Immobilization of porcine pancreatic lipase in zeolite MCM 22 with different Si/Al ratios

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ABSTRACT
The use of zeolites as support for immobilization of enzymes has been a matter of great interest recently due to the potential properties of these materials. In this work, the MCM 22 zeolite with different Si/Al ratios (15, 25 and 50) was used as support for immobilization of porcine pancreatic lipase, assessing the effect of its structure on the immobilization yield and enzyme activity. Results showed that the material composition influenced significantly the immobilization process. Higher yields of immobilization and enzymatic activities were achieved when MCM 22 with Si/Al ratio of 25 was used as support. In general, results demonstrate the potential of MCM 22 as support for porcine pancreatic lipase immobilization.

1. Introduction

Recently, the use of zeolites as support for enzymes immobilization has been a subject of growing interest due to the potential properties of this class of materials [1–6]. Zeolites are microporous materials that present an important role in several technological areas [7,8], mainly due to their high specific area, adsorption ability and presence of active acid centers [9]. These compounds are crystalline aluminosilicates with a structure based on the tridimensional combination of tetrahedrons TO4 (T = Si, Al), linked by oxygen atoms. The zeolite MCM 22 has a precursor of lamellar structure, constituted by lamellas of 25 Å of thickness with only one system of pores formed by sinusoidal channels, constituted by opening rings of 10 members (10 MR). After treatment at 540 °C (calcination of the material), a tridimensional structure (MWW) is obtained, producing a second system, limited by pores of 12 member rings (12 MR) [10]. Fig. 1 presents a typical schematic diagram of these structures. The aluminosilicate MCM 22 presents high specific area, thermal stability and acidity, desirable characteristics for use as support for immobilization of biomolecules.

Lipases catalyzing lipid modifications have attracted considerable attention over the last years. The attractive aspects of this catalyst over chemical methods include the high specificity of some lipases, the mild conditions required for the reactions to take place, thereby requiring minimal energy inputs, reduced levels of by-products generated during the reaction and more efficient conversion of thermo sensitive substrates. Lipases catalyze hydrolysis of long chain, insoluble triglycerides and other insoluble esters of fatty acids with varying chain length specificity. The ability of catalyzing reactions also in micro-aqueous ambient make these catalysts largely used to produce esters from fatty acids and alcohols. At this point, it is worth to mention that lipases are also able to catalyze reactions of transesterification, making possible their use as catalyst for reactions of strong scientific, technological and economical point of view, such as production of biodiesel and emulsifiers for food industries [11].

Despite the potential of lipases as biocatalysts for reactions of interest, the high costs for application in industrial processes are related to their reduced stability under adverse conditions. Thus, the enzyme stabilization is a quite desirable process from an economical point of view, as lipases in soluble form can lose the catalytic activity in a batch system, making difficult their reuse and their application as catalyst in continuous mode. Also, the presence of residual enzyme in the products of the reaction may represent an undesirable contamination. The immobilization of biocatalysts...
inert supports (without damage to the enzyme activity) can ensure the use for several batches, resulting in economy for industrial processes. The main advantages of immobilized enzymes are related to their higher stability and easy separation from the reaction medium. The highest contribution for the good performance of immobilized catalyst is provided by the strategy employed for immobilization [12] and by the characteristics of the support.

Based on the above mentioned aspects, the aim of this work was to evaluate the use of zeolites MCM 22 with different Si/Al ratios as support for immobilization of porcine pancreatic lipase.

2. Experimental

2.1. Enzyme

Lipase (EC 3.1.1.3, crude, from porcine pancreas) was purchased from Sigma–Aldrich (São Paulo, São Paulo, Brazil) and used for immobilization in MCM 22 support. The enzyme preparation used is a lyophilized powder. The enzyme is soluble in organic solvents and has optimum pH varying from 6.5 to 7.5.

2.2. Supports

The zeolites MCM 22-15, MCM 22-25 and MCM 22-50, with Si/Al ratios of 15, 25 and 50, respectively, were synthesized following the methodology described in the literature [13,14]. Table 1 presents the gel composition, temperature and time used for preparation of lamellar precursors of MCM 22.

2.3. Characterization of the supports

Table 1 Gel composition, temperature and time used for preparation of lamellar precursors of MCM 22.

| Si/Al | 15  | 25  | 50  |
|-------|-----|-----|-----|
| SiO₂/Al₂O₃ | 30  | 50  | 100 |
| OH/SiO₂ | 0.11| 0.10| 0.10|
| Na/SiO₂ | 0.18| 0.14| 0.18|
| R/SiO₂ | 0.50| 0.35| 0.50|
| H₂O/SiO₂ | 45  | 45  | 45  |
| Temperature (°C) | 150 | 150 | 135 |
| Time (days) | 7   | 9   | 11  |

2.4. Experimental procedure for lipase immobilization

The lyophilized preparation was solubilized in sodium phosphate buffer (0.05 M, pH 7.0) (2 g of the enzyme and 60 mL of the buffer) and submitted to preferential immobilization by physical adsorption on each support presented before. Enzyme immobilization was performed at an enzyme to support mass ratio of 2:1. Immobilization was performed with magnetic stirring in an ice cooler and aliquots were sampled periodically until 120 min for protein content measurement. The supernatant and carrier with enzyme were also assayed for measurement of esterification activity, following the methodology described in the next section.

2.5. Analytical methodology

2.5.1. Protein content and yield of immobilization

The protein content in the inlet and outlet solutions was measured by the methodology proposed by Bradford [15], using serum bovine albumin as standard. Samples were analyzed in spectrophotometer (Agilent Technologies 8453, Santa Clara, California, USA) at 595 nm and the protein content was determined by Eq. (1).

\[
\text{protein} = \frac{\text{Abs.}}{\text{Abs.}_{\text{d}} \times 10^d}
\]  

(1)

The yield of immobilization was calculated as follows, 

\[
\eta(\%) = \frac{P_a}{P_o} \times 100
\]  

(2)

where \(\eta\) = yield (%), \(P_a\) represents the amount of protein adsorbed, \(P_o\) denotes the amount of protein used in the immobilization (inlet solution).

2.5.2. Lipase esterification activity

The enzyme activity was determined as the initial rates in esterification reactions between oleic acid and ethanol at a molar ratio of 1:1 using 0.4 g of enzyme (immobilized in each support tested or in its free form). At the beginning of the reaction, samples containing the mixture of oleic acid and ethanol were collected, and the oleic acid content was determined by titration with 0.04 M NaOH. After the addition of the enzyme to the substrates, the mixture was kept at 40 °C and 150 rpm for 40 min. Then, the oleic acid consumption was determined [16]. One lipase activity unit (UE) was defined as the amount of enzyme necessary to consume 1 μmol of oleic acid per minute at the established experimental conditions presented previously. All enzymatic activity determinations were replicated at least three times. Results presented here are in fact mean values of the measurements performed. Standard errors lower than 5% were obtained in all determinations.
3. Results and discussion

3.1. Characterization of the supports

Fig. 2(a) presents the X-ray diffractograms of the precursors of MCM 22 with different Si/Al ratios and Fig. 2(b) the diffractograms of the materials after calcination. From this figure, one can observe that the bands assigned as (0 0 1) and (0 0 2) enhance for materials with higher Si/Al ratio, due to an enhancement of the crystallinity. After calcination, an increase of crystallinity is observed as a result of the formation of tridimensional phase of MCM 22.

Table 2 presents the chemical and textural analyzes of the supports, respectively. The specific area BET ($A_{BET}$) corresponds to the total area of the material, calculated by the BET method.

![Figure 2](image1.jpg)

![Figure 3](image2.jpg)
The area representative of micropores ($A_{\text{micro}}$) is obtained by t-plot method. The difference between these values corresponds to the contribution of external area ($A_{\text{ext}}$), which includes meso- and micropores. The total volume ($V_{\text{total}}$) is the volume adsorbed until $P/P_0 \sim 0.99$ and the micropore volume ($V_{\text{micro}}$) is also calculated by t-plot method. The volume of mesopores (from 17 to 300 Å) is obtained by BJH method. From Table 2 one can note a mean specific BET area of 450 m$^2$/g, about 310 m$^2$/g corresponding to the microporosity. As the Si/Al ratio increases, an enhancement on microporosity and reduction of $A_{\text{ext}}$ is observed, due to the increase of crystallinity and length of crystals, also observed by MEV (data not shown).

3.2. Yield of immobilization

Fig. 3 shows the kinetic of adsorption of commercial lipase in zeolites (a), protein content in the inlet and outlet solutions (b), yield of immobilization (c) and esterification activity of the immobilized lipase (d). The difference in protein content of inlet solutions observed in this figure is probably associated to variations on the protein for porcine pancreatic lipase [17]. The solution with higher initial enzyme concentration resulted in higher adsorption by the support. The amount of adsorbed protein by MCM 22-25 was about 4 times higher than for other supports, leading to higher immobilization yield when this support was used. From Fig. 3(b) one can observe that higher adsorption was verified from 90 to 120 min.

The immobilization process can be directly influenced by the chemical composition of the support, in this case by the Si/Al ratio, and indirectly by the acidity of the surface. An enhancement in the amount of enzyme immobilized was observed for lower Si/Al ratio, comparing samples using MCM 22-50 and MCM 22-25. However, when the acidity is increased it was not observed an increase in the yield of immobilization, probably due to the occurrence of the effect of buffering of the pores, hiding the diffusion of the protein in the structure of the material. With the increase of acidity, the acid sites are next to the pores, immobilizing the enzyme and buffering the pores, reducing the amount of enzyme adsorbed in the support. In this way, one can conclude that, aiming at the efficiency of the immobilization, materials with intermediate acidity (in this specific case, MCM 22 Si/Al 25) can be considered more adequate.

3.3. Esterification activity

Fig. 3(d) presents the esterification activities of free and immobilized lipases. Higher activities were obtained for lipase supported in MCM 22-25 (378.75 U/g). MCM 22-15 and MCM 22-50 presented good yields and amount of lipase adsorbed, but lower esterification activities, of 86.71 and 231.27 U/g, respectively.

After the immobilization process, the mass transfer of the enzyme to the substrate can be reduced, due to the difficult of substrate to achieve the active site of the enzyme [18]. However, from Fig. 3(d) one can affirm that the use of zeolite MCM 22 as support for immobilization of porcine pancreatic lipase, mainly that with Si/Al ratio of 25, led to promising results, since good esterification activities were obtained for the immobilized lipase, compared to the free one. One can also observe that the support used here can be considered a low cost one compared to the most usable supports (ion exchange resins, for example) presented in the literature for lipases immobilization.

4. Conclusions

Results showed the potential of application of MCM 22 with different Si/Al ratios as support for lipases immobilization, as higher esterification activities were obtained for MCM 22-25, explained by the chemical compositions of the material. The increase in pH values (related to the Si/Al ratio) leads to higher yields of immobilization, reaching a maximum value for Si/Al ratio of 25. Materials with low Si/Al ratio cause buffering of the pores, leading to lower amounts of protein adsorbed and hence lower esterification activities.

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