Notification No. ***F.NO.COE/Ph.D./(Notification)/554/2024***

Notification Date: ***14-02-2024***

File No. ***CHEM/224/2023***

Name of Candidate: **Shalu Aggarwal**

Name of Supervisor: **Prof. (Dr.) Saiqa Ikram**

Name of Department: **Department of Chemistry**

Faculty: **Faculty of Natural Sciences**

Research Topic: ***Surface Functionalization of Renewable Resources for Protein Immobilization***

Findings

The fundamental objective of the thesis entitled " ***Surface Functionalization of Renewable Resources for Protein Immobilization"*** is to design the heterofunctionalized biocatalytic nanoconjugate system chiefly concentrating on the renewable matrices and their surface functionalization for the impactful immobilization of proteins aiming to augment the enzymatic efficiency and operational stability of enzymes in the far-flung industrial and commercial perspectives. The thesis is divided into 6 chapters and contents of each chapter are briefly summarized herein.

Chapter 1 represents a detailed comprehensive review based on the classification of the remarkable renewable carriers as potential and evolving contenders for enzyme immobilization and thereof strategies. This chapter highlights the recent advancements and developments in the functionalized scaffolds employed for enzyme conjugations and their preferable mode of immobilization viz. physical adsorption, covalent binding, encapsulation, entrapment, ionic binding, and cross-linking by which enzymes are selectively bound to the activated surface of the matrices that can be feasibly accessible to the specific substrates. Moreover, formidable applications of the developed biocatalytic systems and innovations in the biotechnological sectors and relevant emerging fields have also been emphasized and discussed in this chapter which may help forthcoming young researchers and scientists to identify intriguing ideas, proposals, and suggestions in this era. This chapter also describes the aims and objectives of the entire proposed initiative.

Chapter 2 deals with the successful immobilization of Trypsin (Try, EC. 3.4.21.4) on the fragile surface of glutaraldehyde(GA)-activated chitosan-coated zinc-oxide nanoparticles (ZnO/Chitosan nanocomposite) via Schiff-base covalent linkages. The chemical structure of synthesized ZnO nanoparticles and ZnO/Chitosan nanocomposite was determined through UV-visible spectroscopy, Fourier-Transform spectroscopy (FTIR), Powder X-ray diffraction technique (XRD), Transmission electron microscopy (TEM), Particle size distribution, Field emission scanning electron microscopy (FE-SEM), and Energy-Dispersive-X-Ray microanalysis (EDAX) techniques. The immobilization parameters viz. incubation time (16 h), enzyme concentration (1.8 mg/ml), and pH (7.8) were optimized to acquire maximum expressed catalytic activity of the immobilized trypsin. Under the optimized conditions, immobilized trypsin retained 67% of catalytic activity at 50°C during 2h of incubation time and 50% of enzymatic activity after 90 days of storage at 4°C while the activity of solubilized trypsin was completely lost in the same duration. Furthermore, the immobilized counterpart has sustained 50% of catalytic activity after the 9th cycle of recurring sessions revealing that the synthesized bio-nanocomposite is a competent biocatalyst having the potential for several industrial pursuits. The Kinetic modules (Km, Vmax) of solubilized (25.76 μM, 4.16 μM/min) and immobilized trypsin (27.12 μM, 8.82 μM/min) demonstrated that the catalytic efficiency of the trypsin has further improved after the immobilization. Hence, the synthesized bionanocomposite (ZnO/Chitosan-GA-Try) would be a viable biocatalyst for substantial industrial and biotechnological applications.

Chapter 3 discusses the significant immobilization of Aspergillus niger Lipase (E.C.3.1.1.3) on the epoxy (EPI)-activated chitosan-coated silver oxide nanoparticles (Ag2O/Chitosan) via covalent linkages under the established optimized conditions. Numerous analytical techniques viz. UV-visible, FTIR, XRD, TEM, FE-SEM, and EDAX were employed for the structural determination of the silver oxide nanoparticles (Ag2O NPs), Ag2O/Chitosan nanocomposite, and Ag2O/Chitosan-EPI@Lip bionanocomposite respectively. The maximum expressed enzymatic activity of Lipase was obtained under the optimized immobilization conditions. Immobilized lipase preserved 76.47% of catalytic activity at 60°C during 2h of incubation and retained more than 50% of enzymatic activity after three months of storage while solubilized lipases lost most of the enzymatic activity in the same duration. Moreover, immobilized lipases have shown remarkable reusability, storage stability, and thermal stability characteristics over their native counterparts. The obtained kinetic parameters have also demonstrated the catalytic

proficiency and applicability of immobilized lipases have further enhanced rendering them more appealing and approachable towards sustainable and advanced technologies.

Chapter 4 reports the effective immobilization of porcine pancreatic α-amylase (E.C. 3.2.1.1 1,4-α-D-glucan glucanohydrolase) on the glyoxal-activated alginate-coated cerium oxide nanoparticles via covalent Schiff base linkages under the optimized conditions. The structural determination, composition, and morphologies of synthesized nanoparticles (CeO2 NPs), nanocomposites (CeO2/Alginate), and enzymeimmobilized bionanoconjugate (CeO2/Alginate-Gly@α-amy) were evaluated through UV-visible, FTIR, TEM, XRD, SEM, and EDAX analytical techniques respectively. The maximum catalytic activity of the α-amylases was obtained under the established optimized immobilization conditions. Immobilized α-amylases have retained 80% of initial catalytic activity after 75 days of storage while native ones have lost most of the enzymatic activity within the same duration. Furthermore, immobilized counterparts have retained nearly 55% of catalytic activity until the 13th repetitive cycle of utilization within the same working day. The kinetic modules (Km & Vmax) were also investigated whose results demonstrated that catalytic efficiency and affinity to the substrates were improved after immobilization of the enzyme on the prefabricated carrier matrices rendering them more accessible to the food and pharmaceutical industrial applications.

Chapter 5 describes the covalent immobilization of Jack Bean Urease (Ure, E.C. 3.5.1.5) on the glutaraldehyde-activated amino-functionalized Al2O3/SiO2 nanocomposite via Schiff base linkages under the established optimized conditions. Synthesized nanoparticles (Al2O3 NPs), nanocomposites (Al2O3/SiO2), and bio nanocomposite (Al2O3/SiO2-APTES-Glu-Ure) were characterized by UV-visible, FTIR, TEM, XRD, FESEM, and EDAX analytical techniques. The maximum catalytic activity of immobilized and solubilized ureases was expressed under the optimized immobilization conditions. Immobilized ureases have exhibited excellent thermal stability, reusability, and storage stability characteristics over their native counterparts. Moreover, the obtained kinetic parameters have revealed that the efficiency of urease was enhanced by immobilization rendering the bionanoconjugate more liable for proteomic research, agriculture, and environmental monitoring related to urea-based fertilizers.

Chapter 6 deals with the entire summary of the thesis and assessment analysis of the employed immobilization parameters and optimum catalytic activities of the synthesized biocatalytic systems. Based on outcomes, the present work has promoted the functionalization and

activation of biopolymer-based nanoparticulates for the multi-point immobilization of proteins by embracing advanced immobilization strategies rendering the novel bio-catalytic model to be employed in the diverse sectors of biotechnology.

Conclusively, the present research has been primarily focused on designing functionalized biopolymer-based scaffolds to immobilize the bioactive species (enzymes) via the appropriate mode of immobilization with the objective of developing biocatalytic models having excellent catalytic activities and stabilities. Intensive efforts have been made to figure out the numerous incredible pillars or platforms for effective enzyme immobilization with appropriate orientation and inhibition of steric hindrances that can suppress the hurdles of denaturations, enzyme instabilities, conformational amendments, leaching from the matrices, autocatalysis, and protein-protein aggregations. Thus, embracing sustainable green demeanors of enzyme immobilization in a quest to reinforce the site-selectivity, specificity, catalytic efficiency, and structural integrality that trigger the catalytic performances of immobilized enzymes for a longer duration over repeated consecutive cycles.