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Carbon–ceramic composites for enzyme immobilization

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Abstract

Tunable carbon nanofiber-coated monoliths as carriers for enzyme adsorption are presented. Carbon-nanofibers (CNFs) were grown on monoliths with different microstructure. “Classical” cordierite monoliths were compared to novel acicular mullite (ACM) monoliths, with a more open wall structure. This open structure allows for a higher CNF-loading without affecting the open structure of the monoliths. The composites were used as a carrier for lactase from *Aspergillus oryzae*. ACM monoliths allow for a higher CNF loading, and thus for a higher enzyme loading. Lactase adsorption per gram of carbon increases by 15–20% compared to cordierite samples. The total enzyme adsorption capacity for CNF-coated ACM and cordierite monoliths was found to be 350 and 300 mg g_{Carbon}^{-1} respectively. Oxidation treatment of the CNFs affected the surface chemistry, while the porosity was not affected. The growth catalyst could be removed completely from both the support and the CNFs by treatment in HCl or HNO_3, without destroying the carbon structure.

Keywords: Ceramic monolith; ACM monolith; Enzyme adsorption; Carbon nanofibers; Characterization

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1. Introduction

Carbon nanofibers have been known for a long time as a nuisance that often emerges during catalytic conversion of carbon containing gases [1]. Recently, carbonaceous fiber structures have been studied because of their use as catalyst support materials [2–4], selective adsorption agents [5,6], energy storage devices [7], composite materials, nanoelectric devices [8], field emission devices [1,9,10], and filter materials [11]. This work deals with the application of CNFs for physical adsorption of enzymes. Generally, enzyme carriers must meet certain criteria; the support must have a high adsorption capacity, and hold the enzyme firmly on the surface. It must also retain and stabilize the biological activity of the immobilized material. Finally, operational characteristics (mechanical strength, hydrodynamics, resistance to degradation) must be good and cost should be low [5].

An important advantage of physical adsorption is that it is reversible. Denatured enzyme can be replaced by changing the pH or the ionic strength of the reaction medium, followed by adsorption of fresh enzyme. However, desorption can also be a major drawback if it occurs during reaction. The efficiency of the physical adsorption depends on several parameters. The size of the protein to be adsorbed, the specific surface area of the carrier, the texture (porosity, pore size), and the surface chemistry are crucial. Typically the use of a porous support is preferred since the enzyme will not be adsorbed only at the outer surface of the material but within the pores as well. The pH of adsorption is equally important since ionic interactions can either increase or decrease final enzyme loading and can also influence residual activity. Usually, the maximum adsorption is observed at a pH close to the isoelectric point of the enzyme [12]. In the past decades, physical adsorption has been studied in combination with different types of

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particulate carrier materials including different polymers [13,14], molecular sieves [15–18], silica and silica-alumina composites [19–21], and carbonaceous materials [22–24].

Under reaction conditions, these systems are often diffusion limited, leading to a considerable fraction of unused enzymatic activity [25,26]. A thin layer of carrier material on a monolithic support could be an interesting alternative for these particulate carriers by increasing mechanical stability and decreasing diffusion distance.

The honeycomb monolith support offers several advantages over particulate supports, including a high geometric external surface, structural durability, easy catalyst separation, a low pressure drop, and uniform flow distribution within the matrix [27]. The classical honeycomb has square parallel channels with hardly permeable walls: reactants cannot readily enter the wall structure and active material must be deposited on a washcoated egg-shell layer that allows better access to active sites. We have been exploring a new type of structured monolithic support having the same macroscopic geometry as classical cordierite monoliths. This material was developed by The Dow Chemical Company as a new catalyst support. This support is a highly porous acicular mullite (ACM). The unique open microstructure is shown in Fig. 1 [28,29].

By controlling the synthesis conditions, the mean pore size can be tailored on the micrometer length scale. The open pore structure allows access of reactants to catalysts deposited within the monolith wall [30]. In short, the new ACM supports allow us to further fine-tune the interplay of diffusion and reaction. Elimination of diffusion problems affects reaction time and – more importantly for intensifying fine-chemical processes – reduces the extent of unwanted side reaction: diffusion generally acts as an equalizer that favors the (usually slower) side reactions.

The present work is concerned with the preparation of different carbon–ceramic composites by growing carbon nanofibers (CNFs) on monoliths over deposited Ni. The effects of different types of structured monolith supports (cordierite and ACM) are studied. For some applications of CNFs, for example as catalyst support in food or pharmaceuticals production or in filtration, the growth catalyst Ni is an unwanted component. Therefore, the removal of Ni from the fiber tips by acid treatment and the effects on surface chemistry is a relevant topic. Thus, in the present work, the removal of Ni by using different methods is also included. In addition, the supported CNFs were applied as support material for a β-galactosidase (lactase) from *Aspergillus oryzae*. Lactase catalyzes the hydrolysis of lactose to glucose and galactose. Lactose is the main carbohydrate in milk and whey. The consumption of foods with a high lactose content is problematic for almost a 70% of the world population. Together with the relatively low solubility and sweetness of lactose, this has led to an increasing interest in the development of industrial processes to hydrolyze lactose.

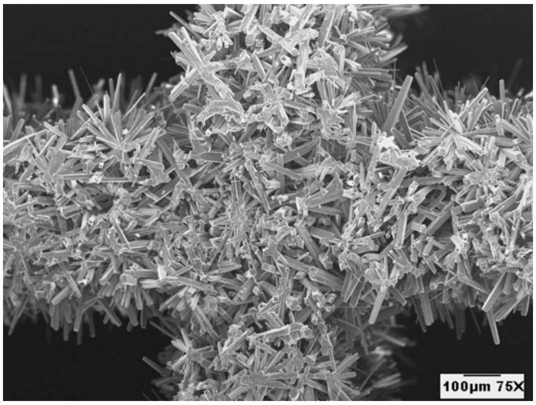

Fig. 1. SEM micrograph of ACM (400 cpsi), showing the open structure of the channel walls.

2. Experimental

2.1. Materials

β-Galactosidase from *A. oryzae* (E.C. 3.1.2.23, lyophilized), oxalic acid, and *o*-nitrophenyl-β-galactopyranoside (oNPG) were purchased from Sigma. HNO_3 (65%), HCl (38%), and $Ni(NO_3)_2$ were purchased from Baker. Ludox AS-30 colloidal silica solution was from Aldrich. ACM monoliths of mullite, 200 and 400 cpsi (cells/inch^2, 31 and 62 cells/cm^2) were prepared by a proprietary Dow process. Cordierite monoliths were provided by Corning, Inc. The samples had a length of 5 cm and a diameter of 4.3 cm. The key properties of the monoliths are given in Table 1.

Prior to coating, the monoliths were calcined for 4 h at 1273 K in a static air oven to remove possible contaminants. In order to prevent fiber growth inside the ceramic structures and to provide sufficient surface area for deposition of the growth catalyst, a SiO_2 washcoat was applied on the monolith channels.

2.2. Support preparation

Monoliths were washcoated [31] with a Ludox AS-30 colloidal Silica layer (calcination 5 K/min, 723 K, 4 h). Ni (0.75 g $Ni(NO_3)_2$ per g SiO_2) was deposited at 363 K for 8 h [32] in 300 ml 0.5 M urea, pH 2. The carriers were dried for 16 h at 323 K, followed by calcination at 673 K (2 h, 2 K/min). Ni was reduced at 823 K (1 h) in 20%v/v H_2 in N_2 (total flow rate 150 ml/min), and carbon fibers were grown at 823 K in CH_4 (120 ml/min) and H_2 (10 ml/min) in N_2 (70 ml/min).

Table 1
Nominal values of the key properties of monoliths employed in this study

	ACM (“medium”)	C (cordierite)
Cell density	200/400 cpsi	200/400 cpsi
Wall thickness	0.35/0.24 mm	0.3/0.18 mm
Wall porosity	60%	30%
Pore diameter	9 µm	2.7 µm