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Amino silicones finished fabrics for lipase immobilization: Fabrics finishing and catalytic performance of immobilized lipase

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ABSTRACT

Finishing of silk fabric was achieved by using amino-functional polydimethylsiloxane (PDMS) and lipase from *Candida* sp. 99-125 was immobilized on the treated silk fabrics. Hydrophobic fabrics were obtained by dipping the native fabric in 0.125–0.25% (w/v) PDMS solution and dried at 70 °C. The direct adsorption on PDMS-treated fabric was verified to be a better strategy for lipase immobilization than that by covalent binding. Compared to unfinished fabrics, the hydrolytic activity of immobilized enzyme on the finished fabric was improved by 1.6 times. Moreover, the activity of immobilized enzymes on hydrophobic fabrics was significantly improved in different concentrations of strong polar solvents such as methanol and ethanol, and in common organic solvents with different octanol–water partition coefficients (Log *P*). Enzymatic activity and stability in 15% water content system (added water accounted for the total reaction mixtures, v/v) showed more than 30% improvement in each batch. The amino–silicone finished fabric surface was investigated by scanning electron microscopy and X-ray photoelectron spectroscopy. The hydrophobic fabric immobilized enzyme could be recycled for more than 80 times with no significant decrease in esterification activity. PDMS-treated woven silk fabrics could be a potential support for lipase immobilization in catalytic esterification processes.

1. Introduction

Enzyme immobilization is an important aspect in biocatalytic processes used for sustainable chemical manufacturing. In contrast to soluble enzymes used in industrial processes, immobilized enzymes often offer advantages in terms of stability, volume-specific biocatalyst loading, recyclability, and simplified downstream processing [1,2]. Volume-specific biocatalyst loading has rarely been studied and it refers to the solubility of an enzyme in comparison to the solubility of its substrate. In aqueous buffer or in solvent-free biotransformations, immobilization offers the possibility of overcoming the problem of catalyst density by increasing the enzyme loading per volume element, and dispersion and retention of the biocatalyst are achieved [1]. Among the diverse immobilization techniques including covalent attachment, entrapment, cross-linking, and adsorption [3–6], the use of nanoparticles as immobilization supports is achieving an increasing importance [7,8]. Fernández-Lafuente exemplified that both coupling chemical modification and immobilization approaches were entirely complementary resulting in a synergism in the final catalytic performance of enzymes [9–11]. Rodrigues et al. presented and discussed the facts that could promote improvements in enzyme activity, specificity, and selectivity after immobilization [3]. Although numerous methods for enzyme immobilization have previously been described, relatively few processes employing immobilized enzymes have been successfully commercialized [12].

Microbial lipases have received significant attention now that enzyme technology is rapidly developing. Consequently, we have thoroughly examined both upstream and downstream processing of the extracellular lipase from *Yarrowia lipolytica* (YlLip2, also named *Candida* sp. 99-125 lipase). Extensive research efforts have been devoted to the study of lipases which not only provide new insights into the structure-function relationships of the protein [13,14], but also offer potentially beneficial ideas to use lipase as catalysts for capacity applications [15–17]. In 2007 in Shanghai, China, immobilized YlLip2 was used as catalyst for biodiesel commercialization on an enzymatic production line with a capacity of 10,000 tons [17]. However, further improvements in immobilization techniques affording higher enzymatic activity and stability still need to be explored.

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Verger explained that an important aspect of lipolytic enzymes was the unique physicochemical character of the reactions they catalyzed at lipid–water interfaces, involving interfacial adsorption and subsequent catalysis *sensu stricto* [18]. Previously, the lipolytic enzymes were defined in kinetic terms, based on the concept of 'interfacial activation'. Subsequently, with neutron crystallographic evidence, Hermoso et al. concluded that lipase activation was not interfacial; however, it occurred in the aqueous phase and was mediated by colipase and a micelle [19]. Immobilization of lipases by selective absorption on the surface of a specific support through hydrophobic interactions has been proven to be a more effective method for the improvement of the activity and stability of immobilized enzymes [20,21]. Because of the characteristics of the interfacial activation, the open conformation of immobilized lipases is stabilized through the strong hydrophobic interaction between the hydrophobic areas around the active site of the enzyme and the surface of the carrier, thus improving the stability and activity of the immobilized enzyme [5,20,22].

According to the literature the extremely hydrophobic styrene-divinylbenzene beads [23–26] acted as an effective support and improved the enzyme performance (in particular, the operational stability) in transesterification synthesis of biodiesel and in esterification reactions involving the synthesis of esters such as ethyl/butyl butyrate or butyl acetate. Moreover, octyl-agarose support has been used for long time with optimal results [27–29]. However, highly hydrophobic matrix is not always advantageous [26], for example, excessive hydrophobic carriers can easily lead to poor biocompatibility on the surface of carriers, due to enhanced nonspecific interactions between the carrier and enzyme protein, resulting in a decrease in enzymatic activity [30]. In contrast, the use of hydrophilic carriers leads to water accumulation which results in the decrease in enzymatic activity [31]. However, the problem of water retention in operation is a different matter to immobilization. Séverac et al. showed the method for the prevention of water accumulation in biodiesel production and permitted the simple reuse of lipase B from *Candida antarctica* (CALB) [32]. Water significantly affects the stability of the enzyme compared to its activity. Therefore, the control of the microenvironment on the surface of the carrier and modifying hydrophobicity to enhance the activity of immobilized enzymes is becoming a temporal and promising area of research.

Typically, hydrophobic carriers can be prepared by two methods [33]. The first method involves the synthesis of polymer carriers from hydrophobic monomers for example, the commercial Novozym 435 resin, an immobilized enzyme, was synthesized using methyl methacrylate with weak hydrophobic properties. The second method involves the modification of the surface of hydrophilic carriers by introducing hydrophobic groups (e.g., incorporation of hydrophobic alkyl groups). The examples of second method include functionalization of mesoporous silica with octyltriethoxysilane [34], and coating native fibers with methyl groups through treatment with amino-functional polydimethylsiloxane (PDMS) [35,36]. Modification of the properties and introduction of functional groups, such as changing the carbon chain lengths of hydrophobic alkyl groups to control the hydrophilicity/hydrophobicity of the microenvironment is an extensively used common technique [37,38].

Increase in the number of reaction cycles of immobilized enzymes for its recycling and reuse is highly desirable. A proper immobilization system should provide a sufficiently strong immobilization to avoid the release of enzyme that may contaminate the product and result in loss of enzyme (and catalytic activity); therefore, covalent attachment method (involving covalent attachment between the enzyme and the support) was conducted [9]. As a widely used intermolecular crosslinker, glutaraldehyde can modify primary amino groups of proteins, although may eventually react with other groups (such as thiols, phenols, and imidazoles [40,41]) Aqueous solution of glutaraldehyde was used in the production of cross-linked enzyme aggregates [39]. Moreover, glutaraldehyde has been used to covalently immobilize enzymes in preexisting supports [28,42,43] or for the pretreatment of immobilized lipase [27]. However, the exact reaction mechanism is still under discussion [41]. Fibrous materials might be ideal for immobilization due to high specific surface area, good mechanical strength, chemical stability, and microbial resistance [44,45]. However, use of fibrous materials for immobilization has rarely been reported in the literature and is limited to covalent attachment only, which involves a variety of chemical reactions, such as sodium periodate oxidation [46] and β-sulfuric acid ester ethyl sulfone aniline [47]. Covalent attachment is a complex and expensive method which results in the production of non-renewable immobilized enzyme, leading to its limitations in industrial environments [48].

In a previous study [49], silk fabric from silk scraps in the form of woven were PDMS-treated and used to immobilized a lipase. The enzyme immobilized in this modified fibers exhibited better hydrolysis/esterification activity when compared to the native fiber. As an extensively investigation, this paper analyze effect of the hydrophobic finishing agent for silk fabrics as a support to immobilize lipases. The biocatalysts were characterized by activity and stability in different organic solvents, and the direct adsorption of the lipase via interfacial activation in the hydrophobic support was compared to the covalent immobilization using glutaraldehyde (a method useful to stabilize enzyme via covalent multipoint attachment).

2. Experimental

2.1. Materials

Silk woven fabrics were donated by Beijing CTA New Century Biotechnology Co., Ltd., Beijing, China. The raw silk from *Bombyx mori* was degummed and then industrially woven. Further special surface treatment was not carried out. Lipase (from *Candida* sp. 99-125, crude powders) was prepared in our laboratory [50] (7.000 U g^{-1}). The amino-modified PDMS (2-8040 polymers, viscosity: 0.8–5.0 Pa s) was purchased from Dow Corning (Shanghai) Management Co. Ltd, Shanghai, China. Finishing agents for fabric, namely amino silicone microemulsion C220A, Organic fluorine microemulsion Fk515, and emulsified wax EP-1 were donated by the China textile academy of sciences. Other reagents and solvents used were of analytical grade.

2.2. Surface modification of silk woven fabric

Prior to treatment and immobilization, the woven fabrics were cut into small pieces (3 cm × 3 cm squares for each piece, weighing about 0.15 g) and immersed in deionized water for 12 h, and then dried at 50 °C for 4 h. Dried fabric films (~1.0 g total) were treated by dipping in amino-modified PDMS liquid [0.5% in hexane (w/v)], or in other finishing agents solution including amino silicone microemulsion C220A, organic fluorine microemulsion Fk515, and emulsified wax EP-1 at 35 °C for 1 h in a water bath with slight shaking. Finally, the treated fabric films were rinsed with fresh hexane and then dried in an oven at 70 °C for 1 h to remove the residual solvents.

Glutaraldehyde cross-linked fabrics were prepared by immersing fabrics in hydrochloric acid (4 M) and performing the hydrolysis at 50 °C for 1 h at 120 rpm on a horizontal shaker. Subsequently, the treated fabric films were rinsed with plenty of water and phosphate buffer (100 mM, pH 8.0) for three times successively. The fabrics were immersed in glutaraldehyde solution [5% (v/v)] at 35 °C for 1 h.