Supporting information

Magnetic cross-linked enzyme aggregates based on ionic liquid modification as novel immobilized biocatalyst for phytosterol esterification

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1. Thermogravimetric analysis (TGA) images.

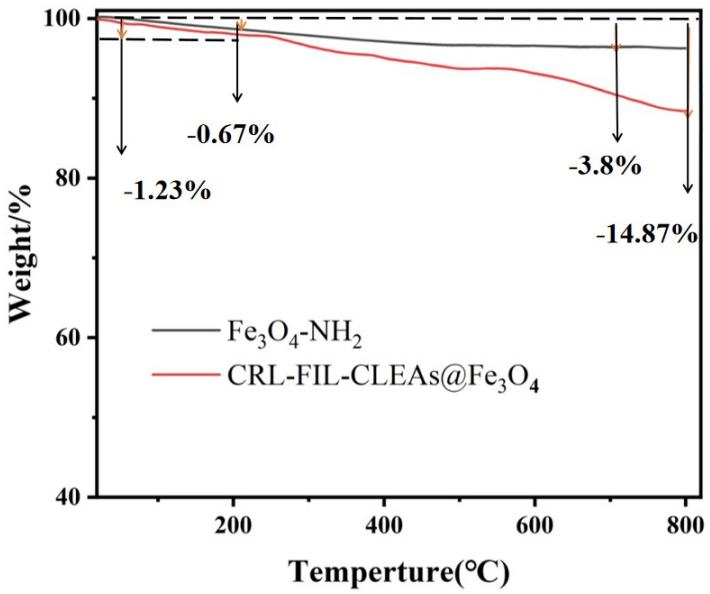

Fig. 1s Thermogravimetric analysis (TGA) of Fe_3O_4-NH_2 and CRL-FIL-CLEAs@Fe_3O_4.

TGA curves of Fe_3O_4-NH_2 and CRL-FIL-CLEAs@Fe_3O_4 under nitrogen were studied (**Fig.1s**). The first weight loss of Fe_3O_4-NH_2 and CRL-FIL-CLEAs@Fe_3O_4 occurred at low temperatures (< 250°C) due to the evaporation of adsorbed and bound water. At 300–800°C, part of the mass lost was attributed to the decomposition of APTES. Fe_3O_4-NH_2 magnetic nanoparticles showed excellent thermal stability, and the residual mass was about 96.4%. The second weight loss of CRL-FIL-CLEAs@Fe_3O_4 occurred after 250°C, which was due to the decomposition of lipase protein molecules. The loading of CRL was about 0.16 mg protein/mg CRL-FIL-CLEAs@Fe_3O_4.

2. Reuse of different catalysts in the esterification reaction.

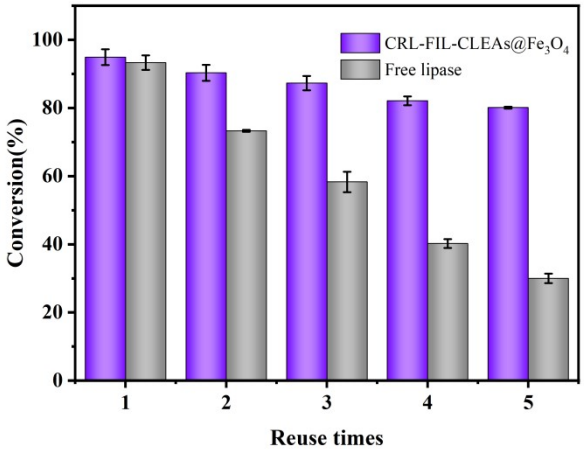

Fig.2s Reuse of different catalysts in the esterification reaction.

As shown in **Fig. 2s**, the phytosterol conversion of CRL-FIL-CLEAs@Fe_3O_4 system remained at 80.02% after five cycles, while that of free lipase system was only 29.91%. The loss of immobilized lipase activity may be due to the separation of CRL-FIL-CLEAs and some Fe_3O_4 nanoparticles in a long-term repetitive experiment. Compared with the free lipase, CRL-FIL-CLEAs@Fe_3O_4 has good reusability in the biocatalytic system. Combined with the catalytic activity and thermal stability of the immobilized lipase, CRL-FIL-CLEAs@Fe_3O_4 was a catalyst with excellent performance.

3. Confocal laser scanning microscopy (CLSM) images.

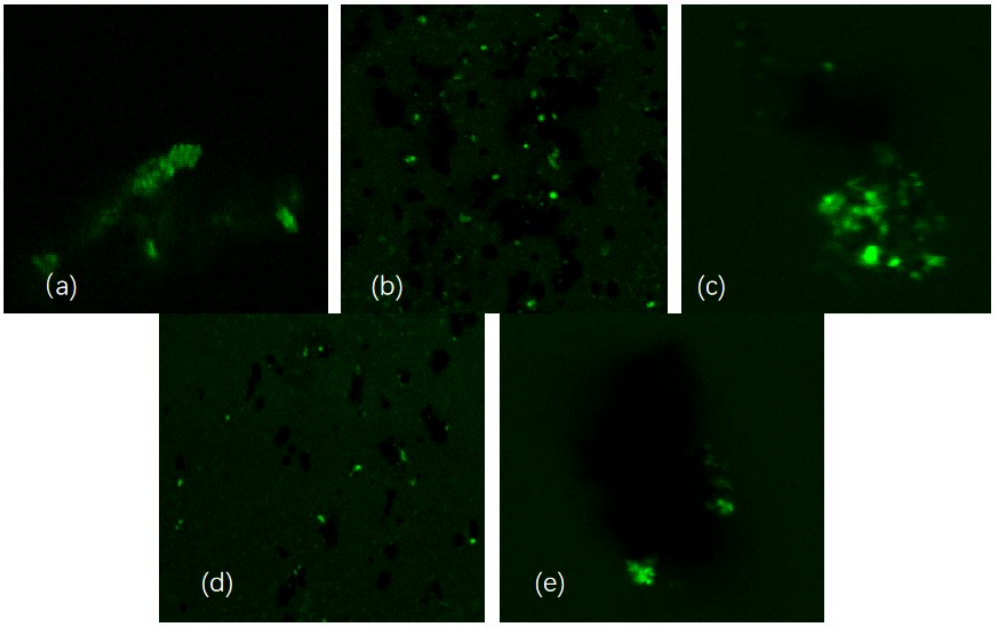

Fig.3s (a) Free lipase; (b) CRL-FIL-CLEAs@Fe_3O_4; (c) CRL-FIL-CLEAs@Fe_3O_4 (5X); (d) CRL-FIL-CLEAs @Fe_3O_4 (repeat-ed 5 times); (e) CRL-FIL-CLEAs@Fe_3O_4 (repeated 5 times, 5X).

The conformational changes of immobilized lipase before and after the reaction were observed by CLSM. As shown in **Fig. 3s(a)**, the free lipase emits green fluorescence after FITC staining. It can be observed that the aggregation morphology of lipase molecules was connected to magnetic nanoparticles before using (**Fig. 3s(b)(c)**). After repeated use five times (**Fig. 3s(d)(e)**), due to mechanical loss and other factors, it can be observed that some cross-linked enzyme aggregates fall off from magnetic nanoparticles. This explains the decrease in CRL-FIL-CLEAs@Fe_3O_4 activity after reuse.

4. Conversion of phytosterols with CRL-FIL-CLEAs@Fe_3O_4 at different times (Table 1s).

Table 1s Conversion of β-sitosterol with CRL-FIL-CLEAs@Fe_3O_4.

Time (h)	Conversion (%)
2	11.03
4	17.29
6	31.92
8	49.20
10	60.48
12	68.58
14	75.35
16	84.23
18	91.23
20	92.01
22	93.24

5. Comparison of enzyme-catalyzed esterification of phytosterols (Table 2s)

Table 2s Comparison of enzyme-catalyzed esterification of phytosterols and fatty acids (FA) with other reported references.

Substrate	Enzyme	Parameters [a]	Conversion	Time	Ref.
Phytosterols /Lauric acid	CRL	[Bmim]PF 6 /Tween20/H_2O, 50℃, A/P 2: 1	87.9%	24 h	54
Phytosterols /Oleic acid	CRL	n-Hexane, 30℃, A/P 5: 1	80%	24 h	55
Phytosterols /Linoleic acid	Immobilized CRL	Isooctane，50℃，A/P 2:1	85.8%	24 h	56
Phytosterols /linolenic acid	NMC/MoS_2@CRL	60% PBS, 55℃, A/P 2: 1	75%	42 h	57
Phytosterols /Oleic acid	CRL-FIL-CLEAs@Fe_3O_4	Solvent-free, 48℃, A/P 10:1	93.24%	22 h	This work

[a]: A/P: molar ratio of acids to phytosterols.

6. Secondary structures for free lipase and CRL-FIL-CLEAs@Fe_3O_4 (Table 3s)

Table 3s Secondary structures for free lipase and CRL-FIL-CLEAs@Fe_3O_4.

Samples	β-sheet(%)	Random coil(%)	α-helix(%)	β-turn(%)
Free lipase	26.57±0.19	35.65±0.23	25.33±0.11	12.45±0.18
CRL-FIL-CLEAs@Fe_3O_4	31.34±0.21	33.57±0.13	19.42±0.15	15.67±0.20