The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 20(1): 285-290, 2025 www.thebioscan.com

Pectinase immobilization on modified glass beads through salinization and its application in juice purification as a commercial activity

Dr. Hiral D. Trivedi[1], Dr. Dinesh S. Patel[2]

[1]Assistantn Professor, Shree P. M Patel College of P.G. Std. & Research in Science. Anand, Recognized Research Center for Ph.D. (Applied Chemistry, Forensic Science & M.Phil. in Chemistry), Gujarat, India

[2]Professor (Professor and Principal)[2], Shree P. M Patel College of P.G. Std. & Research in Science. Anand, Recognized Research Center for Ph.D. (Applied Chemistry, Forensic Science & M.Phil. in Chemistry), Gujarat, India

DOI: https://doi.org/10.63001/tbs.2025.v20.i01.pp285-290

KEYWORDS
glass beads,
pectinase,
enzyme immobilization,
FTIR & SEM analysis

Received on:

29-11-2024

Accepted on:

26-12-2024

Published on:

01-02-2025

ABSTRACT

The Pectinase enzyme was immobilized on glass beads in the current work using covalent coupling. Glass beads were utilized; they were purchased from scientific stationary. Covalent coupling rendered pectinase immobile. Using pectinase - loaded glass beads, different parameters were tuned and comparative studies were conducted following the immobilization procedure for more research. To further show that the support bead's enzyme was immobilized, FTIR & SEM analyses were conducted. Pectinase from it was used to purify fruit juice, specifically pineapple, watermelon, and sugarcane juice. Furthermore, because the immobilized enzyme can be reused in its whole, this study presents a novel development on the environmentally beneficial effects of immobilized enzymes.

INTRODUCTION

Under moderate circumstances, enzymes exhibit great selectivity and catalytic activity. They can therefore be used in a wider range of economic fields. Their nature is eco-friendly and efficient. Because free enzymes are difficult to separate and repurpose, and because they are unstable when exposed to heat, organic solvents, and pH levels, their industrial applications are still limited. However, compared to free enzymes, immobilized enzymes offer a number of benefits. These are stability, ease of separation from the reaction solutions, and reusability.

These days, a variety of techniques have been developed and are widely used in the process of immobilizing enzymes. Physical adsorption, ion exchange, matrix entrapment, covalent bonding, and micro capsulation are some of these methods.

These techniques are based on the properties of different support materials, both synthetic and natural. synthetic supports such as poly vinyl alcohol (PVA), glass beads, hydrogels, HEMA, carrageenan, and agar. The most crucial requirements for using biocatalysts in industrial settings include their operational stability, various types of immobilized microbial cells, and immobilized enzymes.

This paper reviews the literature on immobilized pectinase on glass beads, emphasizing its stability and activity. primarily concentrated on pectinase's activity and reusability as benefits at the commercial level. This study project used multiple reaction cycles as opposed to just one, which produced positive outcomes.

2. Experimental Methods:-

2.1 Materials

Typically used in chemistry labs, glass beads Concentrate H_2SO_4 (used in the chemical lab), The organic lab provided NaOH, the medical supply store provided H2O2, Pectinase (200U/mg), and Sisco Research Laboratories Pvt. Ltd. provided 3-aminopropyl-trirthoxysilane (3-APTES).

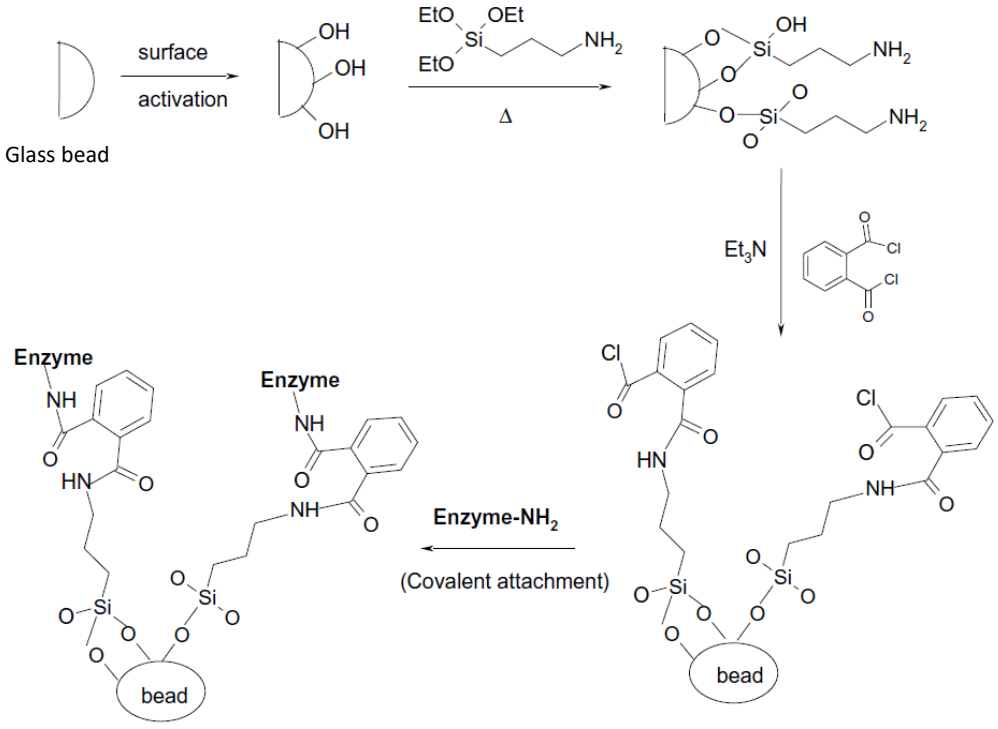
2.2 Surface-based glass bead activation:-

For surface activation of glass beads take 50 gm of glass beads in beaker then 15 ml 30% v/v NaOH was added (15 gm of NaOH pallets in 50 ml of distilled water) then 15 ml 6% H_2O_2 , in next heat the whole contain for 15 min then filter and dry the beads. After this experiment was followed by treatment with piranha solution. (H_2So_4 30 % v/v + H_2O_2 6%) and then heated for 60 min at 90 °C. Then beads were dried with suitable process and then organofunctionalized process of glass beads was done

2.3 Glass beads with organo - functionalization synthesized

Following the extraction of 50g of glass beads from the activated glass beads, five milliliters of 3-aminoPropyl-tiethoxysilane and 100 milliliters of toluene were applied. The mixture of materials was stirred while in reflux for eighteen hours. After being filtered out of the mixture, the beads were kept in the oven for two to three hours at 110 C.

Glass beads made of activated glass weigh 50g. Four grammas of triethylamine and a volume of A 250 ml round-bottom flask with three necks was filled with 150 ml of dry cyclohexane. The next phthaloyl had chloride added drop by drop, and the mixture was well agitated for 30 minutes before being heated to 25 °C for three hours.



Scheme 1. Representation of the mechanism of enzyme immobilization on functionalized support.

2.4 Immobilization of Pectinase (covalent immobilization)

After the cleaning and surface activation processes were finished, glass beads were gradually combined with a 20% V/V glutaraldehyde solution in a reaction flask. For two hours, the stirring operation was conducted at room temperature, or between 23 and 25˚ C. The glass beads were then immersed in a 1:14 diluted pectinase solution (6 ml of enzyme solution in 24 ml of buffer solution) using sodium acetate buffer (pH=4) at 4˚ C. After that, beads were applied and used to take more measurements.

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

2.5 Preparation of juice.

Pineapples that had just been plucked were taken and stored at room temperature. After the pineapple had been thoroughly cleaned and its outer shell removed, fresh juice was prepared using a standard recipe without the addition of any other ingredients. 150 cc of juice was separated out in a beaker to carry out the assay procedure after the filtering operation was finished.

2.6 Enzyme Assay and Protein Estimation

The amount of reducing sugar generated under test conditions was measured using a standard colorimetric technique to evaluate the activity of both free and immobilized pectinase (Nelson 1944; Somogyi 1952). The substrate used in the experiment was 0.9% (w/v) PGA, or 0.9 g of PGA in 100 mL of 0.05 M citric acid-sodium citrate buffer at pH 5.5. One unit of enzyme activity was defined as the amount of enzyme required to release one µmol of galacturonic acid per milliliter per minute under standard test conditions. The protein concentration was determined by utilizing the Lowry et al. (1951) method with bovine serum albumin as a reference.

2.7 Characterization

FT-IR Spectrometer was used to record the FTIR characterization. at Changa, Gujarat, at CHARUSET UNIVERSITY. At CHARUSET UNIVERSITY Changa, a scanning electron microscope was used for the SEM electron microscopic analysis.

2.8 pH activity profile

Since enzymes are made of proteins, the pH of the aqueous media in particular has a significant impact on their catalytic activity. Thus, knowledge of how immobilization of enzymes alters pH-activity behavior is helpful in understanding the link between the composition and capabilities of enzyme proteins. Thus, free and immobilized enzymes were incubated at 27 ˚C for 30 minutes in 50 mm phosphate buffers with varied pH values between 5 and 9, using ethanol as a substrate. This made it possible to measure the activity of the enzymes. The absorbance of the reaction mixture, which was measured at 520 nm, was correlated with the enzyme content. The enzyme's plot activity was computed using calibration.

2.9 Thermal stability

Since the immobilization of the enzyme increases its thermal stability, which is advantageous for the industrial usage of immobilized enzymes, it is critical to determine whether immobilized enzymes are possible for a given application. It was therefore chosen to investigate the thermal stability of both immobilized and unbound enzymes. The enzyme's activity was measured as previously described after soaking free and immobilized enzymes in the optimal pH buffer for varied times at temperatures ranging from 30 to 70 ˚C. The thermal deactivation constant (Kd) was calculated using the following formula:

Ln At = Kd (t) - In Ao where "At" is the activity following a minute-long heat treatment, and "Ao" is the beginning activity. 2.10 Integrity of storage The experiment measured the amount of residual activity in both free and immobile enzymes that were kept at room temperature (30 ˚C). The expression of residual activity maintenance at different times was determined by the actions of the enzymes. Every seven days, the amount of residual metabolism was also recorded while the immobilized beads were stored at 5 ˚C for a duration of 56 days.

3. Conclusion and Talk (Result)

3.1 pH Outline

Every enzyme has a pH range where it functions at its best. pH activity of free and constrained is displayed in Figure 1. We found that the maximum pH displayed by both free and imprisoned enzymes was between 5 and 8. The data indicate that there is no structural change during immobilization.

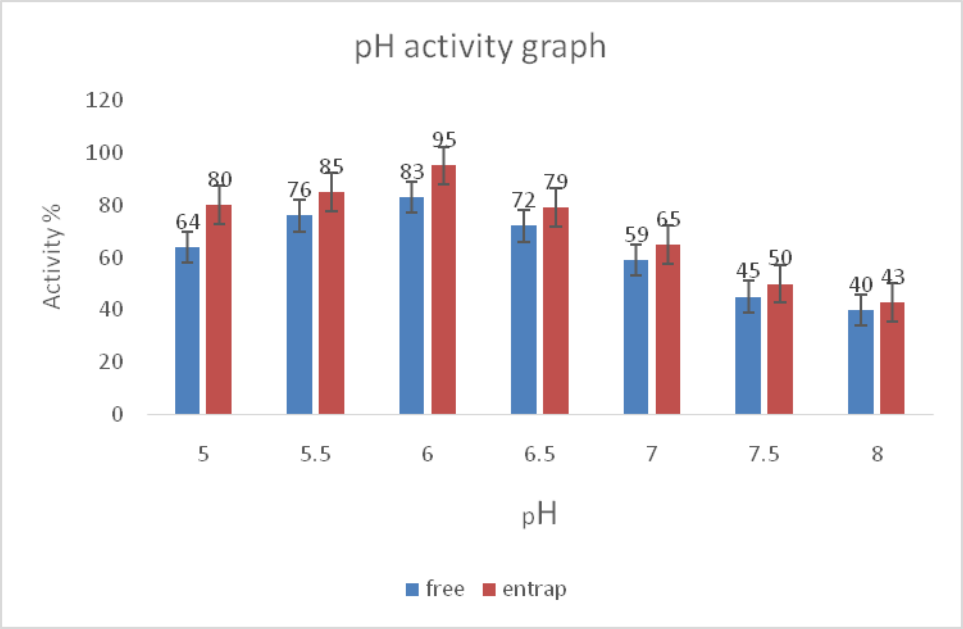


Figure. 1: An immobilized pectinase pH activity graph on glass beads

3.2. Warmth Stability

Enzymes are influenced by climate. Temperature increases the reactivity of enzymes, and at a certain degree, the enzyme loses its activity. Figure 2 shows that the entrapped enzyme has better thermal stability than the free enzyme. The enzyme has more heat stability because it is encased in beads.

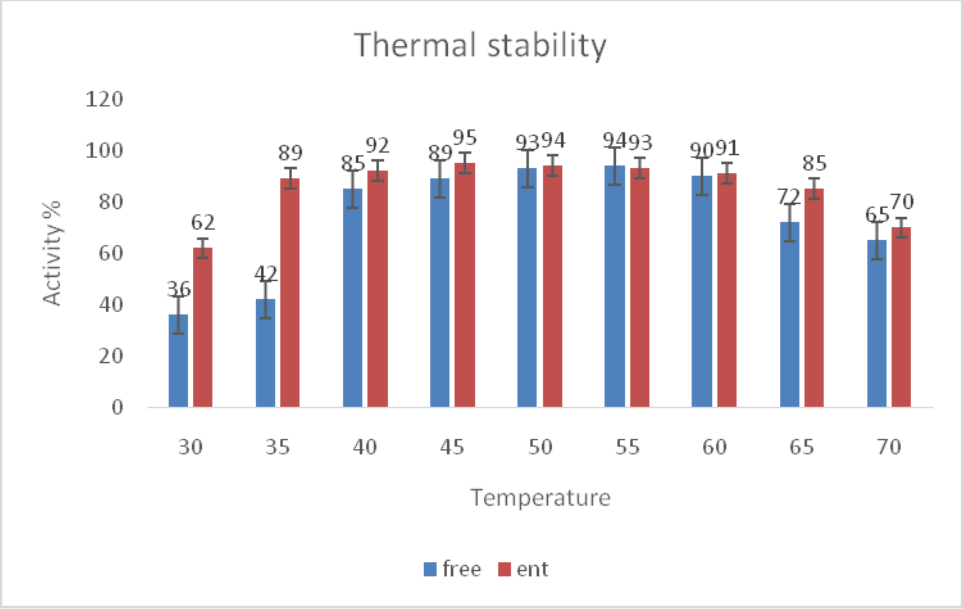


Figure. 2: An immobilized pectinase thermal stability graph on glass beads

3.3 FTIR examination

FTIR analysis was done on a bead. Specific enzyme groups' chemical presence can be ascertained by FTIR analysis. The range of the spectra that were recorded was 4000–500 cm-1. α-amylase's structure includes an amide group. The peease peak, which appears at 1651.58 m-1, indicates the stretching of -C=O, while the bond starching vibration of the amide is displayed at 1026.07 cm-1. At 3395.48 cm-1, there is a -OH starching vibration.

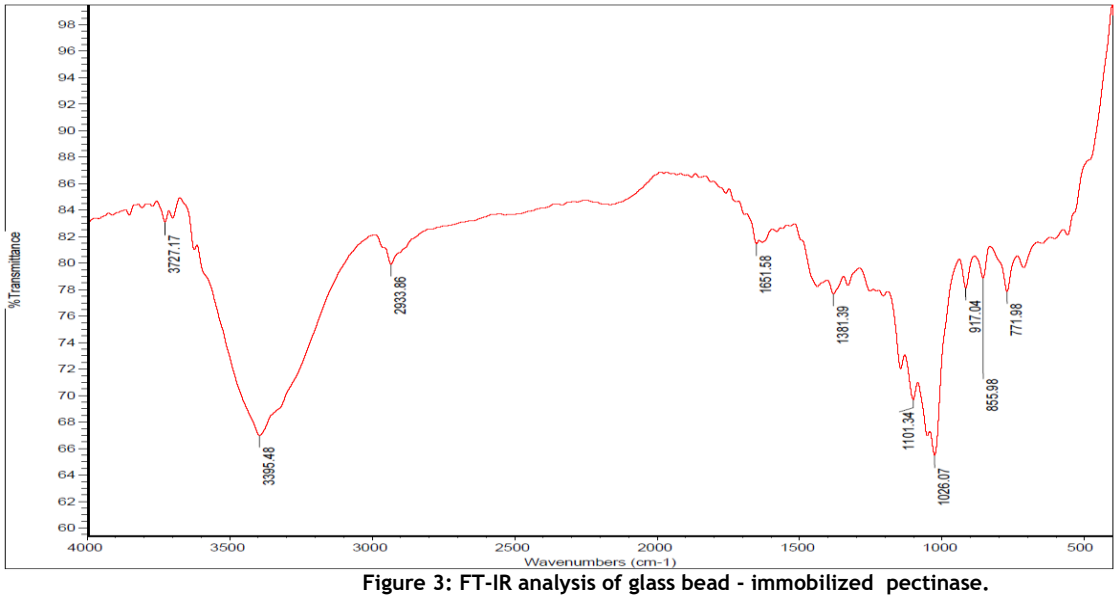


Figure 3: FT-IR analysis of glass bead - immobilized pectinase.

3.4 SEM analysis (Scanning electron microscopy)

The objective of this study was to characterize the support beads topographically. To do this, a SEM analysis of a bead containing an enzyme was conducted. SEM images were taken at room temperature with the appropriate magnification using a scanning electron microscope. Maintaining the operating distance of 9.5 mm while applying an acceleration voltage of 20.00 KV

.

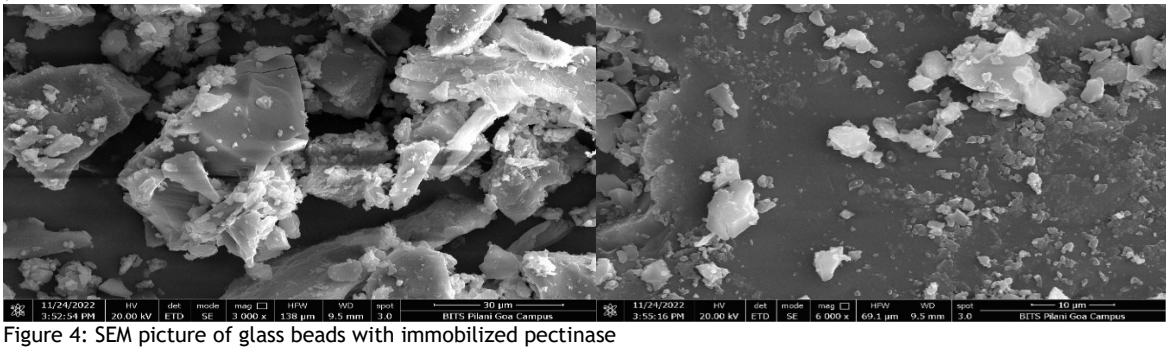


Figure 4: SEM picture of glass beads with immobilized pectinase

3.5 Stability of storage

At room temperature (30 °C), the residual activities of both free and immobilized enzymes were tested and expressed as a percentage retention of their residual activities at different intervals. Furthermore, the immobilized beads were kept at 5 °C for 56 days, with a check on the remaining activity conducted every 7 days.

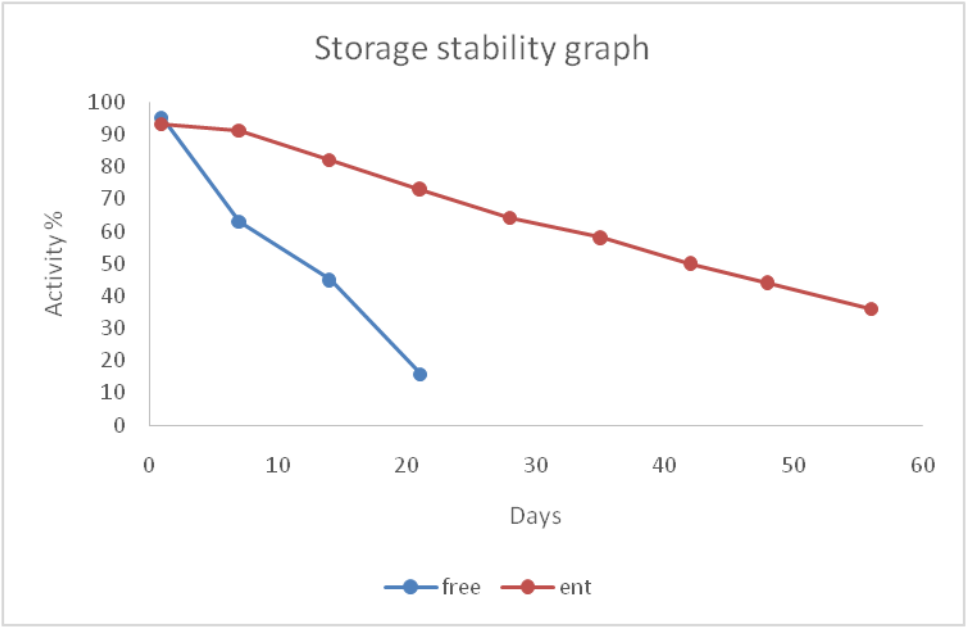


Figure. 5: An immobilized pectinase thermal stability graph on glass beads

3.6 Juice purification using immobilized pectinase

Watermelon, sugarcane, and fresh pineapple all lose their juice with time. For this, five milliliters of juice were extracted and placed in the vial. The entrapped enzyme beads were then added to the test vial along with 1 ml of casein solution and 5 ml of buffer solution. A 30-minute incubation period at 35 ˚C in a shaker incubator was followed by the addition of 1 ml of Folin's reagent and 5 ml of carbonate to the vial. and kept the test vial at 35 ˚C for 30 minutes once more. In the practical, both a free and an entrapped enzyme were utilized. For further computation, an optical density (OD) measurement was taken at 520 nm.

3.7 Reusability of bead

The ability of an imprisoned enzyme to be reused is essential for both industry and biological activities. By using beads in place of the free enzyme solution during the test method, it was confirmed. It was found that entrapped enzyme beads were reusable, as shown in. The bounded enzyme in Figure 6 showed 50-40% activity after 8 cycles, indicating the advantage of immobilized enzyme and increasing its applicability. After 5 or 6 rotations, it used 75% of its enzyme reactivity.

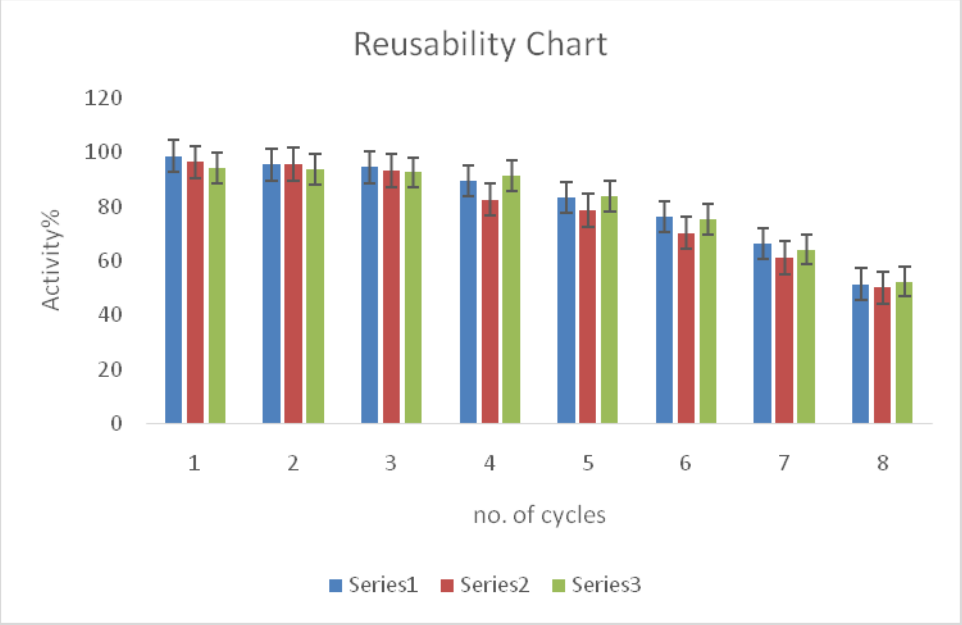


Figure 6: Reusability chart for glass beads with immobilized pectinase.

Acknowledgement

I am very grateful to my institute, **Shree P. M Patel College of P.G. Std. & Research in Science. Anand**, which is a recognized research center for Ph.D. programs in forensic science, applied chemistry, and chemistry, and to my mentor, Professor (Dr.) Dinesh S. Patel, for their support and encouragement. I would want to thank Mr. Dhavalkumar Trivedi, my life partner for giving me this great chance and for making it possible for our institute to have research facilities.

REFERENCES

- *Rawlings ND, Barrett AJ (February 1993). "Evolutionary families of peptidases". The BiochemicalJournal. **290** (Pt1):205218. doi:10.1042/bj2900205. PMC 1132403. PMID 8439290.*
- Sojitra, Uttam V., Shamraja S. Nadar, and Virendra K. Rathod. "Immobilization of pectinase onto chitosan magnetic nanoparticles by macromolecular cross-linker." *Carbohydrate polymers* 157 (2017): 677-685.
- Jump up to:[a] [b] *Rawlings ND, Barrett AJ, Bateman A (November 2011). "Asparagine peptide lyases: a seventh catalytic type of proteolytic enzymes". The Journal of Biological Chemistry. **286** (44): 38321-38328. doi:10.1074/jbc.M111.260026. PMC 3207474. PMID 21832066.*
- Patel, Vashishtha B., Somak Chatterjee, and Abhishek S. Dhoble. "A review on pectinase properties, application in juice clarification, and membranes as immobilization support." *Journal of Food Science* 87.8 (2022): 3338-3354.
- Jump up to:[a] [b] *Rawlings ND, Barrett AJ, Bateman A (January 2010). "MEROPS: the peptidase database". Nucleic Acids Research. **38** (Database issue): D227-D233. doi:10.1093/nar/gkp971. PMC 2808883. PMID 19892822.*
- *Mitchell RS, Kumar V, Abbas AK, Fausto N (2007). Robbins Basic Pathology (8th ed.). Philadelphia: Saunders. p. 122. ISBN 978-1-4160-2973-1.*
- Ma, Xiaobin, et al. "Characteristics of pectinase treated with ultrasound both during and after the immobilization process." *Ultrasonics Sonochemistry* 36 (2017): 1-10
- Oluwaseun, Garuba Emmanuel, Oladele Temitope Fredrick, and Abiodun Onilude. "Characteristics of Luffa sponge-immobilized pectinase from Aspergillus niger FTR 002 and its use in the continuous clarification of orange juice in packed-bed column bioreactor." *Applied Food Biotechnology* 10.4 (2023): 245-256.
- *Rodriguez J, Gupta N, Smith RD, Pevzner PA (January 2008). "Does trypsin cut before proline?". Journal of Proteome Research. **7** (1): 300-305. doi:10.1021/pr0705035. PMID 18067249.*
- *Renicke C, Spadaccini R, Taxis C (2013-06-24). "A tobacco etch virus pectinase with increased substrate tolerance at the P1' position". PLOS ONE. **8** (6): e67915. Bibcode:2013PLoSO...867915R. doi:10.1371/journal.pone.0067915. PMC 3691164. PMID 23826349.*
- *van der Hoorn RA (2008). "Plant Protease: from phenotypes to molecular mechanisms". Annual Review of Plant Biology. **59**: 191-223. doi:10.1146/annurev.arplant.59.032607.092835. hdl:11858/00-001M-0000-0012-37C7-9. PMID 18257708.*
- Seenuvasan, Muthulingam, et al. "Immobilization of pectinase on co-precipitated magnetic nanoparticles for enhanced stability and activity." *Research Journal of Biotechnology* 8.5 (2013): 24-30.

- *Zelisko A, Jackowski G (October 2004). "Senescence-dependent degradation of Lhcb3 is mediated by a thylakoid membrane-bound Protease ". Journal of Plant Physiology. **161** (10): 1157-1170. doi:10.1016/j.jplph.2004.01.006. PMID 15535125.*
- *Sims GK (2006). "Nitrogen Starvation Promotes Biodegradation of N-Heterocyclic Compounds in Soil". Soil Biology & Biochemistry. **38** (8): 2478-2480. doi:10.1016/j.soilbio.2006.01.006.*
- Wu, Rina, et al. "Immobilization of pectinase on oxidized pulp fiber and its application in whitewater treatment." *Carbohydrate polymers* 97.2 (2013): 523-529.
- *Sims GK, Wander MM (2002). "Proteolytic activity under nitrogen or sulfur limitation". Appl. Soil Ecol. **568**: 1–5.*
- *Tong L (December 2002). "Viral Protease ". Chemical Reviews. **102** (12): 4609-4626. doi:10.1021/cr010184f. PMID 12475203.*
- *Skoreński M, Sieńczyk M (2013). "Viral Protease as targets for drug design". Current PharmaceuticalDesign. **19** (6):11261153. doi:10.2174/13816128130613. PMID 23016690.*
- *Kurt Yilmaz N, Swanstrom R, Schiffer CA (July 2016). "Improving Viral Protease Inhibitors to Counter Drug Resistance". Trends in Microbiology. **24** (7): 547-557. doi:10.1016/j.tim.2016.03.010. PMC 4912444. PMID 27090931.*
- Jump up to:[a] [b] *Giménez MI, Cerletti M, De Castro RE (2015). "Archaeal membrane-associated Protease: insights on Haloferaxvolcanii and other haloarchaea". Frontiers in Microbiology. **6**: 39. doi:10.3389/fmicb.2015.00039. PMC 4343526. PMID 25774151.*
- *Maupin-Furlow JA (December 2018). Robinson NP (ed.). "Proteolytic systems of archaea: slicing, dicing, and mincing in the extreme". Emerging Topics in Life Sciences. **2** (4): 561-580. doi:10.1042/ETLS20180025. PMC 7497159. PMID 32953999.*
- Bahrami, Atieh, and Parisa Hejazi. "Electrostatic immobilization of pectinase on negatively charged AOT-Fe3O4 nanoparticles." *Journal of Molecular Catalysis B: Enzymatic* 93 (2013): 1-7.
- *Barrett AJ, Rawlings ND, Woessnerd JF (2004). Handbook of proteolytic enzymes (2nd ed.). London, UK: Elsevier Academic Press. ISBN 978-0-12-079610-6.*
- *Hooper NM, ed. (2002). Protease in biology and medicine. London: Portland Press. ISBN 978-1-85578-147-4.*
- *Feijoo-Siota L, Villa TG (28 September 2010). "Native and Biotechnologically Engineered Plant Protease with Industrial Applications". Food and Bioprocess Technology. **4** (6): 1066–1088. doi:10.1007/s11947-010-0431-4. S2CID 84748291.*
- Wang, B., Cheng, F., Lu, Y., Ge, W., Zhang, M., & Yue, B. (2013). Immobilization of pectinase from Penicillium oxalicum F67 onto magnetic cornstarch microspheres: Characterization and application in juice production. *Journal of Molecular Catalysis B: Enzymatic*, *97*, 137-143.
- *Southan C (July 2001). "A genomic perspective on human Protease as drug targets". Drug Discovery Today. **6** (13): 681–688. doi:10.1016/s1359-6446(01)01793-7. PMID 11427378.*
- Aksu, Aysun, et al. "Immobilization of pectinase on chitosan-alginate-clay composite beads: Experimental, DFT and molecular docking studies." *Journal of Molecular Liquids* 390 (2023): 122947.
- *Puente XS, López-Otín C (April 2004). "A genomic analysis of rat Protease and pectinase inhibitors". Genome Research. **14** (4): 609-622. doi:10.1101/gr.1946304. PMC 383305. PMID 15060002.*
- Zhang, Li, et al. "Supplementation of citrus pectin with whole-cell pectinase PG5 on Pichia pastoris promotes recovery of colitis and enhances intestinal barrier function in DSS-treated mice." *International Journal of Biological Macromolecules* 264 (2024): 130476.
- Tan, Zhongbiao, et al. "Designing multifunctional biocatalytic cascade system by multi-enzyme co-immobilization on biopolymers and nanostructured materials." *International Journal of Biological Macromolecules* 227 (2023): 535-550.