CARBON COATED MONOLITS AS CARRIERS FOR ENZYME IMMOBILIZATION

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Introduction

Immobilized enzymes are increasingly used as catalysts in fine chemical and specialty chemical production. Although activity usually decreases slightly upon immobilization, they possess important advantages over dissolved enzymes, e.g. the possibility of recovery and reuse, simple operation, and improved stability. Many supports have been studied for enzyme immobilization, including polymers and resins [1,2], silica and silica-alumina composites [3,4], and carbonaceous materials [5,6]. These materials are mostly used in the form of powders, beads, or chips. These systems are often severely diffusion limited, leading to a considerable fraction of unused enzymatic activity [7]. Carbon is a well-known material in adsorption and catalytic processes. Carbon is stable in acidic and basic media and the textural properties and chemical characteristics of carbonaceous materials can easily be tailored. Hence, these materials have a wide range of applications in different areas, including catalysis and pollutant removal. The main drawback of using carbon as catalyst support is the low mechanical strength; this can be improved by applying a thin layer of carbon on a suitable ceramic carrier.

Honeycomb catalyst supports, originally developed for use in automotive emission control systems where low pressure drop and high surface area are paramount [8], are an interesting alternative for conventional support materials in heterogeneous catalysis. These porous macro structured supports consist of small square channels on which a thin layer of active material can be applied [9].

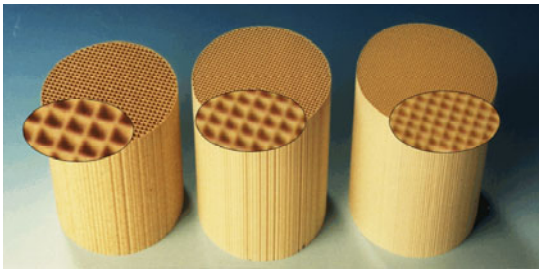


Figure 1. Monoliths with different cell densities

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. The present study is concerned with the application of monolith reactors in the field of biotechnology. The aim of the present work is to combine ceramic monolith materials with different carbonaceous materials and compare the prepared carriers in terms of enzyme adsorption capacity, stability, and catalyst performance.

Experimental

Ceramic honeycomb monoliths were functionalized with different carbon coatings, prepared by sucrose carbonization, polymer carbonization, and growth of carbon nanofibers.
Sucrose-based carbon carriers were prepared [10] by dipping monoliths in a 65% sucrose solution in water, followed by cleaning the channels by air-blowing and horizontal drying. Drying was completed at 393 K for 4 h. The sucrose coat layer was carbonized for two hours under H_2 at 823 K.
Polyfurfuryl alcohol (PFA) based carbon coatings were prepared by [11] coating monoliths with freshly prepared PFA solution and drying at 353 K. Carbonization was performed at 823K under Ar for 2 h.
Carbon nanofibers (CNF) were grown on an alumina or silica washcoat layer [9]. Ni was deposited on the support at 353 K from a 1 M aqueous urea solution. After reduction for 1 h at 973 K, carbon nanofibers [12] were grown under propene or methane in N_2.

Enzymes were adsorbed on the functionalized supports in a recycle reactor. The enzyme concentration was determined using UV-VIS. After immobilization, the samples were washed and stored at 278 K. Upon removal from storage, the biocatalysts were equilibrated for 30 min. in 150 ml 0.1 M tris buffer, pH 7 before activity measurement. Lipase activity for the hydrolysis of pNPP was followed by measuring absorbance at 348 nm. Experimental conditions were 0.4 mM in DMSO/tris buffer, pH 7 (1:9) at 293 K. Enzyme desorption was followed by measuring the enzyme concentration in the supernatant liquid spectrophotometrically for various added concentrations of NaCl.

Results and Discussion

The preparation of carbon coated monoliths yields carbon carriers with different properties. The surface characteristics and carbon loading depend on the preparation method. Some important properties are displayed in table 1.

Table 1. Properties of carbon coated monoliths

Carrier	Type	Y_c Wt%	Sbet [m^2/g]	Pore size [nm]	Pore volume [cm^3/g]
S19	Sucrose	4.3	23	12	0.02
S20	Sucrose	4.8	33	10	0.023
P9	PFA	13.9	64	<2	0.028
F25	CNF	2.8	64	8	0.06
F21	CNF	2.8	70	7-8	0.05

The carriers were prepared as supports for the globular α/β protein lipase, with approximate dimensions of 4.0 x 4.0 x 5.0 nm and relative mass 40 kD. The pore size of the carriers must be substantially larger then 4 nm for adequate enzyme adsorption. The sucrose-based carriers have a moderate carbon yield of 4 wt%, this can be increased to 7-8 by concentrating the precursor or perform two subsequent coating steps. The surface area seems relatively low, but per gram of carbon S_{BET} varies

between 500 and 700 m^2. The average pore size is 11 nm, therefore this carrier type appears to be suitable for lipase adsorption.
The microporous PFA carriers have a large Y_C, caused by the high viscosity of the polymer precursor. The surface area is high, but it mainly consists of microporous (pore size <2 nm) surface area. These carriers are expected to have a lower lipase adsorption capacity.
The low Y_C for the CNF supports results in a large surface area per g/C (>300 m^2/gC). Combined with the open, network like structure of the fibers, these carriers should display a high lipase adsorption capacity.

To study the morphology of the carriers, SEM micrographs were recorded. Figure 2 shows SEM images of (a) CNF and (b) sucrose coatings on cordierite monoliths.

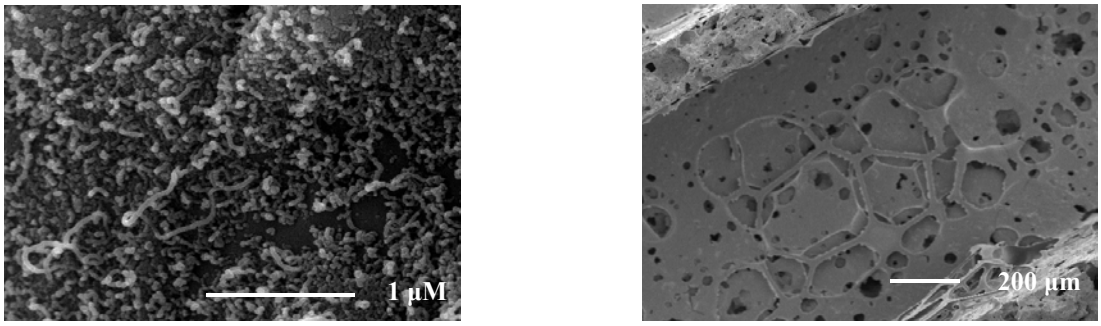


Figure 2. SEM images of prepared carbon coatings. a:carbon fibers, b:sucrose

The CNF completely cover the surface, forming a uniform layer of fibers up to 1 um length The Sucrose coating seems to consist of graphite-like layers that form a 3-D network of carbon.

Results of adsorption of lipase onto the carbon carriers are shown in table 2.
As expected, the CNF supports have the highest adsorption capacity. The microporous PFA carriers have a higher lipase yield then the sucrose supports. The reason for this is not clear.

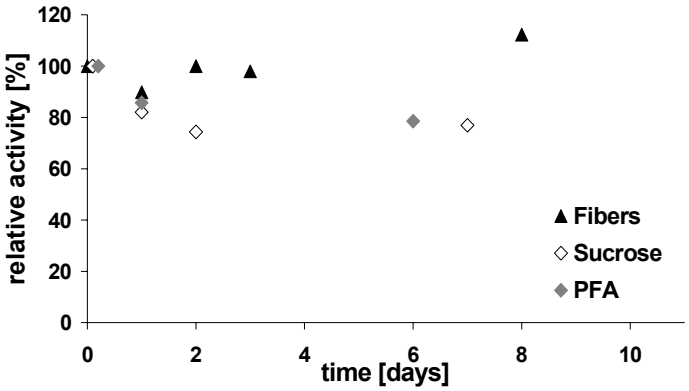
Table 2: results lipase adsorption

Carbon type	Yield of lipase	Enzymatic activity	
	(g)	(mmol/$m^3_{monolith}$/s)	(mmol/g_{enzyme}/s)
Carbonized sucrose	0.05	1.35	$1.8x10^{-3}$
Carbonized sucrose	0.04	1.00	$2.0x10^{-3}$
Carbonized PFA	0.08	1.46	$1.3x10^{-3}$
Carbonized PFA	0.09	1.87	$1.4x10^{-3}$

The activity per unit monolith volume corresponds to the amount of adsorbed enzyme. The activity per unit enzyme seems to be dependent on the carbon type. The activity per unit enzyme is slightly higher for the sucrose-based carriers, compared to the PFA supports. This can be caused by adsorption in the narrow pores, so that the enzyme is not available for substrate. Another reason can be the nature of the surface groups of

the different carbon types that can cause the enzyme to ‘spread out’ over the surface and deactivate.

The stability of the biocatalysts during storage at 278 K is shown in figure 3.



The CNF catalysts remain without significant activity loss for the first 10 days. Both PFA and sucrose carriers show a decrease in activity to 80% of its original activity. Apparently the active configuration of the enzyme is best preserved on CNF.

Figure 3. Stability of the biocatalysts

Conclusions

The preparation of carbon ceramic composites with three different carbon precursors yields carrier materials with completely different properties. The carriers have tunable surface properties such as surface area, pore size distribution and hydrophobicity. Surface area, pore volume and pore size can be influenced by the carbonization method (temperature, atmosphere, optional activation under O_2).
The adsorption of lipase on the carbon-ceramic composites yields active biocatalysts. The carbon type influences the adsorption process and the activity per unit enzyme, due to pore volume, surface area and surface groups. As expected, fibrous supports have the highest adsorption capacity. Adsorption on PFA-based carbon is lower, sucrose supports show the lowest adsorption capacity.
The activity of the biocatalysts depends on the carbon type and the enzyme loading. CNF coated monoliths have the highest stability during storage.

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