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Different organic components on silica hybrid matrices modulate the lipase inhibition by the glycerol formed in continuous transesterification reactions

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ABSTRACT

Silica hybrid materials, functionalized via incorporation of three organic components: β -cyclodextrin (β CD), carboxymethyl-cellulose (CMC) and hydroxyethyl-cellulose (HEC) were synthesized by the sol-gel technique and results were compared to a well-defined matrix (silica-polyvinyl alcohol-SiO₂-PVA) with respect to immobilizing *Burkholderia cepacia* lipase. The main objective was set to select organic components that can replace the PVA to obtain a hybrid composite with greater hydrophobic character, without compromising the remarkable features of SiO₂-PVA. All the three selected compounds allowed obtaining matrices that presented similar textural and morphological properties and gave high activity (1451–1661 U g⁻¹) and thermal stability ($t_{1/2} > 70$ h) upon immobilization. Regarding glycerol affinity, all matrices had lower ability than SiO₂-PVA to adsorb glycerol, with the SiO₂- β CD matrix showing the lowest affinity due to the cyclic structure of the β CD. Transesterification reactions of palm kernel oil with ethanol mediated by *B. cepacia* immobilized on SiO₂- β CD performed in a packed bed reactor under continuous flow confirmed the efficiency of the SiO₂- β CD matrix in increasing the operational stability of system, revealing a half-life ($t_{1/2} = 1040$ h), 2.5 times greater than that achieved in the same system using lipase immobilized on SiO₂-PVA ($t_{1/2} = 430$ h).

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Introduction

The use of immobilized enzymes has been shown to offer several advantages over the use of enzymes in their free forms [1,2]. Immobilized enzymes are more robust and more resistant to environmental changes [2]. More importantly, the heterogeneity of these immobilized systems allows an easy recovery of both the enzymes and products, and hence multiple reusing of enzymes, continuous operation of enzymatic processes, and greater variety of bioreactor designs [3,4]. Furthermore, immobilization often leads to enhance the thermal stability of enzymes, and it is essential to perform reactions in non-conventional media [5,6].

Immobilization of enzymes has been carried out using different techniques, including adsorption, covalent attachment, or entrapment [2,7] on several matrices [5,8,9]. The choice of the matrix

usually depends on its desired features for a potential industrial application, such as its mechanical strength, chemical and physical stability, hydrophobic character, loading capacity, and cost [3,10,11]. It is well known that the properties of a supported enzyme are governed by the properties of both the enzyme and the carrier material [12]. The interaction between the two components provides an immobilized enzyme system with specific chemical, biochemical, mechanical, and kinetic properties [2,12,13].

The support (carrier) can be a synthetic organic polymer, a biopolymer, or an inorganic polymer [5,8,9]. An alternative approach uses a combination of inorganic and organic components to produce matrices with specific features for a particular application or to achieve distinct supports that are not found in conventional materials [14,15]. A convenient method to obtain organic-inorganic hybrid materials is the sol-gel technique [16–18]. This method is employed to generate materials with a wide range of inner chemical surface. Hence, these materials are suitable to immobilize enzymes and allow the combination of the chemical and physical properties of the guest component with the excellent

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optical, thermal, and chemical stability of the host silicon oxide matrices [17–19].

The silica-based hybrid materials can be obtained via hydrolysis and condensation of the silicate precursor, such as tetraethyl orthosilicate (TEOS), and several organic compounds, including polyvinylalcohol [19], hidroxipropil cellulose, glucomannan, xanthan gum; acetylcellulose [17], pectin [20], chitosan [21], beta-cyclodextrin [22] and cellulose [23]. Among them, polyvinyl alcohol has been demonstrated in many articles to be a favorable organic component in the synthesis of hybrid matrices [19], which is suitable for immobilizing lipase from many microbial sources [24–26]. In these studies, polysiloxane–polyvinyl alcohol hybrid composite has been prepared by the sol–gel process involving three steps: hydrolysis, subsequent polycondensation of tetraethoxysilane, and SiO₂–PVA hybrid matrix formation [19,24]. The interpenetration of the PVA in the polysiloxane network during the matrix synthesis using this method has resulted in a porous structure with a higher surface area, which can be further activated, mainly with epichlorohydrin, to obtain epoxy hybrid matrices [24,27,28]. The resulting biocatalysts have been applied successfully in biotransformation reactions in batch mode to produce goods of industrial interest, such as sugar esters [26], monoglycerides [29], structured lipids [30] and biodiesel [24,25,31].

Despite the good performance in the mentioned applications, limitations of these catalyst systems have been reported in the synthesis of biodiesel by transesterification reactions carried out in a packed bed reactor (PBR) running on a continuous basis [32–35]. This limitation has mainly arisen from the retention of glycerol, formed as a by-product, in the catalytic bed [9,33,36]. Glycerol is adsorbed on the surface of the biocatalyst and forms a hydrophilic layer that causes the immobilized lipases to be inaccessible to hydrophobic substrates (such as residual triglyceride, diglycerides, monoglycerides) [9,37,38]. Additionally, unreacted alcohol migrates from the reaction mixtures to the glycerol layer and further covers the surface of the immobilized lipase, resulting in lipase deactivation due to the local alcohol concentration [37,38]. The accumulation of glycerol may also cause column clogging, and may thus increase the pressure drop inside the PBR [37,39]. Several approaches have been proposed to overcome these problems, such as elimination of glycerol by dialysis or extraction using a polar solvent [37,40]. Other solutions have involved adding organic solvents (e.g., *n*-hexane or *tert*-butanol) to make the reaction mixture more homogeneous and less viscous [9,41], or alternatively using a support that prevents adsorption of glycerol such as highly hydrophobic adsorbents [9,42,43].

Several hydrophobic matrixes are commercially available and have already obtained excellent results, as published by several researchers [42,43]. However, the attention was given in the current work to modulate the properties of a well-defined matrix SiO₂–PVA; previously developed in our lab [31,35]. The major goal was to find organic components that can replace the polyvinyl alcohol in order to attain a hybrid composite with a greater hydrophobic character, without compromising the remarkable features of SiO₂–PVA. It is expected that decreasing the matrix polarity may prevent the biocatalyst from being clogged due to glycerol formation [32,33]. This in turn increases the half-life of the immobilized enzyme, allowing the maintenance of a stable reactor operation for longer periods.

To achieve this target, the selected lipase was *Burkholderia cepacia* (BLC), since it is one of the most widely used biocatalysts in biotransformation processes [44]. The extensive applications of this lipase originate from its useful properties such as heat resistance, ability to recognize a wide variety of substrates, as well as tolerance to multiple solvents, including short-chain alcohols [44,45]. BCL exhibits high hydrolytic activity toward triglycerides

regardless of the chain length of the fatty acids and does not have a particular positional specificity.

The organic components chosen to replace polyvinyl alcohol (PVA), which is a highly hydrophilic polymer [19], were β -cyclodextrin (β CD), carboxymethyl-cellulose (CMC), and hydroxyethyl-cellulose (HEC). The resulted hybrid matrices were fully characterized in terms of their textural and morphological properties. The catalytic properties of both the free and immobilized lipases were assessed, and the immobilized lipase with the lowest affinity for binding glycerol was used to perform continuous transesterification of palm kernel oil in a packed bed reactor.

Experimental

Materials

A commercial lipase from *B. cepacia* (Batch number: 01022TD) was purchased from Amano Pharmaceuticals (Nagoya, Japan) and used as received without further purification. Tetraethoxysilane (TEOS); β -cyclodextrin, carboxymethyl-cellulose (MW 250,000) were acquired from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) hydrochloric acid (minimum 36%), ethanol (minimum 99.8%); polyethylene glycol (PEG 1500 g/mol) and glycerol (99.7%) were from cromoline (São Paulo, SP-Brazil). Polyvinyl alcohol (PVA, MW 88,000) was bought from Sinapse Biotechnology (São Paulo, SP-Brazil) and hydroxyethyl-cellulose was acquired from Fluka (Buchs, Switzerland). Commercial olive oil (low acidity, purchased in a local market) was used to determine the hydrolytic activity. *n*-butanol (99%) and butyric acid, both purchased from Merck KGA (Darmstadt, Germany), were used as starting materials for the esterification reactions. Reagents for the transesterification reactions were anhydrous ethanol (99.8%, cromoline) and refined palm kernel oil kindly donated by Agropalma (Belem-PA, Brazil), having the following composition in fatty acids (wt%): 4.3% caprylic; 3.8% capric; 48.0% lauric, 16.0% mirystic, 8.25% palmitic, 2.15% stearic, 15.50% oleic and 2.0% linoleic.

Support synthesis

The hybrid matrices: SiO₂– β -cyclodextrin (SiO₂– β CD); SiO₂–carboxymethyl-cellulose (SiO₂–CMC); SiO₂–Hydroxyethyl-cellulose (SiO₂–HEC) and SiO₂–polyvinyl-alcohol (SiO₂–PVA) were prepared by the hydrolysis and polycondensation of tetraethyl orthosilicate (TEOS). The synthesis were carried out according to the methodology previously described [25,27]: 50 mL of TEOS, 50 mL of ethanol and 60 mL of an aqueous solution containing 2% w/v of the organic component (β CD, CMC, HEC or PVA) were carefully mixed and stirred for 5 min at 60 °C, followed by the addition of 1000 μ L of concentrated HCl, in order to catalyze the reaction. The mixture was then incubated for a period of 40 min, and the material was transferred to micro wells of tissue culture plates (disc shape) and kept at 25 °C until complete gel solidification (formation of an interpenetrated network of SiO₂–organic compounds). Then, the spheres were grounded in a ball mill and classified to attain particles with 0.308 mm of diameter (–40/+60 MESH Tyler standard sieves). The proposed mechanisms for the hybrid matrices formation, is shown in the Supplementary material (S).

Immobilization procedure

The prepared matrixes were activated with epichlorohydrin [27] and used to immobilize the lipase according to the methodology reported by Da Rós et al. [46] as briefly described. Activated hybrid particles were soaked into hexane under stirring (100 rpm) for 1 h at 25 °C. Then, excess hexane was removed and

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