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Immobilization of Alcohol Dehydrogenase on Organofunctionalized Glass Beads and Comparative Studies: It's Beneficial Uses

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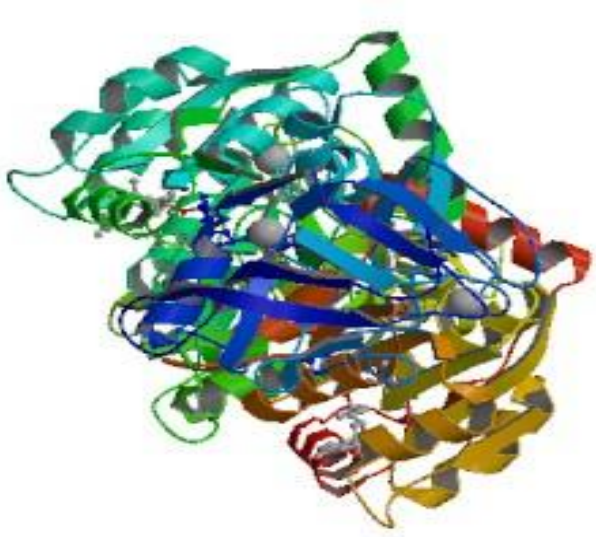
Abstract:

In this Research Study immobilization of Alcohol Dehydrogenase enzyme on glass beads was done. k-carrageenan is a natural polysaccharide like cellulose obtained from seaweeds. ADH was immobilized by Covalent coupling. Then for further study, various parameters like Thermostability ,$_p$H study, storage stability and Reusability were optimized and carried out the comparison studies. Furthermore, FTIR analysis was done to prove that the enzyme was immobilized on the support bead. And the application was done with 3 different fruit juices. i.e. sugarcane juice, pineapple juice, and watermelon juice. In addition, this study introduces a new achievement of the eco-friendly effect of immobilized enzyme.

Keywords: Alcohol Dehydrogenase, Glass beads, Enzyme Immobilization, FTIR & SEM study of alcohol dehydrogenase, Organofunctionalized.

1. Introduction: -

The counterweight of biocatalysts debris one of ultimate real questions in differing fields of their applications. The hold of enzymes is individual of the low methods secondhand for this purpose. Immobilization leads to commercially reasonable requests of arrangements in industry and many different circles. ADH is an catalyst which is intensely appealing for technical delay aimed at conversion of coenzymes, fine natural combining and analytical perseverance of intoxicating in differing publishing. ADH from yeast and mare liver have happened connected to natural and artificial aircraft carriers. Many studies have happened stated on the stability of disabled ADH towards heat, continuos movement, depository and different substrates. Different methods like covalent sticking, cross-connecting and entanglement of ADH are used for limit. In Present study immobilization of alcohol dehydrogrogenase was carried out and in next step different parameters were studied and achieve beneficial results, which is really useful to many industries. so is connecting to economics and green chemistry.



Crystallographic Structure of Alcohol dehydrogenase.

2. Experimental: -

2.1 Chemical:-

Alcohol Dehydrogenase **(200U/mg)** from sisco research laboratories Pvt. Ltd. , Phenol, Hypo chloride solution from Qualikems Fine Chem. Pvt. Ltd., PEI polyethyleneimine solution from TCI. Glass beads from chemistry lab. Hydrogen peroxide from Atul-scientific Store.

2.2 Covalent coupling of YADH on Glass beads.

Covalent coupling of enzyme on glass beads were done by , following process. Enzyme Solution was mixed with phosphate buffer containing 0.05 mg of NADH. Further step Various coupling conditions were optimized for the maximum retention of enzyme activity . For this enzyme solution was mixed with phosphate buffer with (pH=6.0 – 8.0) Containing 0.05mg of NADH. Then prepared enzyme solution was added to 400 mg of glass beads in experimental beaker. In next step the contain of glass beads and enzyme solution was mixed at 277 K for 3h by using rotator. The mixture was dried under suitable condition in desiccator containing silica gel.

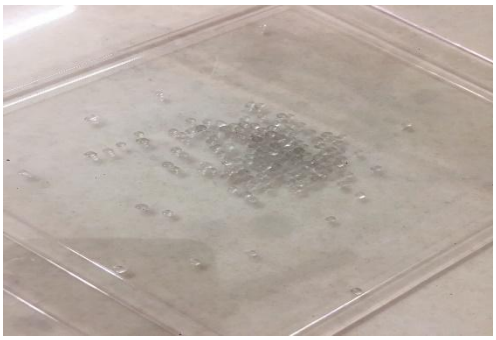


Image of ADH immobilized glass beads.

2.3 Characterization of Support.

To prove enzyme that enzyme is immobilized on support beads analysis Characterization of Support beads like FTIR and SEM were carried out. FTIR is carried out for glass beads by making pallet with KBr . In next step resolution of 1 cm^{-1} between 4000 and 500 cm^{-1} was obtained atchanga Laboratory, in charuset University. SEM analysis by coating the bead with suitable compound were done at BIRLA INSTITUTE OF THECHNOLOGY AND SCIENCE. GOA.

2.4 pH activity profile.

pH is most effective parameter like temperature in present study, activity of immobilized beads were tested with different pH by using assay method. The catalytic activity is markedly affected by environmental conditions, especially the pH of aqueous medium. Thus, information on changes in pH-activity behavior caused by immobilization of enzymes is useful for an understanding of the structure-function relationship of enzyme protein. Hence the activity of the free and immobilized YADH was measured by incubating free and immobilized enzyme at 27 °C for 30 min in the 50 mM phosphate buffers of different pH ranging from 4 to 10 and using ethanol as a substrate. The absorbance of the reaction mixture was measured at 340 nm and correlated to the concentration of enzyme. From the calibration plot activity of enzyme was determined.

2.5 Thermal stability

After immobilization heat stability of enzyme is enhanced , this is most beneficial for 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 enzymeswas investigated.Free and immobilized enzymes were placed in the optimum pH buffer andincubated at different temperatures (40 to 70 °C) for different time intervals. Theactivity of the enzyme was then determined as described earlier. Thethermodeactivation constant (K<j) was calculated by using following equation :

$\ln At = \ln Ao - Kd(t)$

where ‘Ao’ is the initial activity and ‘At’ is the activity after heat treatment for ‘t’ minutes.

2.6 Storage stability.

For study of residual activities of the free and immobilized enzymes stored at room temperature (35 °C) were determined and the activities were expressed as percentage retention of their residual activities at different times. Immobilized beads were also kept at 4 °C to 5 °C for 45 days and residual activity was examine after every 7 days.

$$\text{Storage efficiency (\%)} = \frac{\text{ADH activity after storage}}{\text{Initial ADH activity}} \times 100$$

2.7 Reusability of immobilized ADH

The reusability of immobilized enzymes is one of the most important factors affecting the success of industrialization of an immobilized system. To evaluate reusability of the immobilized YADH it was washed with water and buffer after each use and then suspended again in a fresh reaction mixture to measure the enzymatic activity. This procedure was repeated for ten cycles. Reusability of immobilized YADH was examined by using ethanol as substrate. Leakage of the enzyme, if any was determined by measuring the enzyme activity in the washings.

$$\text{Storage efficiency (\%)} = \frac{\text{ADH activity in } n^{th} \text{ cycle}}{\text{ADH activity in } n^{th} \text{ cycle}} \times 100$$

2.8 Determination of kinetic constants

The Michaelis constant (*Km)* and maximum reaction velocity constant (V^ax) for the free and immobilized YADH were determined by measuring the velocity of the reaction varying ethanol concentrations from 50 to 500 mM and varying NAD concentrations from 2 to 10 mM. Free and immobilized enzymes in optimum pH buffer were incubated with substrates for 30 min at 27 °C. From the activity of the enzymes, *Km* and *Vmax* were calculated using the Line weaver-Burk plot of 1/s vs. 1/v.

3. Results and Discussion

3.1 Insituentrapment of YADH

*Insitu*entrapment of ADH was done in the natural support like k-carrageenan bead. To the polymerizing solution 10 mL of glycine buffer solution of pH 9.2 containing 900 U of YADH was added after 5 min of initiation time. Reaction was allowed to complete for 1 h. The copolymer containing entrapped YADH was washed with cold water followed by buffer solution and meshed to 400 - 250 p. Dried copolymer containing entrapped enzyme was stored at 4 °C till further use.

Activity of entrapped YADH was measured as per the method reported using ethanol as substrate. Percentage activity was calculated from the total initial activity of YADH before polymerization and the total activity after entrapment. Approximately 90% enzyme activity was observed to be retained after entrapment.

3.2 Comparative account of free and immobilized YADH

3.2.1 pH activity profile

Fig. 3.6 illustrates the effect of immobilization on the optimum pH for the enzyme activity. The free enzyme shows maximum activity at pH 8, whereas ADH enzyme shows it at pH 8.8, indicating that polymer matrix behaves as a polycation. When an enzyme is bound to polycation carrier, positive charge on the enzyme increases and the pH of the immobilized enzyme region becomes more alkaline than that of the external solution. Accordingly the enzyme reaction effectively proceeds on the alkaline side of the external buffer pH, and the optimum pH apparently shifts to the acidic side. However, CB-YADH shows optimum pH between 8-9.

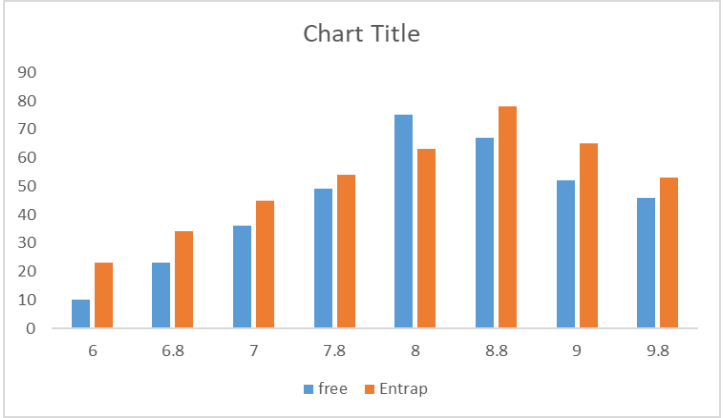


Fig: -1 $_{p}$H stability graph of ADH

3.2.2 Thermal stability

Knowledge of thermal stability of immobilized enzyme is very useful in the investigation of potential applications of enzymes. Fig. 3.7 shows the comparison of relative activity for free and immobilized YADH at 70 °C. It was observed that immobilized enzyme has higher thermal stability than free enzyme at all intervals of time. Free enzyme loses its 90% activity whereas ENT-YADH retained 30% and CB-YADH retained 40% activity over 30 min incubation at 70 °C. From the study the thermodeactivation constants (Kd) calculated as discussed earlier are given . From the data it is observed that rate ofdeactivation increases with temperature for both free and immobilized YADH.However, it can be seen that rate of deactivation is higher for the ENT-YADHsystem in comparison with CB-YADH indicating strong bond formation betweenthe chitosan and YADH.

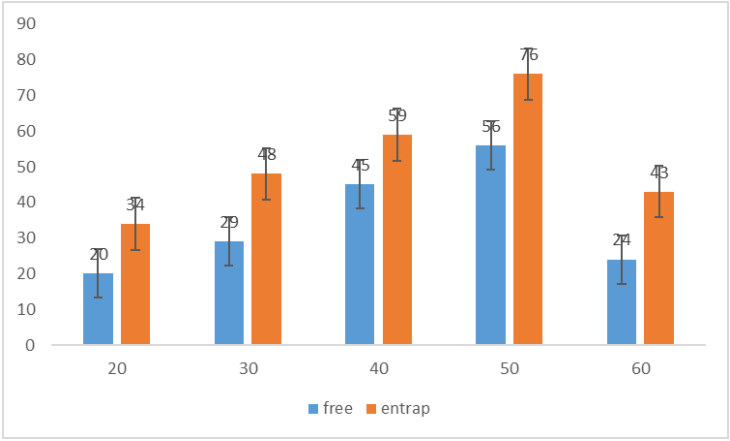


Fig:-2 Thermal stability graph of ADH

3.2.3 Storage stability

Destabilization is considered to be caused by autolysis or microbial growth on the enzyme. Immobilization reduces autolysis and/or prevents microbial growth. The storage stability of free and immobilized YADH has been investigated and results are given in Fig. 3.8. At room temperature (35 °C) free enzyme loses its activity completely after 5 days whereas CB-YADH retain 50% of their activity after 30 days respectively. The stabilization on immobilization is attributed to multipoint attachment of the enzyme to the support and/or it's role as semipermeable membrane creating more rigid enzyme molecule as stated by Glassmayer and Ogle.70 Hence disruption of the active centre becomes less likely to occur. Similar type of results were also observed for YADH immobilized on cyanogen bromide activated sepharose system by

Li et. al.71 However, Millis and Wingard32 observed retention of only 10% activity of YADH immobilized in albumin matrix cross-linked with glutaraldehyde on storage at pH 8.8 at 30 °C after 7 days storage.

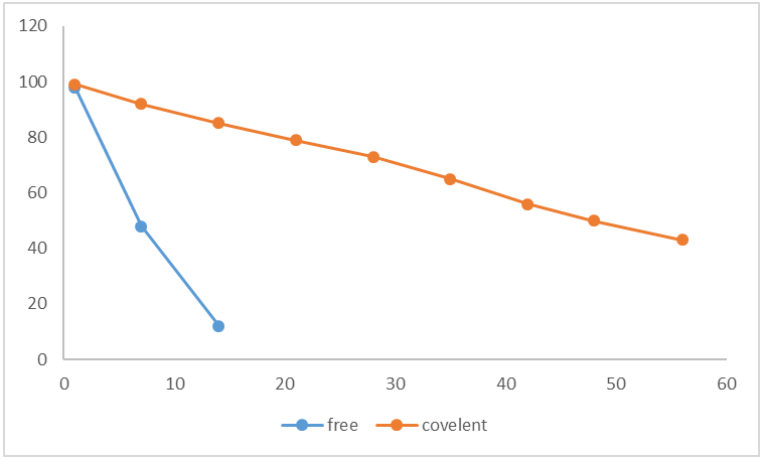


Fig:-3 Storage stability graph of ADH

3.2.4 Characterization of Support

(1) FTIR analysis

FTIR analysis of immobilized k-carrageenan 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. 1639.70 cm^{-1} show the bond starching vibration of amide. 3519.38 cm^{-1} shows OH starching vibration.

(2) 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. Working distance of 6.5 mm was maintained, and the acceleration voltage used was 20 kV, with the secondary electron image (SEI) as the detector.

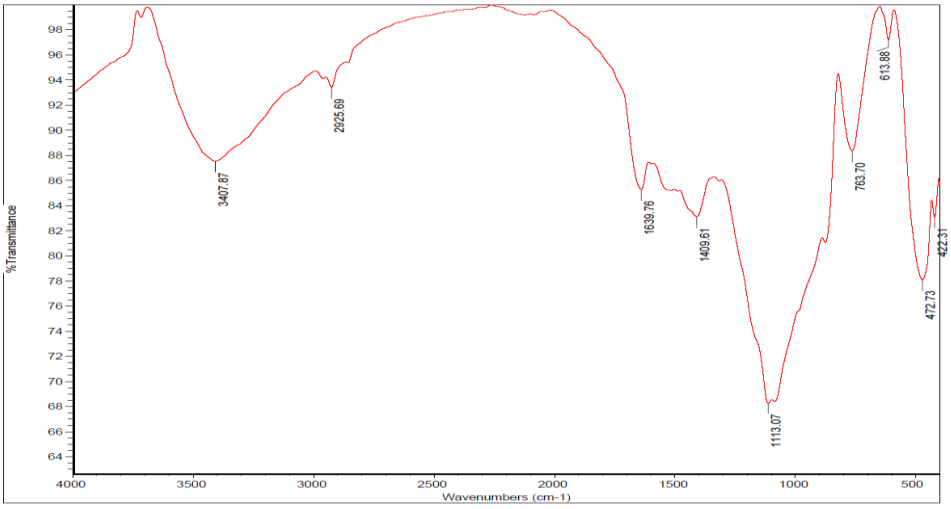


Fig-4 F shows the Spectra of support beads of bounded ADH.

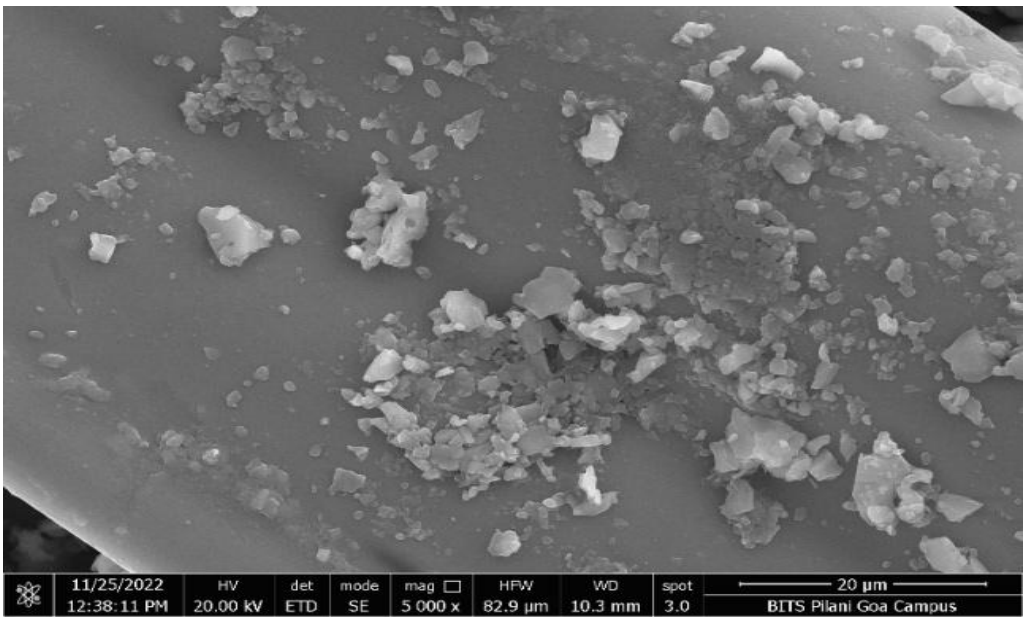


Fig-5 shows the SEM of support beads of bounded ADH.

3.2.5 Reusability

Free enzymes suffer from a major drawback of non-reusability. This is an advantage for immobilized enzymes. The activity of the immobilized systems after successive uses is given in CB-YADH retained 50% of its initial activity after eight cycles respectively. 8 cycles whereas CB-YADH retains 30% of its original activity after ten cycles for ethanol oxidation. Higher reusability of covalently bound YADH further confirms strong chemical bond formation between enzyme and support.

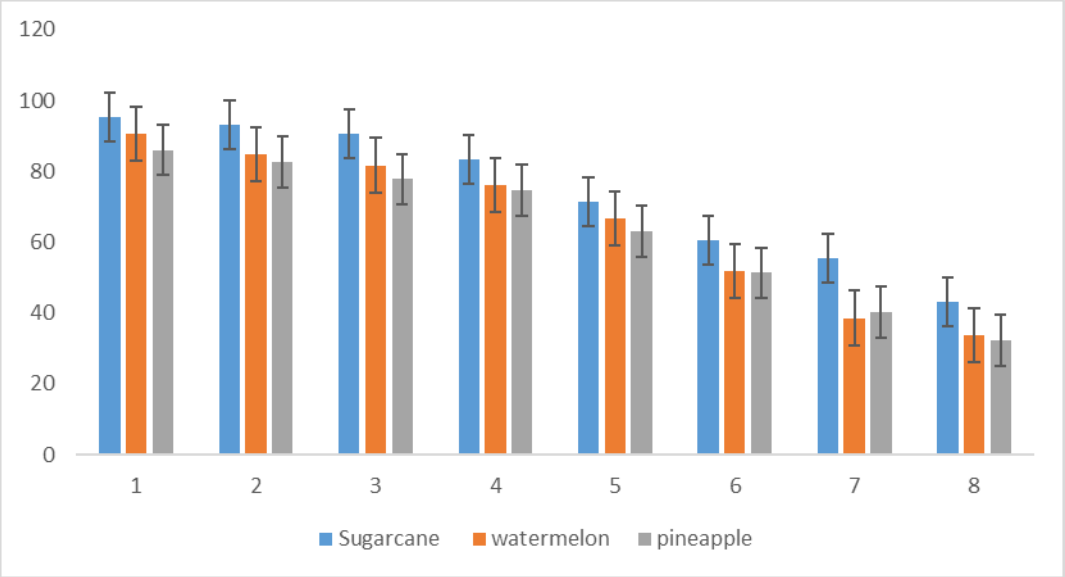


Fig:- 6 Reusability graph of Support beas

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