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HETEROGENEOUS ENZYMATIC CATALYSTS: COMPARING THEIR EFFICIENCY IN THE PRODUCTION OF BIODIESEL FROM ALTERNATIVE OIL

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Abstract

In this study, four heterogeneous enzymatic catalysts were synthesized: three from the immobilization of *Pseudomonas fluorescens* lipase (L_{Pf}) on SBA-15, Ca/SBA-15, and Na / SBA-15, and one using the one-step coprecipitation technique, called LOBE (Low Ordered Biosilicificated Enzyme). The physicochemical properties of these materials were determined by small-angle X-ray scattering (SAXS) and Fourier Transform infrared spectroscopy (FT-IR). The biocatalysts activity was evaluated in the production of biodiesel with different oily raw materials. It was possible to infer from these results that besides enzyme-metal-support synergistic effect (L_{Pf}/Ca/SBA-15 or L_{Pf}/Na/SBA-15), confinement effects influence the substrates diffusion or mass transfer depending on the pore, channel, or cavity architecture, determining the catalytic efficiency. While the SBA-15 material presents one-dimensional channels, the LOBE biocatalyst has interconnected three-dimensional cavities that favor the mixing of reactant phases (oil-alcohol) and interaction with active sites. This characteristic would increase the specific activity of the LOBE biocatalyst approximately five times concerning the other studied biocatalysts, depending on the raw material used.

Results and discussion

In this work, four enzymatic heterogeneous catalysts were used for biodiesel production. Three of them were obtained by the physical adsorption of the lipase on the pure synthesized material SBA-15 or the metal modified materials with sodium or calcium according to [5,14,16]. They were denominated LPS/SBA-15, LPS/Na/SBA-15 or LPS/Ca/SBA-15, respectively. The fourth hybrid biocatalyst was obtained by enzymatic mineralization with an organic silicic precursor according to [12]. This technique, the biosilicification [17], provided the enzymatic heterogeneous catalyst in only one step denominated “Low Ordered Biosilicified Enzyme” (LOBE).

The chemical environment in which a peptide or protein exists influences its structure and stability. For this reason, the FT-IR technique was used to determine the structural characterization and the presence of proteins on the enzymatic heterogeneous catalysts [18]. The characteristic functional groups of free *Pseudomonas fluorescens* lipase can be observed in Figure 1a. The presence of the amide I and amide II bands indicates that the enzyme secondary structure and bioactivity are conserved in the formed nanostructures [22].

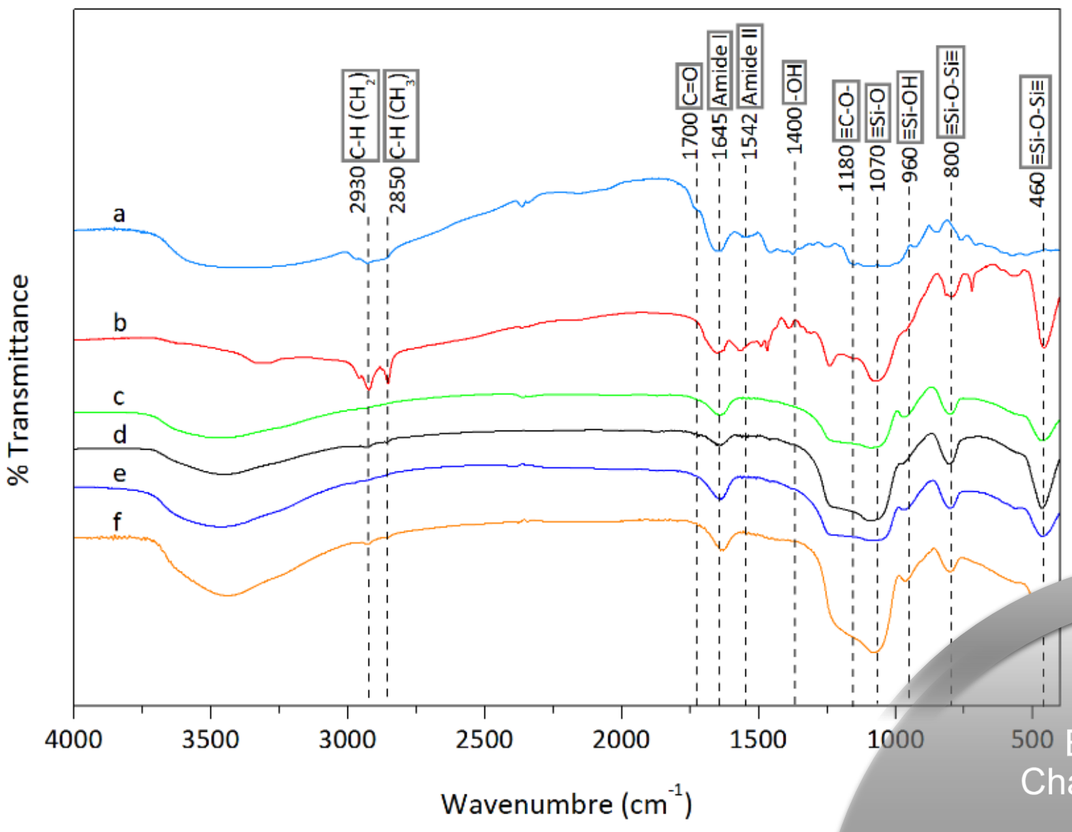


Figure 1: FT-IR spectra of lipase immobilized on the different supports: a) free lipase, b) LOBE, c) L*Pf*/SBA-15, d) L*Pf*/Na/SBA-15, e) L*Pf*/Ca/SBA-15 and f) SBA-15.

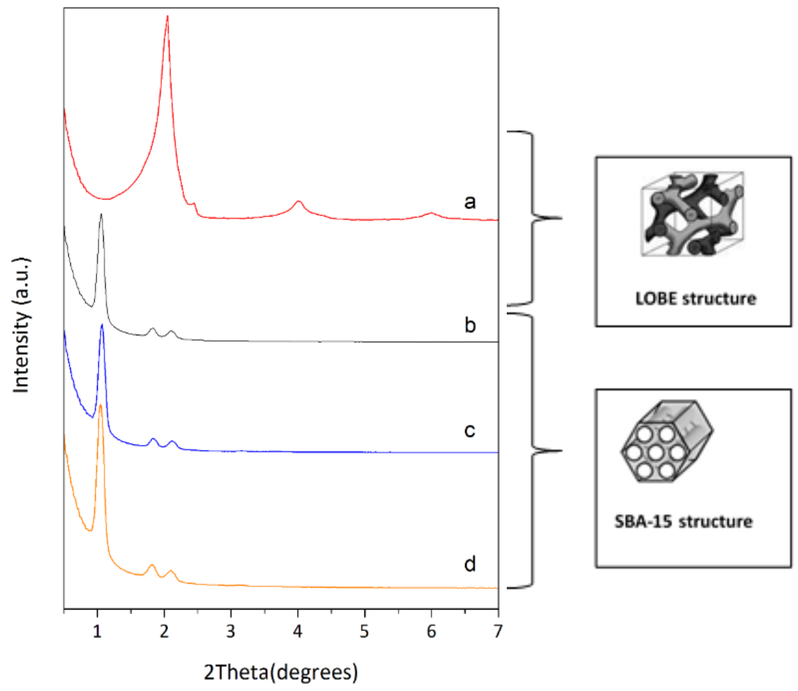
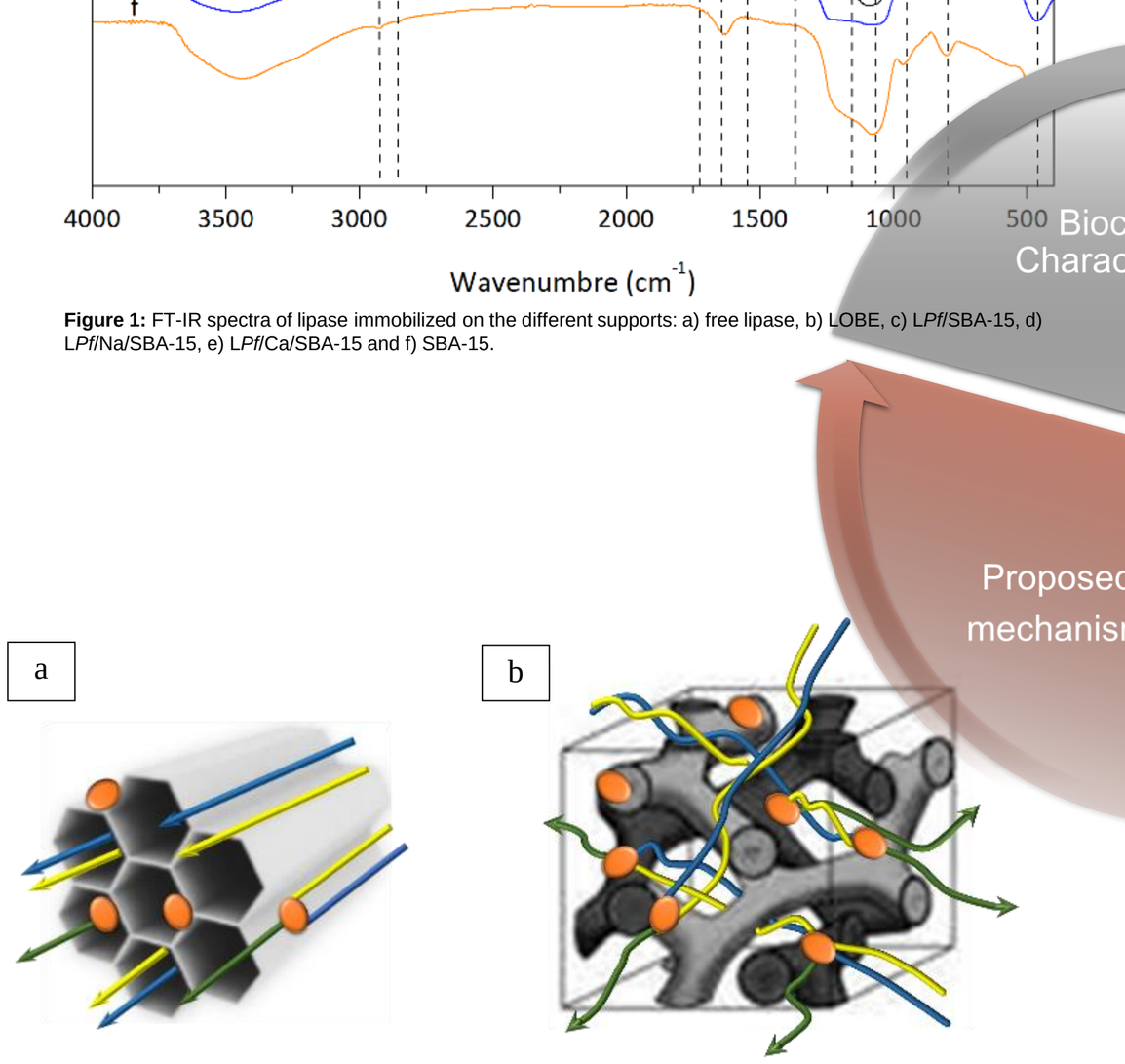
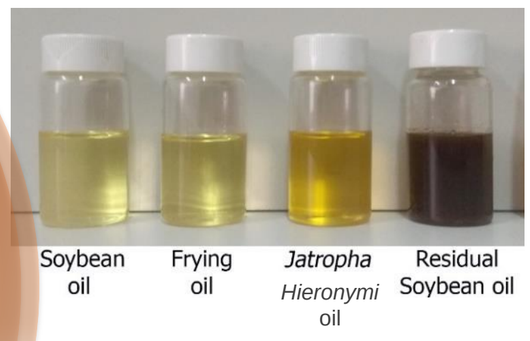


Figure 2: Small-angle X-ray scattering patterns of: a) LOBE, b) Na/ SBA-15, c) Ca/ SBA-15 and d) SBA-15.

To reach an adequate comparison between the different obtained enzymatic heterogeneous catalysts it is key analyzing the nature of the materials used to immobilize the enzyme, measuring both structural and texture properties. Thus, Figure 2 b-d shows SAXS patterns of the pure SBA-15, Ca/ SBA-15 and Na/SBA-15, which present three well-resolved peaks, corresponding to the diffraction of planes (1 0 0), (1 1 0), and (2 0 0) characteristic of the SBA-15 structure. These reflections are typical of a hexagonal ordered and unidimensional pore arrangement [10]. Meanwhile, the LOBE presents a different pattern, with two maxima peaks assigned to (2 1 1) and (2 2 0) reflections. These reflections and the ratio value d220/d2110 (~0.87) are consistent with a tridimensional cubic structure similar to MCM-48 [29] (Figure 2d).

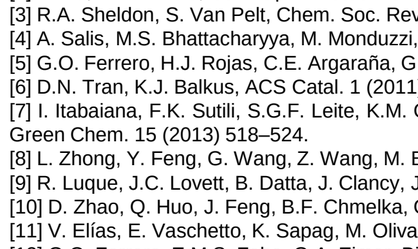




To analyze the performance of biocatalysts with different raw materials, the specific activity of the biocatalysts was determined using soybean oil, waste frying oil, acid oil from soybean soapstock, and Jatropha *hieronymi* oil. Soybean oil is one of the main oils to produce biodiesel in Argentina [23] while the used frying oils, which have low value as food but high energy content, are a domestic and gastronomic industry scrap. Then, biodiesel production from waste frying oils could be a sustainable alternative to reduce the price of biofuel [24]. The acid oil from soapstock is a side-product generated during soybean oil purification. This contains a large amount of free fatty acids (50-80wt% of FFA approx.), and a mixture of phospholipids, tocopherols, sterols, degraded and oxidized components, pigments, salts, color bodies, triglycerides, diglycerides, and monoglycerides in a minor proportion [25]. Converting this acid oil into biodiesel could give it greater added value.

On the other hand, *Jatropha hieronymi* is an endemic and non-conventional oilseed species from the semiarid and arid northwest of Argentina with an oil concentration of approx. 36 wt%. This oil has a 4,07 wt% of FFA and is presumably toxic [24,25]. Then, it does not represent competition with agricultural food crops and diversifies farmland. For these reasons, it also has economic potential as an alternative feedstock to produce biofuels [25].

a



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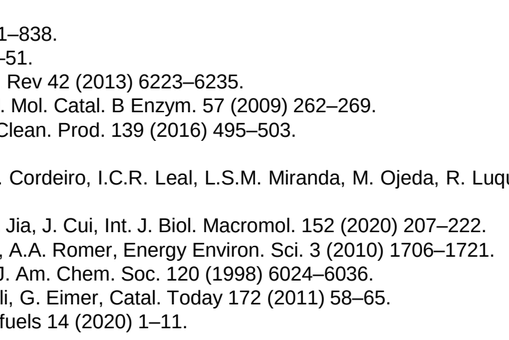

Figure 3. Schematic representation of substrate mixtures and interaction with lipase immobilized on different supports: a) SBA-15, b) LOBE. Yellow flux: oil, light blue flux: alcohol, green flux: biodiesel, orange: immobilized lipase enzyme.

Table 1. Comparison of the biocatalysts specific activity for the different raw materials used.

	Raw material	L_{PF}/SBA-15	L_{PF}/Ca/SBA-15	L_{PF}/Na/SBA-15	LOBE
Specific activity (U/g lipase)	Sunflower oil	0,92	1,24	1,24	7,29
	Soybean oil	0,88	1,14	1,00	6,51
	Frying waste oil	0,94	1,20	1,23	6,41
	Acid oil from soapstock	0,64	0,88	1,02	6,85
	J. Hieronymi oil	0,99	1,06	1,16	6,55

Conclusions

In this work, it was demonstrate how the structure of the material where the enzyme is supported influences its activity. During the biodiesel production reaction using heterogeneous catalysts, three phases must come into contact: oil, alcohol, and biocatalyst. This fact makes the interaction between the phases difficult, and consequently, longer reaction times are needed. According to the results herein presented, the interaction between substrates and mass transfer is favored by the chaotic flow produced by the structure of the LOBE, leading to a specific activity much higher than that of SBA-15, where only one-dimensional flow is possible. That is why the LOBE biocatalyst has reaction rates much higher than those of the rest of the biocatalysts.

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