Electronic supplementary information

Efficient biodiesel production from rice bran oil using magnetite immobilized-recombinant lipase from probiotic *Bacillus licheniformis*

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Characterization:

The secondary structure of the native and codon-optimized lipase was studied using circular dichroism (CD) spectrophotometer (Jasco J-1500). The UV-visible absorbance measurements in the *p*-NPP assay were performed using SpectraMax[®] iD3 plate reader (Make: Molecular Devices). The structural integrity of the magnetite support after the immobilization of lipase from B. licheniformis was confirmed using X-ray diffraction (XRD; Rigaku Ultima IV) at a scan rate of 0.5 °/min, while the enzyme coverage over the magnetite support was characterized using X-ray photoelectron spectroscopy (XPS; Thermofisher K-Alpha) fitted with an Al K_a Xray source. On the other hand, field-emission scanning electron microscopy (FE-SEM; FEI, Apreo S) was employed for verifying any morphological changes during the surface derivatization processes over the magnetite particles. Gas chromatography (GC; GC-2010 Plus, Shimadzu) analyses were performed using the biodiesel with a mixture of nitrogen and air as the carrier gas under a constant pressure of 98.5 kPa and the obtained peaks of methyl esters were compared against the standards. The GC run was performed with the following parameters: injection port temperature – 200 °C; column temperature – 100 °C; total carrier gas flow rate - 60.9 mL/min; split ratio - 1:20; flame ionization detector - 250 °C. Nuclear magnetic resonance (NMR) analyses of the RBO and biodiesel were recorded using Bruker (AV NEO 400 MHz) NMR spectrophotometer. The thermal analysis of the biodiesel was carried out in thermogravimetric analyser (Shimadzu DTG-60) at a ramping rate of 10 °C/min and a nitrogen flow rate of 100 mL/min.

The codon optimized lipase gene sequence from *B. licheniformis* is as follows:



Fig. S1. CD spectra of native and expressed lipases analyzed using BeStSel online tool.

Secondary structure content (%)	Native lipase	Expressed lipase
Helix (regular)	13.9	21.4
Helix (distorted)	9.1	12.8
Antiparallel (left twisted)	0	0
Antiparallel (relaxed)	0	0
Antiparallel 1 (right twisted)	13	4
Parallel	6.4	7.2
Turn	15.3	13.5
Others	42.3	41.1
Total	100.0	100.0

Table S1. Parameters of secondary structure corresponding to Fig. S1.



Fig. S2. XPS survey scan spectra of commercial Fe₃O₄, and its surface derivatized products.



Fig. S3. Composite FE-SEM images of commercial magnetite before functionalization (a, b), and after derivatization with APTES (c, d), glutaraldehyde (e, f) NL immobilization (g, h), and EL immobilization (i, j). The set of two different magnification images shown in (k, l), (m, n), and (o, p) correspond to Fe₃O₄@BSA, Fe₃O₄@BSA@NL, and Fe₃O₄@BSA@EL, respectively.



Fig. S4. XPS survey scan (top left panel) and narrow scan (elements quoted in the respective panels) spectra of the fresh catalysts before subjecting to the recyclability experiments.



Fig. S5. XPS survey scan (top left panel) and narrow scan (elements quoted in the respective panels) spectra of the spent catalysts recovered after eighth cycles.