Modification and characterization of Clinoptilolite for the co-immobilization of Formate dehydrogenase and Glycerol dehydrogenase enzymes

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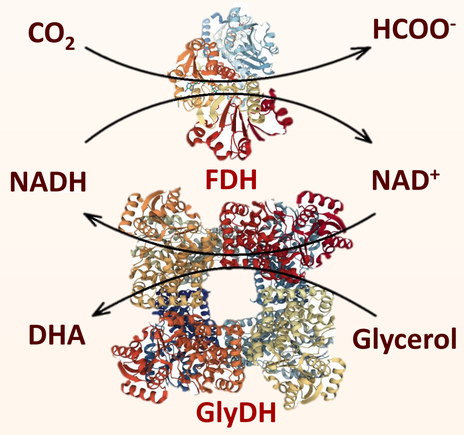
1. Introduction

A sequence of three different enzymatic reactions (catalyzed by FDH, FalDH and ADH) can be employed to convert CO_2 to methanol in atmospheric conditions[1]. The immobilization of enzymes on zeolites allows to re-use them with a consequent reduction of cost. Moreover, the immobilization will increase enzymes stability[2].



Formate dehydrogenase (FDH) was co-immobilized with Glycerol dehydrogenase (GlyDH) (Fig. 1), used for regenerating the cofactor NADH, on Clinoptilolite and modified Clinoptilolite (M-Clinoptilolite), this last one was subjected to dealumination-desilication treatment[3].

Fig. 1 Schematic representation of the two reactions simultaneously catalyzed FDH and GlyDH

2. Methodology

Dealumination-desilication treatment

This treatment has the purpose of modifying the zeolite morphology.
- Dealumination: HCl 1 M at 100 °C for 4 h
- Desilication: NaOH 0.2 M at 100 °C for 30 min

Characterization of Clinoptilolite and modified Clinoptilolite

Characterization analyses:
- X-Ray diffraction
- N_2 physisorption at -196 °C
- FESEM

Functionalization of support

Supports were functionalized with amino and glyoxyl groups to immobilize the enzymes.

Immobilization of enzymes

Reaction between support and enzyme at T = 4 ° C and pH = 7.
Bradford assay was carried out to measure the amount of protein.

Stability and activity tests of enzymes

Activity was correlated to the change in absorbance at 340 nm following the NADH produced by the biocatalyst.

3. Results & Discussion

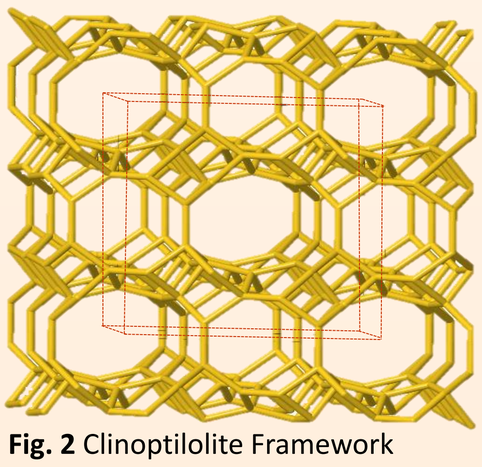


Fig. 2 Clinoptilolite Framework

Clinoptilolite framework (Fig. 2) is composed by two different parallel channels with eight and ten membered-rings. These channels are interconnected by eight membered-rings line-up along a-axis[4].

Clinoptilolite (Fig. 3a) appears to be composed by anhedral particles[5]. This feature seems to be maintained afterwards the dealumination-desilication treatment, but the particles are sharper (Fig. 3b).

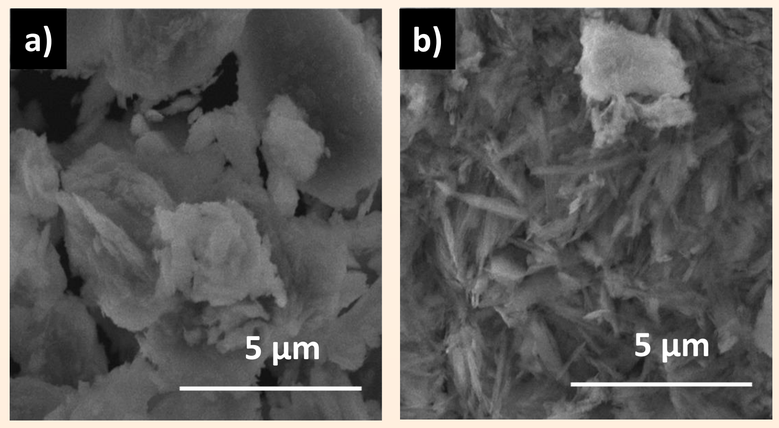


Fig. 3 FE-SEM images for Clinoptilolite (a) and M-Clinoptilolite (b)

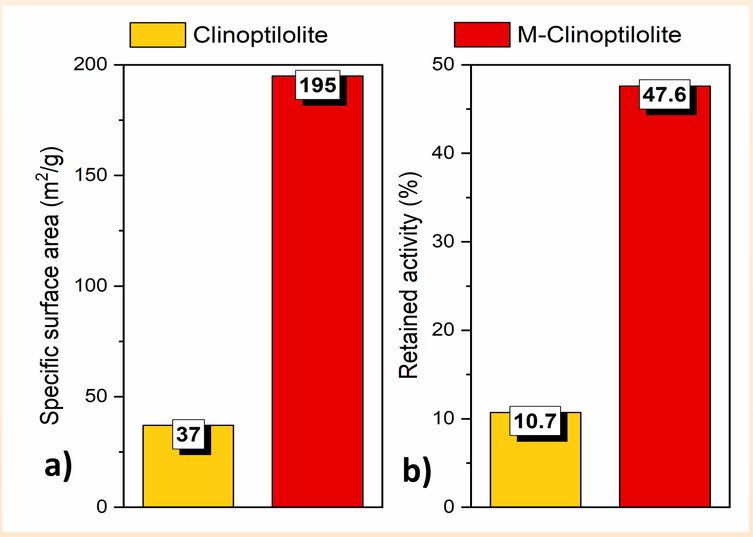


Fig. 4 Specific surface area of Clinoptilolite before and after the dealumination-desilication treatments (a) Retained activity of FDH immobilized on Clinoptilolite and M-Clinoptilolite (b)

In Fig. 4a the specific surface area of Clinoptilolite and M-Clinoptilolite are compared. The subsequent acid and alkaline attacks have successfully modified the morphology of the natural zeolite increasing its specific surface area of 427 %.
The modification of Clinoptilolite results being very effective for the immobilization of FDH (Fig. 4b) in fact, its Retained Activity is much higher (445 %) when supported on M-Clinoptilolite. In addition, the immobilization on M-Clinoptilolite has positive effects on the thermal stability too.

Finally, the co-presence of the two enzymes, FDH and GlyDH, on Clinoptilolite was confirmed by optical fluorescence microscopy.

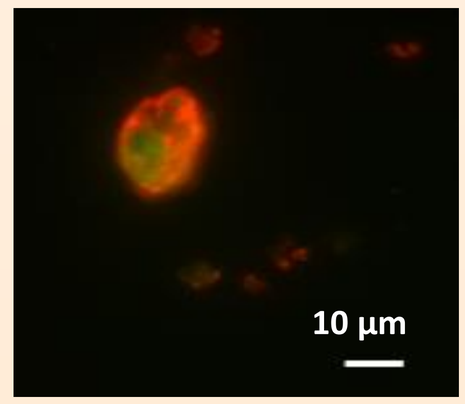


Fig. 5 Optical fluorescence microscopy images of Clinoptilolite with ATTO-488 labelled FDH enzyme in green channel and ATTO-550 labelled GlyDH enzyme in red channel

4. Conclusions

A dealumination-desilication treatment was performed on a natural zeolite with the aim of enhancing its properties as support for FDH used as catalyst for the reduction of CO_2. The increased surface area obtained with the dealumination-desilication process enhances the Retained Activity of the immobilized enzyme. In addition, the immobilization of the enzyme on the zeolite increased its thermal stability, as well as allowing to reuse it.

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