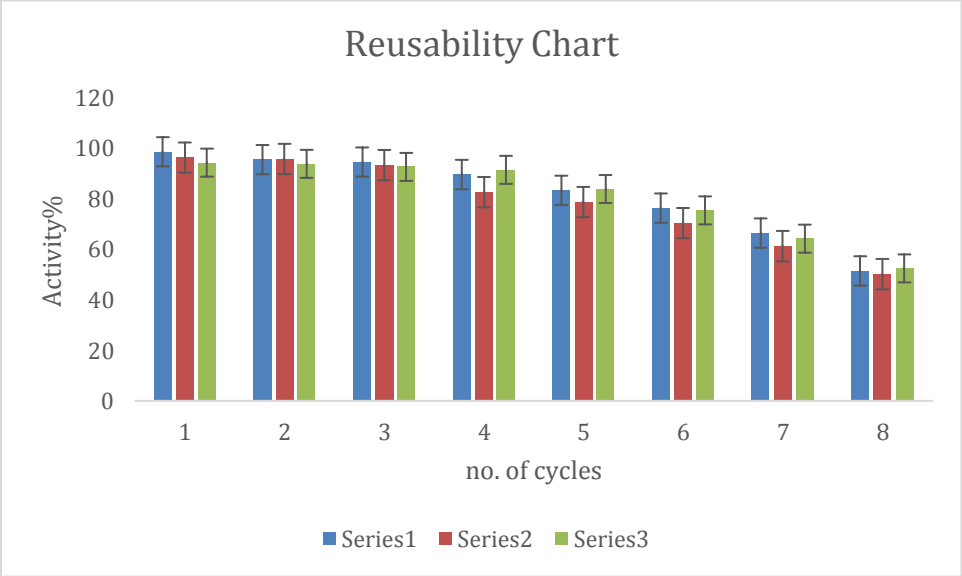

Fig-6 Reusability chart of immobilized protease on glass beads.

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