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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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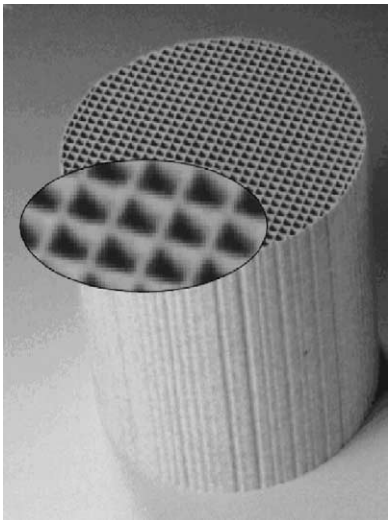

Fig. 1. Monolith structure.

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.

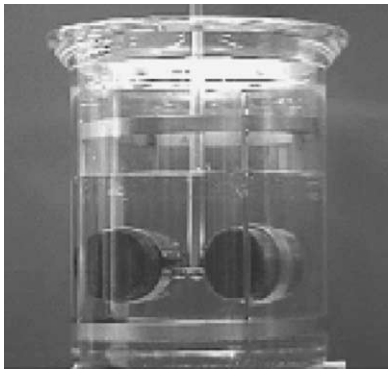

Fig. 2. MSR.

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

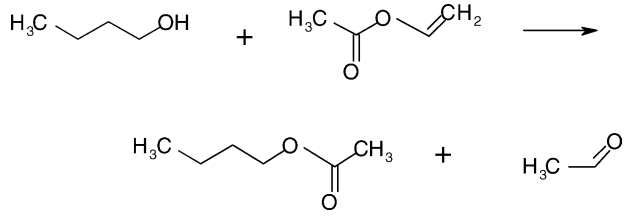

Fig. 3. Acylation of butanol with vinylacetate.

Table 1
Properties of carbon coated monoliths

Carbon type	$Y_{Carrier}$ wt.%	S_{BET} (m^2/g)	S_{BET} (m^2/g_{Carbon})	Pore size (nm)	Pore volume (cm^3/g)
Sucrose	4.3	25	580	10	0.03
PFA	13.9	50	360	<2	0.02
CNF	2.8	26	–	8	0.06

dried at room temperature for 24 h, and stored under air at 278 K.

Catalytic tests were performed in a glass vessel equipped with a stirrer motor. During testing, the vessel was flushed with nitrogen. Two monoliths (diameter 4.3 cm, length 4 cm) were mounted in plane on the stirrer axis. The reaction scheme is given in Fig. 3. Tests were performed at 300 K and varying stirrer rate. The total reaction volume was 2.5 l. Butanol and vinyl acetate concentrations were 0.6 and 1 M, respectively. A slight excess of vinyl acetate was used to reduce the inhibiting effect of butanol, generally observed for lipase catalyzed transesterifications following the Bi-Bi Ping-Pong model [18]. Reactants were dried over molsieve before use. The reaction was followed by GC analysis (323–523 K, 10 K/min) on a Varian CP 3380 gas chromatograph, equipped with an 1177 FID detector and a CP-SIL-8 column (length 60 m, internal diameter 0.25 mm) and H_2 as the carrier gas.

3. Results and discussion

3.1. Support preparation

Preparation of PEI coated monoliths with APTES and GPTMS yields a polymer loading of 6 and 10 wt.% respectively, apparently coupling through GPTMS yields more binding sites for the polymer. It is, therefore, expected that GPTMS functionalized supports have a higher enzyme adsorption capacity.

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.

The carriers were prepared as supports for the globular α/β protein lipase, with approximate dimensions of 4.0 nm × 4.0 nm × 5.0 nm and relative mass 33 kD. The pore size of the carriers must be substantially larger than 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 wt.% by concentrating the precursor or perform two subsequent coating steps. The surface area might seem relatively low, but per gram of carbon S_{BET} varies between 300 and 500 m^2. The average pore size is 10 nm, therefore this carrier type appears to be suitable for lipase adsorption. The microporous PFA carriers have a large carbon loading, 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 CNF supports do not seem to have a large BET surface area, but the absence of microporosity combined with the open, network like structure of the fibers and the large total pore volume, these carriers should display a high lipase adsorption capacity. The BET surface area per gram of carbon was not calculated for these supports, because at this point it is not known how much the underlying silica surface contributes to the total surface area. If we assume that 30% of the surface is covered with CNF (7.8 m^2/g), a carbon content of 4 wt.% gives a BET surface area of 185 m^2/g_{Carbon}. This is in agreement with earlier research [19,20].

3.2. Enzyme adsorption and catalyst performance

Results of lipase adsorption and catalytic tests performed at 150 rpm and 300 K are given in Table 2. Coupling of the enzyme via APTES and glutaraldehyde yielded a carrier with a very low enzyme adsorption capacity, and preliminary activity tests in aqueous environment already revealed a substantial deactivation due the negative effect of

Table 2
Results of lipase adsorption and activity tests

Carrier type	Yield lipase pH 7, 278 K		k_{obs} 150 rpm, 300 K	Enzymatic activity 150 rpm, 300 K	
	g	g/gC	mmol/l s	($mmol/m^3_{monolith}s$)	($mmol/g_{enzyme}$ s)
Carbonized sucrose	0.075	0.18	0.050	1.97	0.95
Carbonized sucrose	0.052	0.18			
Carbonized PFA	0.088	0.05	0.046	1.88	0.85
Carbonized PFA	0.081	0.04			
CNF	0.334	0.01	0.221	9.25	0.96
CNF	0.379	0.01			
PEI (GPTMS)	0.025	–	0.023	0.64	0.93
PEI (GPTMS)	0.032	–			

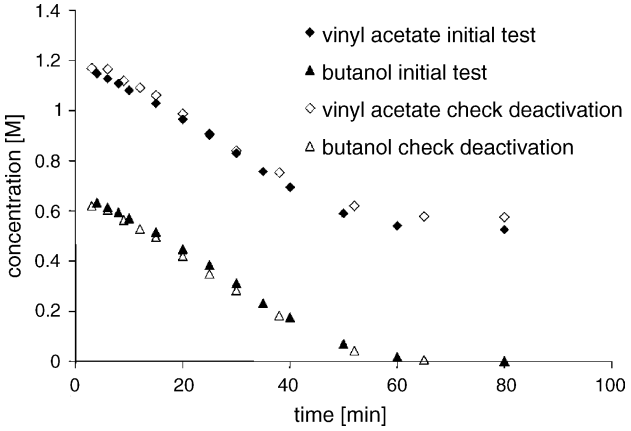

Fig. 4. Typical concentration profiles of different performance tests in the MSR at 150 rpm, 300 K. The initial test is presented in closed markers, a duplicate experiment after 2 weeks is represented by open markers.

glutaraldehyde. Therefore, the APTES-PEI carriers were not included in the catalyst performance study.

The activity per unit monolith volume corresponds to the amount of adsorbed enzyme. The CNF coated supports have the highest lipase adsorption capacity and therefore a higher activity per monolith volume. The activity per gram of lipase is constant at a value of around 0.9 mmol/g_{lipase} s, which corresponds to a turnover frequency of 35 s^{-1}.

Typical concentration profiles of a performance test with CNF based carriers are given in Fig. 4. The reactant concentrations of two tests, performed under the same conditions, are given. The tests were performed at an interval of several weeks, in which the catalysts were used repeatedly. From these results it becomes clear that no deactivation is observed during repeated testing over a period of several weeks.

The stirrer rate was varied between 50 and 400 rpm to check for any mass transfer limitations. The results for the

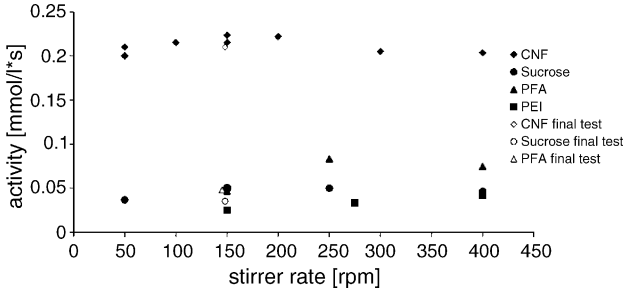

Fig. 5. Effect of stirrer rate on the activity of CNF (◆), Sucrose (●), PFA (▲) and PEI (■) based biocatalysts.

different carbon types are plotted in Fig. 5. The absence of any influence of stirrer rate indicates the absence of external mass transfer limitations. This was checked by calculating the Carberry number, the ratio of the observed rate and the maximum mass transfer rate. Under these conditions, Ca $\ll$0.05, so no external mass transfer limitations are present.

After each run, the monolithic stirrer was replaced by a normal stirrer, and fresh reactants were added to check if any enzyme had desorbed during the test, but no activity could be detected; apparently the lipase does not desorb from the support under the chosen reaction conditions. To investigate the stability of the catalysts, they were tested one more time after completing the series of stirrer rate variation. The time between the first and last test was approximately 2 weeks. In Fig. 5, these runs are represented by open marks. No decrease in activity could be detected. Hence, the biocatalysts are stable for at least 2 weeks, under repeated catalytic testing.

For comparison, a commercial immobilized lipase and free enzyme were also tested. The activity per unit enzyme for each biocatalyst is shown in Fig. 6a. Compared to the free enzyme, the activity of the immobilized lipase is significantly lower. But as stated before, immobilizing the

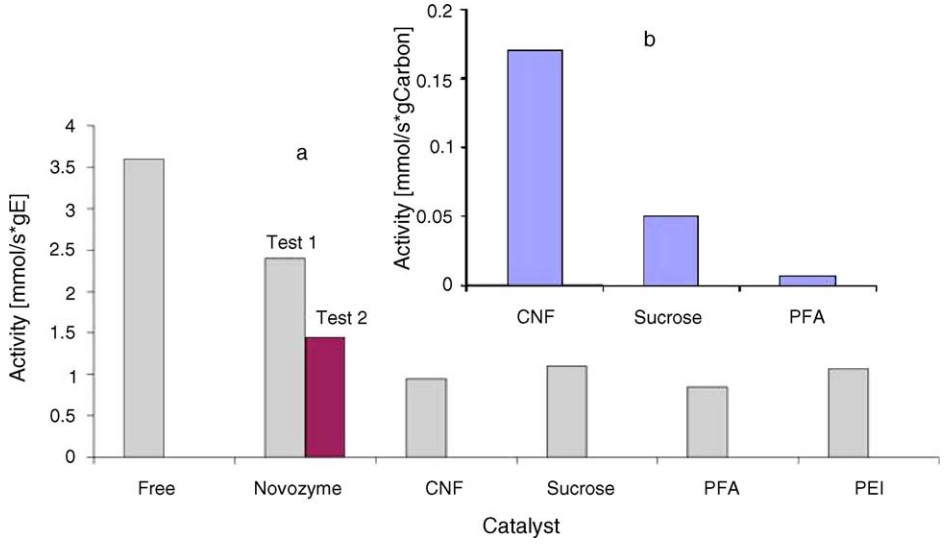

Fig. 6. Comparison of the activity of different biocatalysts (a) per gram of enzyme; (b) per gram of carbon.

enzyme enhances stability and facilitates easy catalyst separation and reuse of the enzyme. Moreover, the free enzyme tends to stick to the wall of glass vessels when used in organic media, deteriorating the handling of the catalyst. In general, the use of a more stable immobilized catalyst is preferred over the use of free enzyme. Compared to the commercial immobilized lipase, the activity of the monolithic biocatalysts is also relatively low, but apparently the Novozyme deactivates much faster. In the second run the activity is only slightly higher than that for the monolithic biocatalysts, for which no deactivation was found in subsequent runs. For the Novozyme, it was not checked if the deactivation was caused by leaching of enzyme from the support or by instability of the support matrix in this combination of solvent and products. To compare the different carbon carriers, the activity per gram of carbon is plotted in Fig. 6b. As expected, the specific activity of the CNF exceeds the activity of the other carbon carriers.

4. Conclusions

Several monolithic enzyme biocatalysts were prepared and characterized. For all prepared biocatalysts, satisfactory immobilization and activity was observed. The carbon nanofiber-based catalysts performed best. They showed the highest activity per volume of catalyst and per gram of carbon, without enzyme leaching. The catalysts are stable for several weeks, without any significant loss of activity. Compared to the commercial immobilized lipase and free enzyme the activity of the monolithic biocatalysts is relatively low, but the Novozyme shows significant deactivation upon reuse. The monolithic stirrer reactor is a useful tool for comparing the catalytic performance of different monolithic immobilized enzyme systems.

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