Review Paper:

Enzyme immobilization by nanoparticles

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Abstract

Recently, cost effective and efficient enzymes have gained popularity among biotechnological industries. Development of novel techniques for enzyme production, its stability and activity to facilitate large-scale and economic formulation is required. Enzyme immobilization provides an outstanding base for increasing availability of enzyme to the substrate with greater output. The nanomaterials have unique physico-chemical properties and act as efficient support materials for enzyme immobilization.

This review provides an updated study of enzyme immobilization by nanoparticles, their advantages, disadvantages and kinetic properties which make it a suitable biocatalyst for potential industrial applications.

Keywords: Nanoparticles, Immobilization, Kinetics, Enzyme.

Introduction

Enzymes are biochemical catalysts over chemical catalysts which have maximum reaction rate under optimum condition, high specificity and lower energy consumption. Enzymes are catalyst and its reusability is an important criterion for industrial utilization. Enzyme immobilization by nanoparticles or nano material is a new technique implemented in the recent years[1]. The unique properties of nanomaterials have shown many applications in industry where enzymes are used[2]. Different types of nano materials are used for enzyme immobilization[3].

Enzyme immobilization by nanomaterial results in excellent immobilization, high enzyme loading due to large surface area, the high enzyme activity, minimum diffusion problem to substrate, hence product can move freely within the porous nanomaterial, which further improves the enzyme-substrate interaction[4-6].

A different type of magnetic and nonmagnetic nanoparticles has been used for enzyme immobilization. κ-carrageenase-immobilized by carboxyl-functioned magnetic iron oxide nanoparticles (CMNPs) exhibits lower thermal stability and improved pH stability, as well as better storage stability[7]. α-amylase immobilized by magnetically nanocomposite can be effectively recovered and reused for maximum utilization, thereby, decreasing cost production of starch hydrolysis[8].

Immobilization of glucose oxidase on nonmagnetic gold nano particles develops disposable biosensors used as a clinical indicator for diabetes and quality control in the food industry[9]. The most advantageous and applicable magnetic nanoparticles are iron oxides, silica-encapsulated magnetic and super paramagnetic Fe_3O_4 because they have low toxicity and good biocompatibility[10].

Enzyme immobilization by nanoparticles

Nanoparticles based immobilization depends on composition, size, morphology and uniformity to make it easier to perform the enzyme immobilization. The selection of nanoparticles for immobilization is very important factor to prevent the loss of enzyme activity by altering the chemical nature or binding site of enzyme. In other words, nanoparticles attach with enzyme without damaging its structure.

Among the various nanostructures of materials like silica, metal nanoparticles, silver, gold nanoparticles and others are very attractive to be used as host matrices. A different variety of immobilization techniques can be used including adsorption, covalent attachment and entrapment in matrices[11]. Table 1 shows different enzyme immobilized with variety of nanoparticles.

Advantages and disadvantages of enzyme immobilization by nanoparticles

Immobilized enzymes are getting significant attention for commercial applications as they can be reused many times as biocatalyst which results in reduction in operational cost. Immobilized enzyme shows more resistant to denaturation, greater thermal and operational stability at various pH values and ionic strengths[28]. In addition to easy handling of the enzyme, it can be easily separated from the product[29].

Enzymes immobilized by nanoparticles provide the followings advantages (i) it allows greater enzyme loading because of higher surface area (ii) lower mass transfer resistance and (iii) separation from the reaction mixture by applying a magnetic field.

In other words, we can say that Nanotechnology motivated enzymes have generated more interest recently because nanomaterials allow the upper limits on enzyme - efficiency factors[30].

Nanoparticles exhibit higher internalization in multiple cell lines *in vitro*, compared with the soluble enzyme. Nanoparticles are biocompatible, high efficient alternative for intracellular delivery of active enzyme[31].

Table 1
Enzymes immobilized on nanoparticles and their biological applications

Enzyme	Nanoparticles used for Immobilization	Application
Lipase	Gold nanoparticles	Esterification and Transesterification[12]
Tyrosinase	Iron oxide (Fe_3O_4) and gold nanoparticles	Biosensor[13]
Chloroperoxidase	Fe_3O_4 magnetic nanoparticles	Dye decolorization[14]
Amylase	Ferric oxide nanoparticles	Surface treatment[15]
Glucose oxidase	Silica nanoparticles	Glucose biosensor[16]
β- Galactosidase	Zinc oxide nanoparticles	Enzyme-based analytical devices[17]
β -Galactosidase	Aluminium oxide nanoparticles	Biosensor[18]
Lipase	Chitosan nanoparticles	Drug delivery[19]
Glutamate oxidase	Pt nanoparticles	Disposable biosensor[20]
Chitosanase	Amylose-coated magnetic nanoparticles	High yield of Oligosaccharides[21]
Urease	Silver nanoparticles	Urease electrodes[22]
α-Amylase	Cellulose-coated magnetite nanoparticles	Starch degradation[23]
Protease	Amino silane coated magnetic nanoparticles	Produce active peptides[24]
Papain	Silica coated magnetite nanoparticles	Meat tenderization[25]
Peroxidase	Magnetic nanoparticles	Bioremediation of textile waste water[26]
Glucose oxidase	Chitosan nanoparticle	Antimicrobial agent[27]

Table 2
Advantages and disadvantages of enzyme immobilization by nanoparticles

Enzyme	Nanoparticles used for immobilization	Advantages	Disadvantages
Xanthine oxidase	Shell magnetic silica nanoparticles	Fe_3O_4@SiO_2 layer effectively improves nanoparticle's affinity to enzyme	Low expression and activity loss is observed during repeated usage.[32]
Lipase	Aminosilane modified magnetic nanoparticles	No loss in enzyme activity. The immobilized enzyme retains 100% activity.	Yield of products is comparatively lower with this enzyme.[33]
Benzoylformate decarboxylase	Magnetic epoxy support nanoparticles	Good agreement with yield by free enzyme	Physical adsorption of the enzyme is necessary to obtain covalent immobilization of the enzyme molecules on the magnetic- epoxy support.[34]
Formate dehydrogenase	Polydopamine coated iron oxide nanoparticles	High loading-capacity efficiency and activity recovery under aqueous conditions, compared to the free enzyme,	Weakening of the bond strength between the particles and the enzymes may lead to leaching of the enzyme from the system.[35]
Horseradish peroxidase	Silica-coated magnetic nanoparticles	These bio-nanocatalysts have advantages of simple and facile preparation involving the good magnetic strength, hydrophilicity and stability	Enzyme-nanoparticle conjugates usually undergo a partial reduction of intrinsic enzyme activity because the active site of the enzyme is often blocked by the immobilization.[36]
Acetyl xylan esterase	Chitosan coated magnetic nanoparticles	Easier to use, better stability at thermal and pH ranges than free enzyme	deformation of the enzyme structure, less accessibility of the substrate to the active site of the enzyme, so reducing its biological activity[37]
Pullulanase	Magnetic chitosan/Fe3O4 composite nanoparticles	Immobilized pullulanase show high activity retention and good thermal and operational stability	After first reuse, immobilized pullulanase undergo a fast decline in catalytic activity[38]

Laccase	Fe_3O_4 nanoparticles	enzyme loading and activity recovery compared with conventional covalent binding	mass transport constraint and by structural changes of the proteins during covalent binding[39]
Trypsin	Magnetic AuNP@Fe3O4 nanoparticles	higher efficiency is observed compared with in-solution digestion using free trypsin	enzyme leakage and slow diffusional mass transfer[40]
Glucose oxidase	Tannic acid modified CoFe2O4 magnetic nanoparticles	The immobilized Glucose oxidase shows excellent reusability and even after 8 consecutive activity assay runs, enzyme activity is found to improve	Lower biological affinity for glucose than that of the free Glucose oxidase.[41]
Pectinase	Fe3O4/SiO2 Carboxymethyl chitosan	The immobilized enzyme exhibits good adaptability to environmental acidity, which retains more than 90% of the original activity.	Temperature causes protein denaturation and decreases the reaction rate.[42]

Table 3
Kinetic study of free and immobilized enzyme and comparisons.

Enzyme	Nanoparticles	Kinetic parameters for free enzyme	Kinetic parameters for immobilized enzyme	Comments
β -Amylase	Chitosan /PVP blend and chitosan coated PVC beads	K_m = 2.18 mg / mL, V_m =714. 28 µ moles/ min. /mg	K_m = 2.26 mg / mL V_m =176.92 µ moles / min. /mg	Limited substrate diffusion to the immobilized enzyme.[43]
α -Amylase	Gold nanorods	K_m = 3 mg/ml V_m = 6.4 µ moles/min	K_m = 3.4 mg/ml V_m = 5.91 µ moles / min	High thermal & pH stability.[44]
Laccase	Copper oxide nanoparticles	K_m = 0.589 mM V_m = 11.03 Unit	K_m = 0.465 mM V_m = 40 Unit	Enhanced enzyme-substrate affinity and catalytic conversion rate[45]
Xylanase	Superparamagnetic iron oxide nanoparticles	K_m = 4.1 mg / mL V_m = 1.7 µ moles/min	K_m = 4.9 mg/ mL V_m = 1.6 µ moles/min	low accessibility and affinity of substrate to the active site of immobilized enzymes.[46]
Lipase	Magnetic Fe_3O_4@chitosan nanoparticles	K_m = 21.25 mM V_m = 5.72 mM/min	K_m = 28.73 mM V_m = 2.26 mM/min	Nanoparticles could be excellent support for immobilization.[47]
α-Galactosidase	Graphene nanosheets	K_m = 0.70 ± 0.02 mM V_m = 2.80 ± 0.12 µM/min	K_m = 0.24 ± 0.01 mM V_m = 0.35 ± 0.02 µM/min	Immobalization may result in structural changes[48]
Invertase	Chitosan nanoparticles	K_m = 65.7 mM V_m = 1670 U/ml	K_m = 205.7 mM V_m = 1830 U/ml	Reduction in the affinity of the enzyme for the substrate[49]
Glucose oxidase	Core-shell gold@calcium phosphate nanoparticle	K_m = 34.22mM	K_m = 34. 23 mM	Relatively complicated preparation and compromised enzyme activity.[50]
Pectinase	Chitosan magnetic nanoparticles	K_m = 3.201 mg/ml V_m = 0.595 µmol of galacturonic acid/min	K_m = 2.680 mg/ml V_m = 0.801 µmol of galacturonic acid/min	Efficient and green method, improved properties of Enzyme.[51]

During the immobilization procedure, enzymes may be denatured and lose their activity due to diffusional problem, mass transfer limitation, block of active site of enzyme. Table 2 shows advantages and disadvantages of enzyme immobilization by nanoparticles.

Kinetic study of enzyme immobilization by nanoparticles: The kinetic study of immobilized enzyme can differ significantly from that of the same enzyme in free form. The properties of an enzyme can be amended by suitable choice of the immobilization protocol whereas the same method may have significantly different effects on different enzymes. These differences may be due to conformational changes within the enzyme due to the immobilization procedure. Immobilization can greatly affect the stability of an enzyme. Table 3 shows kinetic study of free and immobilized enzyme and their comparisons.

Conclusion

Recent advances in biotechnology, particularly in enzymes with immobilization by nanoparticles have shown good stability, enhanced enzyme activity, reusability and cost effective techniques. Different types of nanoparticles have been studied for immobilization with various merits and demerits. The kinetics of immobilized system have showed better enzyme-substrate affinity, catalytic conversion rate due to structural changes in the enzyme introduced by immobilization procedure.

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