catalysts

MDPI

Supplementary Material

Composites of cross-linked aggregates of Eversa® Transform and magnetic nanoparticles. Performance in the ethanolysis of soybean oil

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Supplementary Material

1. Adsorption profile of Eversa Transform 2.0 on magnetic nanoparticles functionalized with octyl and amine groups (SMNPs)

A solution was prepared containing PEI (section 3.4.3) and enzyme with a protein:PEI mass ratio of 1:1 in 5mM sodium phosphate buffer. This mixture was incubated at 25 °C and stirred in a 3D roller agitator (Kasvi, São José dos Pinhais, PR, Brazil) at 150 rpm. After 60 min, an aliquot of the enzyme solution was added to a SMNPs suspension to a SMNPs/enzyme mass ratio of 3:1, and 5 mM sodium phosphate buffer pH 7.0 was added to a final volume of 1 mL. Then, 3 mL of 5 mM sodium phosphate buffer pH 7.0 were added instead of ethanol (precipitating agent in the CLEA preparation). This mixture was incubated at 25 °C and stirred in a 3D roller agitator (Kasvi, São José dos Pinhais, PR, Brazil) at 150 rpm. A solution control was prepared under the same conditions, except for no addition of SMNPs. Hydrolytic activities (using tributyrin as substrate, section 3.2) were measured in the supernatant and enzyme control throughout the reaction. The residual activity in the supernatant was calculated according to the Eq. S1:

$$\text{Residual activity in the supernatant} = \frac{A_S}{A_{ci}} \times 100, \quad \text{(S1)}$$

where A_s is the activity of the supernatant and A_{ci} is the initial activity of the solution control.

For an adsorption assay of the enzyme non-treated with PEI, 5 mg of protein was offered to 1 g of SMPNs in 10 mL of 5 mM sodium phosphate buffer, pH 7.0. This mixture was incubated at 25 °C and stirred in a 3D roller agitator (Kasvi, São José dos Pinhais, PR, Brazil) at 150 rpm. The solution control was prepared under the same conditions, except for no addition of SMNPs. During the reaction, hydrolytic activities (using tributyrin as substrate, section 3.2) were measured in the supernatant and in the solution control throughout the reaction. The residual activity in the supernatant was calculated according to the Eq. S1.

The enzyme non-treated with PEI was fully adsorbed on SMNPs after 30 min reaction, while the enzyme treated with PEI was slowly adsorbed (Figure S1), but after 30 min reaction around 50% of the enzyme was adsorbed on the SMNPs, reaching a adsorption plateau of around 70%.

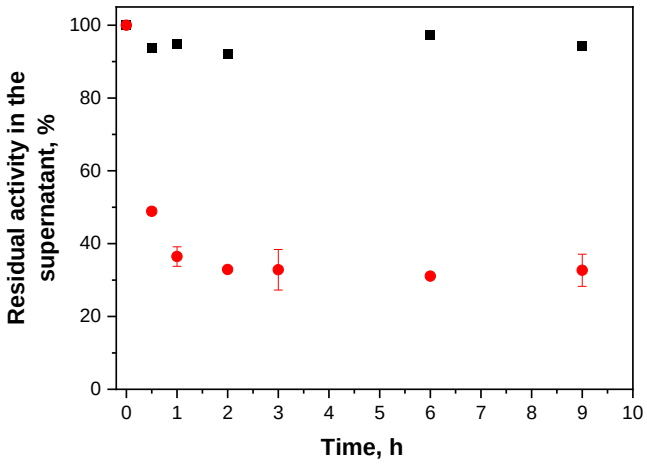

Figure S1. Adsorption profile of Eversa Transform 2.0 treated with polyethyleneimine (Section 3.4.3) on silica magnetic nanoparticles (SMNPs) functionalized with octyl and amine groups at 25 °C under 150 rpm stirring (3D roller agitator). Hydrolytic activities (using tributyrin as substrate, Section 3.2) were measured in a solution control (■) and in the supernatant (●).

2. Inactivation profile of liquid Eversa Transform 2.0

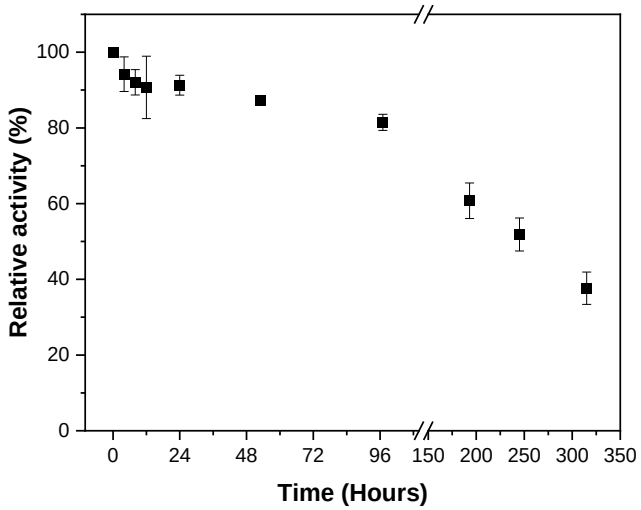

Figure S2. Profile of inactivation of liquid Eversa® Transform 2.0 at 60 °C and pH 7.0 (100 mM sodium phosphate buffer) using an enzyme concentration of 5 mg protein/mL. The activity of the enzyme initial solution was taken as 100%. Hydrolytic activities were measured with tributyrin as substrate (Section 3.2).

3. Water content in transesterification reactions of soybean oil with ethanol using liquid and magnetic CLEA of Eversa Transform 2.0 (Eversa-mCLEA)

Table S1. Experimental conditions (enzyme load and water content) for transesterification reactions using liquid and immobilized Eversa.

Biocatalyst	Eversa-mCLEA				Liquid Eversa	
Enzyme load (U_{est}/g_{oil})	4.0	7.0	7.0	12.0	7.0	7.0
Water (% w/w_{oil})	6.1[a]	6.9[a]	1.6[b]	2.8[b]	2.8[b]	6.5[a]

[a] Amount of water present in the reaction (water from the biocatalyst plus water added to the reaction).

[b] Reactions without adding water. The water present in these reactions comes from the biocatalyst.

Reaction conditions: 15 g refined soybean oil, oil/ethanol molar ratio of 1:6, 40 °C, 1700-2000 rpm stirring in a vortex flow reactor. Biocatalyst activity (esterification activity, U_{est}/g): liquid Eversa = 106.36, Eversa-mCLEA = 106.17. Water content determined by Karl Fischer method.