


<p>P-010 Lipases in hierarchically structured montmorillonite.</p> <p>Kuncova G^{*1}, Sabata S.¹, Kucerova L.², Fuzik T.³, Duchek P.² ¹Institute of Chemical Process Fundamentals ASCR, v.v.i., Prague, 16502, Czech Republic ²The University of West Bohemia, Pilsen 30614, Czech Republic ³Institute of Chemical Technology Prague 16628, Czech Republic *kuncova@icpf.cas.cz</p>	
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INTRODUCTION AND OBJECTIVES

Structurally organized inorganic materials offer the desirable combination of high internal reactive surface area, produced by narrow nanopores and facile molecular transport possible through broad highways leading to and from these pores. The incorporation of enzymes within such organized materials with full preservation of their native structures would result in a biocatalyst with increased level of spatial organization.

The widely used strategy, which provides control of the scale and degree of porosity in inorganic materials, is template-directional assembly. This technique consists of combination of nanoparticles with organic template. The dimension of template; molecule, supramolecular or colloid, determines dimension of a pore (micro-, meso- or macro-) in the final porous material. Template removal, usually by thermal or dissolution treatment yields in hierarchically organized material with a structure that is replica of original template. The application of this process is widespread however there are only few examples of hierarchical enzyme biocatalysts. This is because chemicals used as templates and/or processes of their removal are detrimental to the enzymes.

Ice-segregation-induced self-assembly (ISISA) is a biocompatible process often used for preparation of hybrid materials containing polymer networks and inorganic nanoparticles. The process involves cooling down of an aqueous gel or sol to low temperatures by immersion into liquid nitrogen or dry ice + ethanol bath. The rapid ice formation causes every solute originally dispersed in the aqueous gel to be segregated from the ice phase, giving to rise to a hierarchical assembly characterized by fences of solid matter enclosing empty areas where the ice originally residues (Gutierrez 2008).

Silicates have been widely studied as lipase carriers due to their high specific surface and chemical inertness. Clay minerals are layered silicates with good intercalating properties. Metal, hydroxyl, polymeric cations, alkylammonium ions, polymers, and their combinations have been intercalated into phyllosilicates to form a broad spectrum of materials ranging from pillard clays and organo-clay, to polymer-clay nanocomposites. The intercalated phyllosilicates exhibit good mechanical and thermal stability, controlled pore size and high adsorption capacity (Fuentes 2001).

The aim of this work was to prepare highly active and mechanically stable lipase biocatalyst with hierarchically structured clay carrier.

MATERIALS AND METHODS

Preparation of modified montmorillonite MM

Montmorillonite (Cloisite Na⁺, Southern Clay products USA) was modified with triethoxyoctadecylsilane (OCT). Water ethanol (1:3) suspension of Cloisite Na⁺ was mixed with OCT, 2 hours at 25°C and 5 hours at 50°C and filtrated. The modification of Cloisite Na⁺ with aliphatic chain was confirmed with ¹³C NMR solid state spectroscopy.

Lipase adsorption on MM

Commercial lipases from *Thermomyces lanuginosa* Lipolase 100L (25 mg_{protein}/ mL) (Aldrich) was adsorbed on suspended MM, by described procedure (Hetflejs 2006) to form adsorbates pastes (MMA) that contained 10% of dry mass.

Preparation of ice-templated biocatalyst

MMA pastes, were shaped by three procedures:

- D) The paste was dropped from syringe into liquid N₂ and freeze dried .
- I) The paste was filled into syringe (1 mL or 2 mL) and immersed into liquid N₂ (5 mm/min). After 24 hours storage at -30°C the filled syringes were freeze-dried.
- IP) The paste was mixed with PVA (10% solution) in weight ratio 1:1 or 3:1, filled into syringe (1 mL) and immersed into liquid N₂ (5 mm/min). After 24 hours storage at -30°C the filled syringes were freeze-dried.

Sample characterization

Interlayer distance of MM was determined by X-ray diffraction (XRD) using X'Pert PRO MRD diffractometer (PANalytical B.V). Biocatalyst structures were investigated by SEM (Vega 3 XM Tescan) and scanning confocal optical microscopy (OLYMPUS LEXT OLS 3000).

Enzyme activity test

The new biocatalysts were applied as catalyst of esterification of stearic acid with propanol in flasks at 40°C, 4 hours (Hetflejs 2006).

RESULTS AND DISCUSSION

Structure of OCT modified Cloisite Na⁺ (MM)

The interlayer spacing 12.6 Å as measured for the hybrid is slightly increased compared to the sodium montmorillonite (Cloisite Na; 12.07 Å). Dimensions of (triethoxysilyl)- group itself amount for more than 9 Å, it is very bulky for the determined gallery expansion. Thus, it can be concluded that the organosilicon species does not intercalate but only hydrophobizes the mineral surface, which was described elsewhere (Song 2001)

The biocatalysts structure and activity

The dry droplets of biocatalyst (preparation D)) consist of sheets oriented from centre to surface. The sheets are interconnected with ribs forming pores with average diameter of 1 µm (Fig 2). The structures of directionally frozen ice-templated MMA biocatalysts are on Fig. 3. These biocatalysts have contained deep unidirectionally oriented pores dimensions of 10 - 100 µm separated with ridges of biocatalyst with ten times smaller pores. The dimension and shape of pores have been dependent on composition and velocity of freezing.

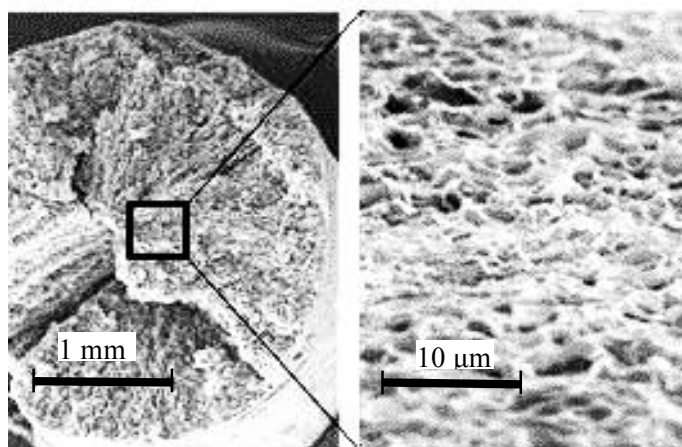


Figure 2: SEM of a droplet of MMA with adsorbed Lipolase 100L

Conversion of stearic acid catalysed with free lipase was 7 mg/mg_{protein}. Hierarchical structure and pore orientation by unidirectional freezing in syringes increased the activity of the lipase biocatalysts up to 17 mg/mg_{protein} (Table 1).

Table 1 :The influence of MMA structure with Lipolase 100L on esterification of stearic acid in hexane

Preparation	D	I	IP
Protein adsorbed on dry carrier (mg/g _{carr})	187	187	192
Conversion of stearic acid (mg _{SA} /mg _{protein})	13	17	15

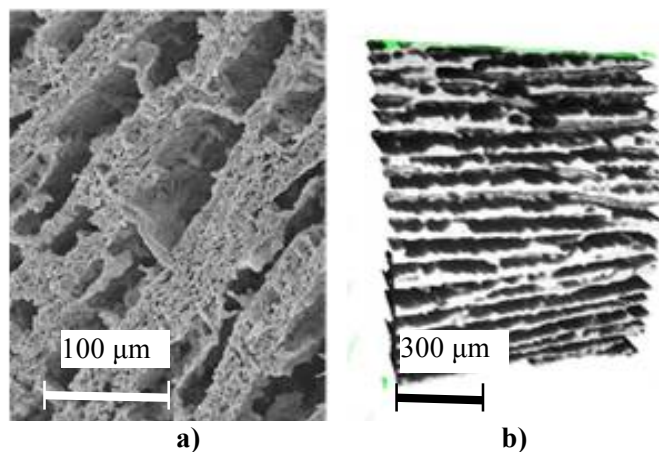


Figure 3: Directionally frozen MMA. a) SEM of cross-section (preparation I), b) OM of a surface of MMA+PVA (3:1) monolith (preparation IP)

The incorporation of PVA led to pore enlargement and the slight improvement of toughness.

CONCLUSIONS

Air-drying of MMA paste results in a powder like biocatalyst that can be used only in flask in laboratories and the activity is dependent on mixing. Hierarchically oriented biocatalysts prepared by directional freezing and freeze-drying can be easily reused and applied in column reactors. In addition to that biocatalyst monolith with pores oriented preferentially in one direction can serve as miniature flow-through bioreactor with potential application in analytical and pharmaceutical chemistry.

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