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Review

Recent trends and valorization of immobilization strategies and ligninolytic enzymes by industrial biotechnology

Muhammad Asgher, Muhammad Shahid, Shagufta Kamal, Hafiz Muhammad Nasir Iqbal*

Industrial Biotechnology Laboratory, Department of Chemistry and Biochemistry, University of Agriculture Faisalabad, Pakistan

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ABSTRACT

From the last several years ligninolytic enzymes find applications in numerous industrial processes. However, their lower catalytic efficiencies and operational stabilities limit their practical and multipurpose applications in various sectors of the current industrial processes. Dependence of lignin peroxidase (LiP) on veratryl alcohol and that of manganese peroxidase (MnP) on Mn^{2+} is another limitation for these enzymes. Therefore to expand the range of natural industrial bio-catalysts, e.g., ligninolytic enzymes, significant progress related to the enzyme biotechnology has appeared and researchers have been redirecting their interests to immobilization engineering processes. Among the diverse immobilization techniques, the use of pre-existing supports (via covalent or physical coupling) and the immobilization without supports (enzyme cross-linked aggregates (CLEAs) or crystals (CLECs) are among the most promising. This review article mainly focuses on recent trends and valorization of immobilization and ligninolytic enzymes, i.e., LiP, MnP and laccase by industrial biotechnology. The information is also given on various immobilization techniques followed by a brief summary about an immobilization of LiP, MnP and laccase. The present review was also focused primarily on recent trends in ligninolytic green biotechnology to suggest the potential industrial applications of ligninolytic enzymes in various sectors of the modern industry.

Contents

1. Introduction

The ligninolytic enzyme system of white rot fungi (WRF) (comprising lignin peroxidase (LiP, E.C. 1.11.1.14), manganese

* Corresponding author. Tel.: +92 41 9200161x3312.
E-mail address: nasir_pk99@hotmail.com (H.M.N. Iqbal).

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peroxidase (MnP, E.C. 1.11.1.13) and laccase (E.C. 1.10.3.2) along with H_2O_2-producing oxidases) has low substrate specificity, non-sterio selectivity and strong oxidative abilities [1,2]. Versatile peroxidase (VP) and many accessory enzymes such as glyoxal oxidase, aryl alcohol oxidase, oxalate producing oxalate decarboxylase (ODC), and P-450 mono-oxygenase have also been isolated from culture filtrates of many WRF strains [3–7]. Despite the extensive applications of ligninolytic enzymes, the use of these bio-catalysts in industrial processes has been limited by several factors, mainly because of the high cost, instability, availability in small amounts, susceptibility to attack by proteases and intermediate inhibition processes, etc. [5]. Therefore, in order to increase their utilization as industrial bio-catalysts, it is mandatory to obtain enzymes with enhanced operational stabilities. In this background below are some potential strategies to develop stable and recoverable enzymes for multipurpose biotechnological applications.

i. The first way to obtain bio-catalysts for a variety of industrial processes is to isolate enzymes from microbes which naturally exist in extreme environments, such as thermophiles from hot springs, fuel tanks and salt saturated waters of Dead Sea [8]. Some major advantages offered by thermo-stable enzymes in industrial processes are increase in the rate of reaction, higher substrate solubility, decrease in viscosity of liquids and less chances of microbial contamination. However, in order to obtain individual hyper-thermo-stable enzymes in the amounts required for their use in industry, new bio-reactor concepts and processing protocols must be developed to provide suitable conditions to the isolated extremophilic microbes.
ii. The second way of obtaining naturally stable enzyme involves genetic engineering which involves the isolation of all or part of the genome of a thermophillic microbe followed by its introduction into the genetic machinery of a suitable mesophile. This general approach is well established and has found applications in the production of pharmaceutically valuable products.
iii. The third strategy of stabilizing enzymes from meso-philes is considered to be the best way to obtain stable bio-catalysts and this is achieved by immobilization, protein engineering and chemical modifications. Protein engineering has been used to produce thermostable enzymes that differ from their native counterparts only in one or several predefined amino acids. This approach (protein engineering) has brought the possibility of stabilizing mesophilic enzymes by changes in amino acid sequences [6,9].
iv. Immobilization by associating the enzyme with an insoluble matrix, so that it can be retained in proper reactor geometry for its economic reuse under stabilized conditions. Immobilization has revolutionized the field of biotechnology because these enzymes provide an alternative tool to traditional chemical technologies [10–12].

The industrial applications of biocatalysts depend on the development of effective and stable immobilized enzymes. The immobilization greatly increases the stability and eases the burden of enzyme cost and thus, is widely pursued for efficient, selective and environmentally friendly catalysis. Immobilized enzymes have ability to catalyze the reactions in wide environmental conditions. Solid support like xerogels, sand, clay or soil are required for the attachment of enzyme without posing any kind of environmental risks and thus are beneficial for many of the industrial application [10]. In industrial reactor columns it allows to decouple the enzyme location from the flow of the liquid carrying the reagents and products. Immobilization helps in the development of continuous processes allowing more economic organization of the operations, automation, decrease of labor, and investment/capacity ratio. Stabilization of enzyme can be achieved not only by using

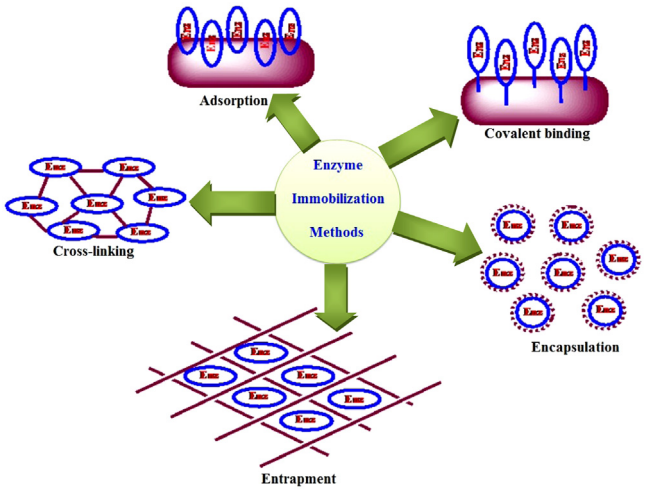

Fig. 1. Basic enzyme immobilization methods.

support immobilization, but also by support-free immobilization, e.g., by formation of cross-linked enzyme aggregates or crystals. While, the increased stability of enzyme–matrix complex could be predicted, e.g., multipoint covalent attachment often increases protein stability, therefore, protein engineering was used to design protein mutants with available functional groups to achieve more intense covalent attachment to the support and stabilize the enzyme in organic solvents [13]. The research on immobilization started from late nineteen and until now various improvements have been made as summarized in Table 1.

2. Enzyme immobilization techniques

The method of immobilization and nature of enzyme supports are very important because equipped steadiness and long term use of the enzyme depends on immobilization method. Fig. 1 illustrating the basic methods of immobilization.

2.1. Adsorption

The enzyme is attached to the outside of an inert material. This method involves the attachment of enzyme through surface binding on glass, alginate beads or matrix. In general, this method is the slowest. As adsorption is not a chemical reaction, the active site of the immobilized enzyme may be blocked by the matrix or bead, greatly reducing the activity of the enzyme. Adsorption of laccase on nano-porous gold (NPG) particles [14], and adsorption entrapment technique has been found to be more effective as compared to surface adsorption [15].

2.2. Covalent binding

The enzyme is covalently bonded to a matrix through a chemical reaction. It involves direct binding of enzyme with solid support by covalent linkages or using a cross linking reagent that binds the enzyme at one side and immobilization matrix on other side (Fig. 1) [16–18]. This method is by far the most effective and enzyme can be immobilized by multipoint covalent attachment of enzyme with immobilization support to enhance activity, stability and reusability of enzymes. As the chemical reaction ensures that the binding site does not cover the enzyme's active site, the activity of the enzyme is only affected by immobility [19,20]. However, the inflexibility of the covalent bonds precludes the self-healing properties exhibited by chemo-adsorbed self-assembled monolayers. Use of