



Novel Immobilization Technique and its Application

Kamal-Alahmad

School of Food Science and Technology, Jiangnan University, Wuxi, China.
Laboratory of Food Enzymology and Food chemistry, Jiangnan University, Wuxi, China
Department of Food Science and Technology, Faculty of Agriculture, University of Alfurat, Deir Ezzor, Syria
Email address: Kamalalani85@yahoo.com (Kamal-Alahmad)

Al-Farga Ammar

School of Food Science and Technology, Jiangnan University, Wuxi, China

Mohammed Abdalbasit A. Gasmalla

School of Food Science and Technology, Jiangnan University, Wuxi, China
Laboratory of Food Enzymology and Food chemistry, Jiangnan University, Wuxi, China

Sameh A. Korma

Department of Food Science, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

Abdellatif A.Sulieman

School of Food Science and Technology, Jiangnan University, Wuxi, China

Abstract – Immobilized enzymes are widely used for variety of applications. Based on the type of application, the method of immobilization and support material can be selected. The immobilized enzymes can be separated from the reaction mixture and reused and also immobilized in order to prevent the enzyme from being exposed to harsh conditions. High temperature, surfactants, and oxidizing agents used in food industry, pharmaceutical industry bioremediation, detergent industry. Enzyme immobilization improves the operational stability and is also due to the increased enzyme loading which causes the controlled diffusion. Several hundreds of enzymes are immobilized and used for various industries.

Keywords – Immobilized Enzymes, Novel Immobilization, Applications of Techniques.

I. INTRODUCTION

The term immobilized enzymes refers to "enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously." Immobilized enzymes are currently the subject of considerable interest because of their advantages over soluble enzymes. In addition to their use in industrial processes, the immobilization techniques are the basis for making a number of biotechnology products with application in diagnostics, bioaffinity chromatography, and biosensors. At the beginning, only immobilized single enzymes were used, after 1970s more complex systems including two-enzyme reactions with cofactor regeneration and living cells were developed. The enzymes can be attached to the support by interactions ranging from reversible physical adsorption and ionic linkages to stable covalent bonds. Although the choice of the most appropriate immobilization technique depends on the nature of the enzyme and the carrier, in the last years the immobilization technology has increasingly become a matter of rational design. As a consequence of enzyme immobilization, some properties such as catalytic activity

or thermal stability become altered. These effects have been demonstrated and exploited (Aehle, 2007). The concept of stabilization has been an important driving force for immobilizing enzymes. Moreover, true stabilization at the molecular level has been demonstrated, e.g., proteins immobilized through multipoint covalent binding.

Enzyme immobilization is confinement of enzyme to a phase (matrix/support) different from the one for substrates and products. Inert polymers and inorganic materials are usually used as carrier matrices. Apart from being affordable, an ideal matrix must encompass characteristics like inertness, physical strength, and stability, and regenerability, ability to increase enzyme specificity/activity and reduce product inhibition, nonspecific adsorption and microbial contamination Immobilization (Cao,2005).

II. DIFFERENT TECHNIQUES USED FOR IMMobilIZATION

Affinity Immobilization : Affinity immobilization exploits specificity of enzyme to its support under different physiological conditions.

It is achieved by two ways: either the matrix is precoupled to an affinity ligand for target enzyme or the enzyme is conjugated to an entity that develops affinity toward the matrix. Affinity adsorbents have also been used for simultaneous purification of enzymes (Gale, 1944).

Complex affinity supports like alkali stable chitosan-coated porous silica beads and agarose-linked multilayered concanavalin A harbor higher amounts of enzymes which lead to increased stability and efficiency.

Bioaffinity layering is an improvisation of this technique that exponentially increases enzyme-binding capacity and reusability due to the presence of non-covalent forces such as coulombic, hydrogen bonding, van der Waals forces, (Langmuir and Schaefer, 1938) etc.



Adsorption : Enzyme adsorption results from hydrophobic interactions and salt linkages where either the support is bathed in enzyme for physical adsorption or the enzyme is dried on electrode surfaces. Adsorbed enzymes are shielded from aggregation, proteolysis and interaction with hydrophobic interfaces (Nelson and Griffin, 1916). Researchers have used eco-friendly supports like coconut fibers having good water-holding capacity and high cation exchange property; microcrystalline cellulose with irreversible binding capacity; kaolin with high enzyme retainability by chemical acetylation; and micro/mesoporous materials having thiol functionalized, large surface area ideally suited for reduction and oxidation reactions.

Covalent Binding : Covalent association of enzymes to supports occurs owing to their side chain amino acids like arginine, aspartic acid, histidine and degree of reactivity based on different functional groups like imidazole, indolyl, phenolic hydroxyl, etc. Peptide-modified surfaces when used for enzyme linkage results in higher specific activity and stability with controlled protein orientation (Grubhofer and Schelth, 1953). Cyanogen bromide (CNBr)-agarose and CNBr-activated-Sepharose containing carbohydrate moiety and glutaraldehyde as a spacer arm has imparted thermal stability to covalently bound enzymes. Highly stable and hyperactive biocatalysts have been reported by covalent binding of enzymes to silica gel carriers modified by silanization with elimination of unreacted aldehyde groups and to SBA-15 supports containing cage-like pores lined by Si-F moieties.

Cross-linking of enzymes to electrospun nanofibers has shown greater residual activity due to increased surface area and porosity (Harkins and Fourt, 1940).

Entrapment : Entrapment is caging of enzymes by covalent or non-covalent bonds within gels or fibers. Efficient encapsulation has been achieved with alginate-gelatin-calcium hybrid carriers that prevented enzyme leakage and provided increased mechanical stability (Barnet, 1959). Entrapment by nanostructured supports like electrospun nanofibers and pristine materials have revolutionized the world of enzyme immobilization with their wide-ranging applications in the field of fine chemistry, biomedicine biosensors and biofuels (Mosbach, 1966). Prevention of friability and leaching and augmentation of entrapment efficiency and enzyme activity by *Candida rugosa* lipase entrapped in chitosan have been reported. This support has also been reported to be non-toxic, biocompatible and amenable to chemical modification and highly affinitive to protein due to its hydrophilic nature. Entrapment by mesoporous silica is attributed to its high surface area, uniform pore distribution, tunable pore size and high adsorption capacity (Bernfel and Wan, 1963).

III. NOVEL METHOD FOR IMMOBILIZATION OF ENZYMES TO MAGNETIC NANOPARTICLES

The value of coupling biological molecules such as enzymes to solid materials has long been recognized. To date, protein immobilization onto such surfaces often

involves covalent coupling, encapsulation, or non-specific adsorption techniques. Here we demonstrate the feasibility of specifically attaching a haloalkane dehalogenase enzyme to silica-coated or uncoated iron oxide superparamagnetic nanoparticles using affinity peptides (Riaz and Anwar, 2009). The unique physical properties of nanoparticles are allowing their application in many fields such as biomedicine. Sensor development water purification and environmental remediation Superpara magnetism of magnetic nanoparticles (MNPs) is a size-dependent property that is useful for applications requiring manipulation of MNPs by an external magnetic field. Such particles do not retain any residual magnetism once the magnetic field is removed. By coupling the unique electrical, optical, and magnetic properties of nanoparticles with the specific recognition or catalytic properties of biomolecules, many novel biotechnological applications have emerged (Tischer and Wedekind, 1992). The large surface-area-to-volume ratio of a nanoparticle allows it to serve as an efficient carrier of biomolecules. This feature has resulted in the development of many biomolecule-nanoparticle (bio-NP) hybrids for biomedical applications in the diagnosis and localized treatment of disease.

Enzymes have long been used in industry as catalysts for catabolic processes or for the production of specific chemical enantiomers. MNP-enzyme conjugates (MNP-Es) represent a specific class of bio-NP conjugates that are of great interest for biotechnological applications where high catalytic specificity, prolonged reaction time, and in some cases the ability to recycle an expensive biocatalyst is required. In addition, magnetic field susceptibility provides a mechanism for efficient recovery of the enzyme complex from reaction products, which is especially important in the pharmaceutical industry where enzyme contamination of the final product can cause detrimental side effects (Salis, Pinna, Monduzzi and Solinas, 2008). Xenobiotic chemical degrading enzymes attached to MNPs also hold potential for use in novel nano-remediation technologies that will allow precise delivery (using electromagnetic probes) of the MNP-E conjugate to the contaminant source in locations such as aquifers while enabling recovery and reuse of the MNP-Es. The fate of biomolecules in natural or human-controlled environments (e.g., sewage treatment plants, aquifers, or soils) could also be traced by tagging the biomolecules with MNPs (Sakai, Yuping, Yamaguchi, Watanabe, Kawabe, and Kawakami, 2010).

IV. APPLICATION OF THE IMMOBILIZED ENZYMES

Biomedical Application : Immobilized enzymes are used in medicine from 1990 immobilized enzymes are used for diagnosis and treatment of diseases in the medical field. The inborn metabolic deficiency can be overcome by replacing the encapsulated enzymes (i.e., enzymes encapsulated by erythrocytes) instead of waste metabolites, the RBC acts as a carrier for the exogenous enzyme drugs and the enzymes are biocompatible in nature, hence there is no immune response (Mendes,



Giordano and Castro, 2011). The enzyme encapsulation through the electroporation is a easiest way of immobilization in the biomedical field and it is a reversible process for which enzyme can be regenerated. The enzymes when combined with the biomaterials it provides biological and functional systems. The biomaterials are used in tissue engineering application for repair of the defect. The advantage of the enzyme immobilization in biomedical is that the free enzymes are consumed by the cells and not active for prolonged use, hence the immobilized enzymes remains stable, to stimulate the growth and to repair the defect (Bergmann and Sanik, 1957).

Food industry Application: In food industry, the purified enzymes are used but during the purification the enzymes will denature. Hence the immobilization technique makes the enzymes stable. The immobilized enzymes are used for the production of syrups. Immobilized beta-galactosidase used for lactose hydrolysis in whey for the production of baker's yeast. The enzyme is linked to porous silica matrix through covalent linkage. This method is not preferably used due to its cost and the other technique developed by Valio in 1980, the enzyme galactosidase was linked to resin (food grade) through cross linking. This method was used for the various purposes such as confectionaries and icecreams (Bruce and Sen, 2005).

Biodiesel Production : Biodiesel is monoalkylesters of long chain fatty acids. Biodiesel is produced through triglycerides (vegetable oil, animal fat) with esterification of alcohol (methanol, ethanol) in the presence of the catalyst. The production of catalyst is a drawback of high energy requirements, recovery of glycerol and side reaction which may affect the pollution. Hence the biological production of liquid fuel with lipases nowadays has a great consideration with a rapid improvement (Fernandes, Queiroz and Abraham, 2006). Lipase catalyses the reaction with less energy requirements and mild conditions required. But the production of lipase is of high cost, hence the immobilization of lipase which results in repeated use and stability. The immobilization of lipase includes several methods entrapment, encapsulation, cross linking, adsorption and covalent bonding.

V. PROPERTIES OF IMMOBILIZED ENZYMES

The stability of the immobilized enzymes is based on the temperature and time. The activity of the enzyme is retained throughout series of cycles. Due to immobilization, the properties of enzymes will be altered such as catalytic activity with respect to the support matrix. The change in the enzyme properties in the immobilized enzyme is due to the enzyme and the substrate reacts in the microenvironment which is different from the enzyme substrate reaction in the bulk solution environment (Tratnyek and Johnson, 2006). The change is also due to the change in the three dimensional conformation of the protein when linked with the support matrix. These conformational changes

are to a lesser extent and these changes occur in the limited enzyme systems. Enzyme immobilization improves the operational stability and is also due to the increased enzyme loading which causes the controlled diffusion. The stabilization as a result of, number of bonds formed between the enzyme and the support matrix. When the immobilized enzyme acts on the macromolecular substrates, active site of the enzymes does not able to access with the substrates, hence the enzyme loses its activity (Whitesides and Grzybowski, 2002).

VI. CONCLUSION

Enzyme immobilization is widely exploited technique in various industries food industry, pharmaceutical industry, bioremediation, detergent industry, textile industry etc. This method is used due to its technical and economical advantage. Large number of enzymes have been immobilized and used in various large scale processes. This Stabilization method can lower the cost of the enzymes. Enzyme immobilization provides operational stability to enzymes.

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AUTHORS' PROFILES



First Author: Kamal-Alahmad, Master student, school of food science and technology, Jiangnan University, Wuxi, China



Second author: Al-FargaAmmar, Assistant Professor, School of Food Science and technology, Jiangnan University, Wuxi, China