Electronic Supplementary Information

Sputtering deposition of magnetic Ni nanoparticles directly onto an enzyme surface: a novel method to obtain a magnetic biocatalyst

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Experimental Section

Deposition of nickel on the enzyme lipase by sputtering

The lipase from *Pseudomonas cepacia* and *p*-nitrophenylpalmitate (p-NPP, N2752-1G) were purchased from Sigma-Aldrich (St. Louis, USA). All other chemicals used were of analytical grade. The nickel target used for sputtering was from Angstrom Sciences, with 99.99% purity.

Around 5 g of the enzyme lipase were placed into a conical aluminum flask specially mounted inside the sputtering vacuum chamber. The chamber was evacuated to a base pressure of $4x10^{-6}$ mbar, and the enzyme powder was maintained at this pressure for 4 h. Next, by inserting argon the pressure in the vacuum chamber was increased to reach the sputtering working pressure ($4x10^{-3}$ mbar). During deposition, the enzyme powder was continuously homogenized by revolving the aluminum flask at a vibration frequency of 24 Hz. The Ni was sputtered onto the revolving lipase at 30 W for 5 min. After deposition, the chamber was ventilated with N_2 and the gray powder was stored for enzymatic tests.

Lipase hydrolytic activity

The enzymatic tests were performed by measuring the increase in absorbance at 410 nm in a visible spectrophotometer (Ultrospec 2000) caused by the release of *p*-nitrophenol after the hydrolysis of *p*-nitrophenylpalmitate (pNPP) at 40°C for 10 min. The reference was a control sample without enzyme. To initiate the reaction, 6 mg of the free lipase (*Pseudomonas cepacia*) or the enzyme decorated with nickel and 94 µL of citrate-phosphate buffer pH 7.0 were added to 0.9 mL of substrate solution containing 3 mg of pNPP dissolved in 1mL 2-propanol and 9 mL of reaction mixture (40 mg of Triton X-100, 10 mg of Arabic gum dissolved in buffer solution).[1, 2] The activity of the immobilized enzyme was measured in the low-density solution at 410 nm, after sedimentation by gravity. One unit of lipase (U) was defined as the amount of enzyme that releases 1 µmol p-nitrophenol.min^{-1} in the assay conditions described previously. The calibration curve was prepared using p-nitrophenol as the standard (1.0 µmol.mL^{-1}). All tests were conducted in duplicate and data regarding lipase activity were processed by central tendency (mean) and dispersion (standard deviation) measurements.

Biodiesel characterization

The HPLC experiments were performed on a Shimadzu LC-20A Prominence liquid chromatograph equipped with an Evaporative Light Scattering Detector (ELSD) and a four-solvent delivery system.

Sample injection volume of 20 µL and a flow rate of 1 $mL.min^{-1}$ were used in all experiments. All samples were dissolved in 2-propanol-hexane (5:4, v/v). All solvents were of HPLC grade and were used as obtained, without further purification.

A column Shim-Pack (C-18, 250 mm, 4.6 mm i.d.) was obtained from Shimadzu. HPLC method: reservoir A contained water, reservoir B contained acetonitrile and reservoir C contained 2-propanol- hexane (5:4, v/v). A 57 min ternary gradient with two linear gradient steps was employed: 30% A+70% B in 0 min, 100% B in 15 min, 50% B + 50% C in 30 min, followed by isocratic elution with 50% B + 50% C for the last 27 min.[3]

Lipase transesterification activity

In a 125 mL flask, soybean oil (0.05 g, 0.057 mmol), distilled water (10 µL) and methanol (275 µL, 0.685 mmol) were added to the free lipase (*Pseudomonas cepacia*) (0.2 g) or to the enzyme decorated with nickel (0.2 g). The reaction medium was stirred for 24 h at 55°C. After decantation, the phase containing the biodiesel was removed and analyzed by HPLC experiments[4]. All tests were conducted in duplicate and data regarding lipase activity were processed by central tendency (mean) and dispersion (standard deviation) measurements.

Biocatalyst reusability

The reusability of the lipase with nickel nanoparticles was determined by hydrolysis and transesterification assays as described above. After the reaction, the biocatalyst was recovered by the application of a magnetic field and used in another reaction. Reusability was tested for ten cycles for hydrolysis and five cycles for the transesterification reaction. The biocatalyst activity was compared with that in the first run (activity defined as 100%). The relative activity (%) was the ratio between the activity of each sample and the maximum activity of the sample (activity defined as 100%).

Characterization of the magnetic biocatalyst

Surface structure of the biocatalyst was observed by JEOL JSM 50800 scanning electron microscopy (SEM) (CME-UFRGS, Brazil). The size, morphology, HRTEM and electron diffraction (ED) patterns of the Ni nanoparticles were investigated by transmission electron microscopy using a TECNAI G20-FEI equipped with an energy dispersive X-ray spectroscopy (EDS) system and a MORGAGNI 268d-FEI electron microscope, operating at an accelerating voltage of 200 and 80 kV, respectively. AFM images were obtained in a Nanoscope IIIa system in the tapping mode using standard Si tips. Samples for electron and force microscopy were prepared by putting a droplet of a diluted aqueous solution of the lipase-Ni composite on a carbon-coated copper grid (TEM) or freshly cleaved mica (AFM).

Magnetic properties of the biocatalysts were investigated at room temperature in a Superconduction Quantum Interference Device (SQUID) from Quantum Design. Due to the small concentration of Ni in the biocatalysts the measurement was performed using the no-overshoot mode for good response.

Characterization of amount of Ni in the biocatalyst

The amount of Ni in the biocatalyst was quantified using a flame atomic absorption spectrometer (Analyt 200, PerkinElmer, Massachusetts, USA).With a deuterium background corrector and calibrated by analytical standards. Hollow cathode lamp was operated at 25 mA. Wavelength was set at 232 nm and the spectral band pass at 1.8 (width) / 1.35 (height) nm. Integrated absorbance (peak area) was used for all measurements. The gases for flame formation were produced by a mixture of 2.5 L.min-1 of the acetylene and 10 L.min-1 of air. For digestion of the free and magnetic lipase about 100 mg of each sample was mixed to 2 mL of concentrated HNO3 (2 mL) in a capped vessel and stirred for 4 h. After digestion, samples were diluted with water to 25 mL and transferred to graduated polypropylene vials. Colorless and clear solutions were obtained for digestion of the free enzyme. However, the magnetic biocatalyst was not digested completely, and in this case only extraction was performed. Blanks were run and analyzed after each 10 measurements in order to check eventual memory effects for all elements.

For the analysis of the amount of nickel released in the aqueous and organic phase during the reuse of the biocatalyst, before introduction of the samples on the spectrometer a dilution procedure was applied. In this sense, all the samples were diluted 5 times in 2.5% (m/v) HNO3

acid. This procedure was necessary because just 500 µL of sample was available for analysis. In addition, the dilution step could be necessary to avoid sample matrix interference.

Thermal stability of free and decorated lipase

The lipase temperature stability was determined by incubating 5 mg of free lipase or lipase decorated with nickel with 0.1 ml citrate–phosphate buffer at pH 7.0 (50 mM) for 1 h at 40, 55 and 70 °C in the absence of a substrate. Enzyme activity was measured by the spectrophotometric assay at 40 °C and pH 7.0. As a blank, 5 mg of nickel without lipase was incubating at tested temperatures for 1 h and measured by the spectrophotometric assay described above.

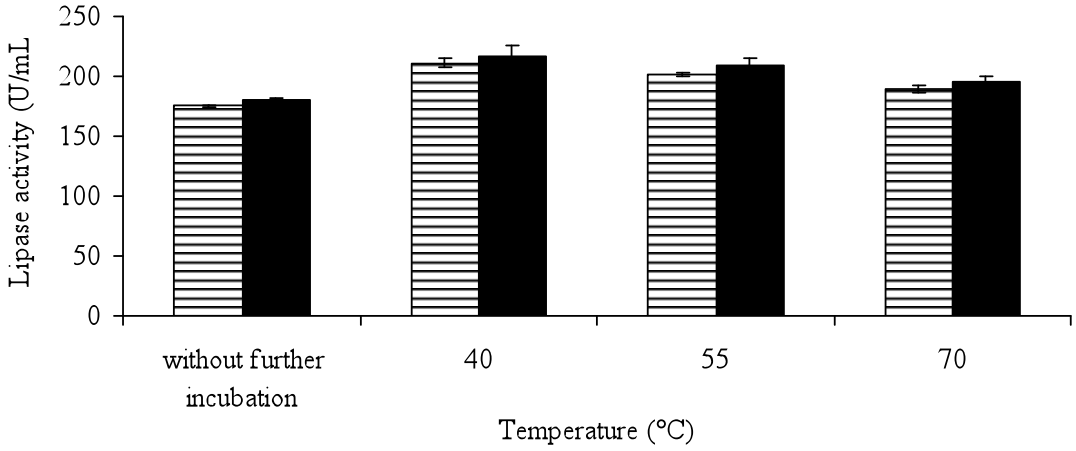

Figure S1: The free lipase (≡) and the lipase decorated with nickel (▉) were incubated at different temperatures for 1 h. The control was done with free and decorated lipase without further incubation.

Stability of free and decorated lipase at different pHs

The lipase pH stability was determined by incubating 5 mg of free lipase or lipase decorated with nickel in buffer solutions of pH values ranging 3.0 to 8.0 (50 mM citrate–phosphate pH 3.0, 4.0, 5.0, 6.0 and 7.0; 50 mM Tris–HCl pH 8.0) for 1h. Enzyme activity was measured by the spectrophotometric assay at 40 °C. As a control, 5 mg of nickel without lipase was incubating at tested buffers for 1 h and measured by the spectrophotometric assay described above.

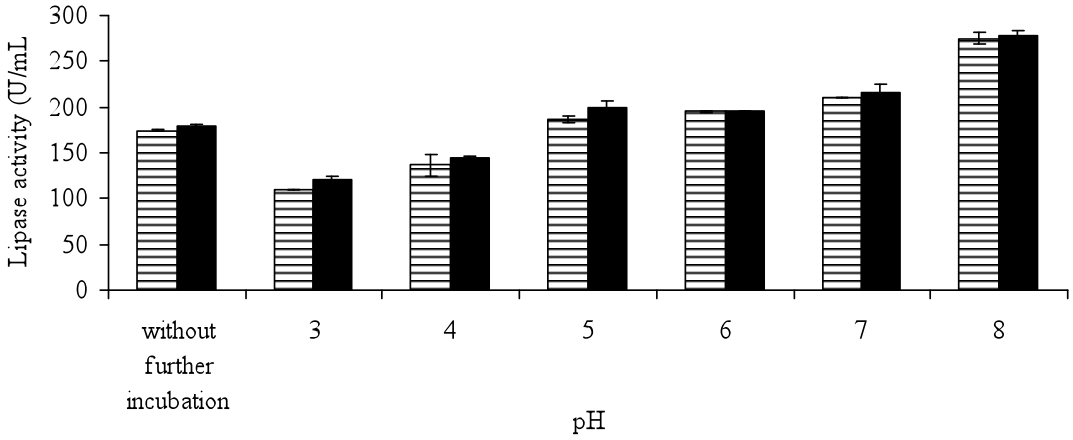

Figure 2: The free lipase (≡) and the lipase decorated with nickel (▉) were incubated at different pHs for 1 h. The control was done with free and decorated lipase without further incubation.

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