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Catalysis Today 105 (2005) 443–447
CATALYSIS TODAY
www.elsevier.com/locate/cattod

A novel structured bioreactor: Development of a monolithic stirrer reactor with immobilized lipase

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Abstract

Cordierite monoliths were functionalized with polyethylenimine (PEI) and with different types of carbon, consisting of carbonized sucrose, carbonized ploy furfurryl alcohol, or carbon nanofibers, in order to create adsorption sites for a lipase from *Candida antarctica*. The prepared supports were compared in terms of immobilization capacity, activity, and stability. The supports with a carbon nanofiber coating displayed the highest enzyme adsorption capacity. The biocatalysts were assayed in the acylation of 1-butanol with vinyl acetate in toluene, yielding butanyl acetate and acetaldehyde. For catalyst performance testing a novel reactor type was employed, the monolithic stirrer reactor, in which monolithic structures are applied as stirrer blades. No profound effect of stirrer rate on the reaction rate was observed, implicating the absence of external mass transfer limitations. For comparison, free enzyme and a commercial (particulate) immobilized lipase were also included in the study. Compared to the free enzyme, the immobilized lipase shows a significantly lower activity. Increased stability, easy catalyst separation and the possibility to reuse the enzyme in immobilized form can overcome this difference. The commercial immobilized lipase initially has a significantly higher activity than the monolithic biocatalysts, but deactivates relatively fast. For the monolithic biocatalysts, no deactivation was observed; the prepared catalysts were stable for several weeks.

Keywords: Biocatalysis; Lipase; Monolithic catalyst; Stirrer reactor

1. Introduction

Driven by environmental regulations and public opinion, the interest in application of enzymes in industrial processes has increased. The use of enzymes has some important advantages over using conventional (inorganic) catalysts, including high selectivity, operation under ambient conditions and in aqueous environment, and in general no production of unwanted side products. The application of enzymes however, brings about several practical problems concerning the fragile nature of the catalyst and expensive downstream processing to prevent loss of catalyst. Immobilizing enzymes on a suitable carrier facilitates easy catalyst separation. Although activity usually decreases upon immobilization, the stability is drastically increased. Many supports have been studied including polymers and resins [1,2], silica and silica-alumina composites [3,4], and carbonaceous materials [5–7]. These materials are mostly used in particulate form. These systems generally have a low mechanical strength and often exhibit severe diffusion limitations, leading to a considerable fraction of unused enzymatic activity [8]. To improve the performance of these immobilized enzyme systems, they can be applied on a macrostructured support material. A thin layer of enzyme carrier, applied on the walls of a structured support material can be an interesting alternative for conventional particulate supports.

Honeycomb catalyst supports were originally developed for use in automotive emission control systems where low pressure drop and high surface area are paramount [9]. For similar reasons, monoliths are also attractive for liquid and gas–liquid heterogeneous catalysis [10]. The classical honeycomb monolith (Fig. 1) has square parallel channels on which a washcoating can be applied.

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doi:10.1016/j.cattod.2005.06.056

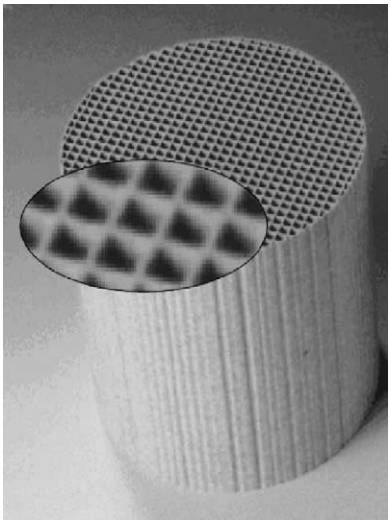

Fig. 1. Monolith structure.

The monolithic stirrer reactor is a novel reactor, designed to implement in a convenient way monolithic structures in existing reactor vessels. In this reactor, monolithic structures are used as stirrer blades. By rotating the monoliths through the liquid, both mixing of the reaction medium and contacting the catalyst with reactants by convection through the monolithic channels is facilitated. The monolithic stirrer set-up is shown in Fig. 2. This reactor is thought to be especially useful in production of fine chemicals and biotechnology [11]. Acylations are very important basic reactions in the production of many pharmaceuticals, agrochemicals and fragrances. To eliminate environmental problems and corrosion, usually associated with conventional catalysts such as $AlCl_3$, many studies were performed [12,13] to find new catalysts. The application of a biocatalyst could be an interesting innovation in this respect.

In this paper, the preparation and catalytic testing of different functionalized ceramic monoliths is presented. Different carbons and an electrolyte polymer coating were applied on the monolithic channels, to create adsorption sites for a lipase from *Candida antarctica*. The objective of this study was to compare the performance of the prepared biocatalysts in the monolithic stirrer reactor and demonstrate the feasibility of enzyme catalysis in this reactor. The biocatalysts were tested in the acylation of 1-butanol by vinyl acetate in toluene. A commercial (particulate) immobilized lipase and free enzyme were also included in the study.

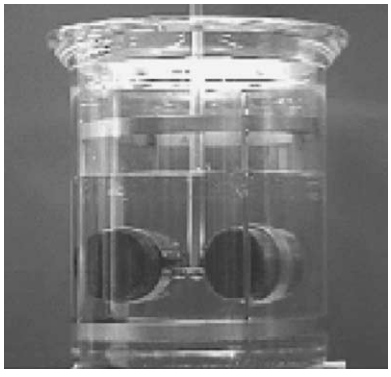

Fig. 2. MSR.

2. Experimental

2.1. Materials

Cordierite monoliths with cell densities of 200 and 400 cells/in.^{2} (31 and 62 cells/cm^2) were provided by Corning Inc. Lipase from *Candida antarctica* was purchased from Roche. Sucrose, polyethyleneimine (high molecular weight), γ-(aminopropyl)triethoxysilane, and (3-glycidoxypropyl)trimethoxysilane were purchased from Sigma.

2.2. Methods

Sucrose-based carbon carriers were prepared following the method of [14]. Monoliths were coated with a 65% sucrose solution in water, followed by drying at 393 K and carbonization for two hours under H_2 at 823 K.

Polyfurfuryl alcohol (PFA) based carbon coatings were prepared by the method of [15]. Monoliths were coated with freshly prepared PFA solution and dried at 353 K. Carbonization was performed at 823 K under Ar for 2 h.

Carbon nanofiber based coatings were prepared by washcoating the monolithic supports with a silica layer, following the method of [16]. Ni was deposited on the support by homogeneous deposition precipitation at 353 K from a 0.5 M aqueous urea solution, starting at pH 2. After reduction for 1 h at 773 K, carbon nanofibers [17] were grown under methane in N_2.

The texture of the prepared supports was analyzed using N_2 and CO_2 adsorption.

Polyethylenimine-functionalized supports were prepared by the method of [3], either using a two step approach via γ-(aminopropyl)triethoxysilane (APTES) and glutaraldehyde or by direct coupling via (3-glycidoxypropyl)trimethoxysilane (GPTMS).

Lipase was adsorbed at 278 K from a 50 mM phosphate buffer pH 7 (2 g/l lipase), using a recycle reactor in which the liquid was recycled over the support in up flow at 40 ml/min. The enzyme concentration was determined using UV–vis at 260 nm. After immobilization, the samples were washed several times with phosphate buffer pH 7, vacuum

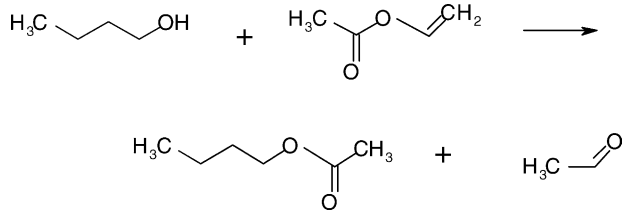

Fig. 3. Acylation of butanol with vinylacetate.