Supporting Information

Large-scale aerosol-assisted synthesis of biofriendly Fe_2O_3 yolk-shell particles: a promising support for enzyme immobilization

Sanjay K. S. Patel,[a1] Seung Ho Choi,[b1] Yun C. Kang,[*b] and Jung-Kul Lee[*a]

[a] Department of Chemical Engineering, Konkuk University, 1 Hwayang-Dong, Gwangjin-Gu, Seoul, 143-701, South Korea

[b] Department of Chemical Engineering, Korea University, Anam-Dong, Seongbuk-Gu, Seoul, 136-713, South Korea

*Corresponding author. Mailing address:

Department of Chemical Engineering, Konkuk University, Seoul 143-701, South Korea. *E-mail: jkrhee@konkuk.ac.kr; Fax: +82-2-458-3504; Tel: +82-2-450-3505*

Department of Materials Science and Engineering, Korea University, Seoul 136-713, South Korea. *E-mail: yckang@korea.ac.kr; Fax: +82-2-928-3584; +82-2-3290-3282.*

Laser Scanning Confocal Microscopic Analysis. 8 mg of fluorescein isothiocyanate (FITC) dissolved in DMSO (2 mg mL^{-1}) was added to 1 mL of laccase in 9.5 pH carbonate buffer (0.5 M) and resulting solution was incubated at 300 rpm for 6 h in dark conditions. The excess FITC was removed through dialysis against distilled water. Further, fluorecently labeled laccase was used for the immobilization on Fe_2O_3 yolk-shell particles. Laser scanning confocal microscope images were taken by FV-1000 Olympus confocal microscope.

Table S1 Cross-linking by glutaraldehyde and leaching of the immobilized laccase on different Fe_2O_3 yolk-shell particles

Fe_2O_3 Particles	Average particle size (μm)	S_{BET} (m^2/g)	Average pore size (nm)	Immobilization yields (IY%)	Immobilization efficiency (IE %) after		Leaching (%) after[a]	
					Immobilization	Cross-linking	Immobilization	Cross-linking
A300	1-3	24.4	19	90.6±6.1	83.5±8.0	90.2±7.7	41.3±4.0	2.8±0.2
A400	1-3	15.1	16	81.5±5.8	83.1±8.1	86.3±7.6	52.6±5.1	4.2±0.4
A500	1-3	19.5	28	84.3±6.2	80.2±7.7	81.1±7.2	54.5±5.3	4.7±0.4

[a] An amount of protein in supernatant after the treatment with NaCl (1 M) for 1 h at 25 °C. Cross-linking was performed with 0.05 M glutaraldehyde at pH 7.0 in phosphate buffer (50 mM) for 2 h.

Table S2 Energy dispersive spectroscopy analysis of immobilized laccase on Fe_2O_3 yolk-shell (A300) particles

Elements	Fe_2O_3 yolk-shell particles composition (%)	
	Before immobilization	After immobilization
CK	4.58	5.77
NK	3.29	4.94
OK	23.14	28.45
FeK	68.99	60.84
Total	100	100

Table S3 Determination of the denaturation constant (k_d) and $t_{1/2}$ values for the free, immobilized, and cross-linked laccase

Temperature (K)	Laccase								
	Free			Immobilized			Cross-linked		
	k_d (h^{-1})	R^2	$t_{1/2}$ (h)	k_d	R^2	$t_{1/2}$	k_d	R^2	$t_{1/2}$ (h)
303	0.075±0.004	0.99	9.24±0.58	0.016±0.001	0.97	43.3±3.3	0.005±0.001	0.95	138±12
313	0.096±0.006	0.99	7.22±0.54	0.027±0.002	0.99	25.7±2.2	0.012±0.001	0.96	57.8±4.3
318	0.220±0.014	0.99	3.15±0.28	0.031±0.002	0.99	22.4±1.9	0.017±0.001	0.99	40.8±3.2
323	0.312±0.024	0.86	2.22±0.16	0.130±0.008	0.99	5.33±0.48	0.031±0.004	0.99	22.4±1.7
333	3.22 ± 0.29	0.91	0.22±0.02	0.444±0.032	0.98	1.56±0.11	0.176±0.020	0.99	3.94±0.22
343	6.36 ± 0.52	0.99	0.11±0.01	4.140±0.365	0.99	0.17±0.01	1.723±0.140	0.99	0.40±0.01

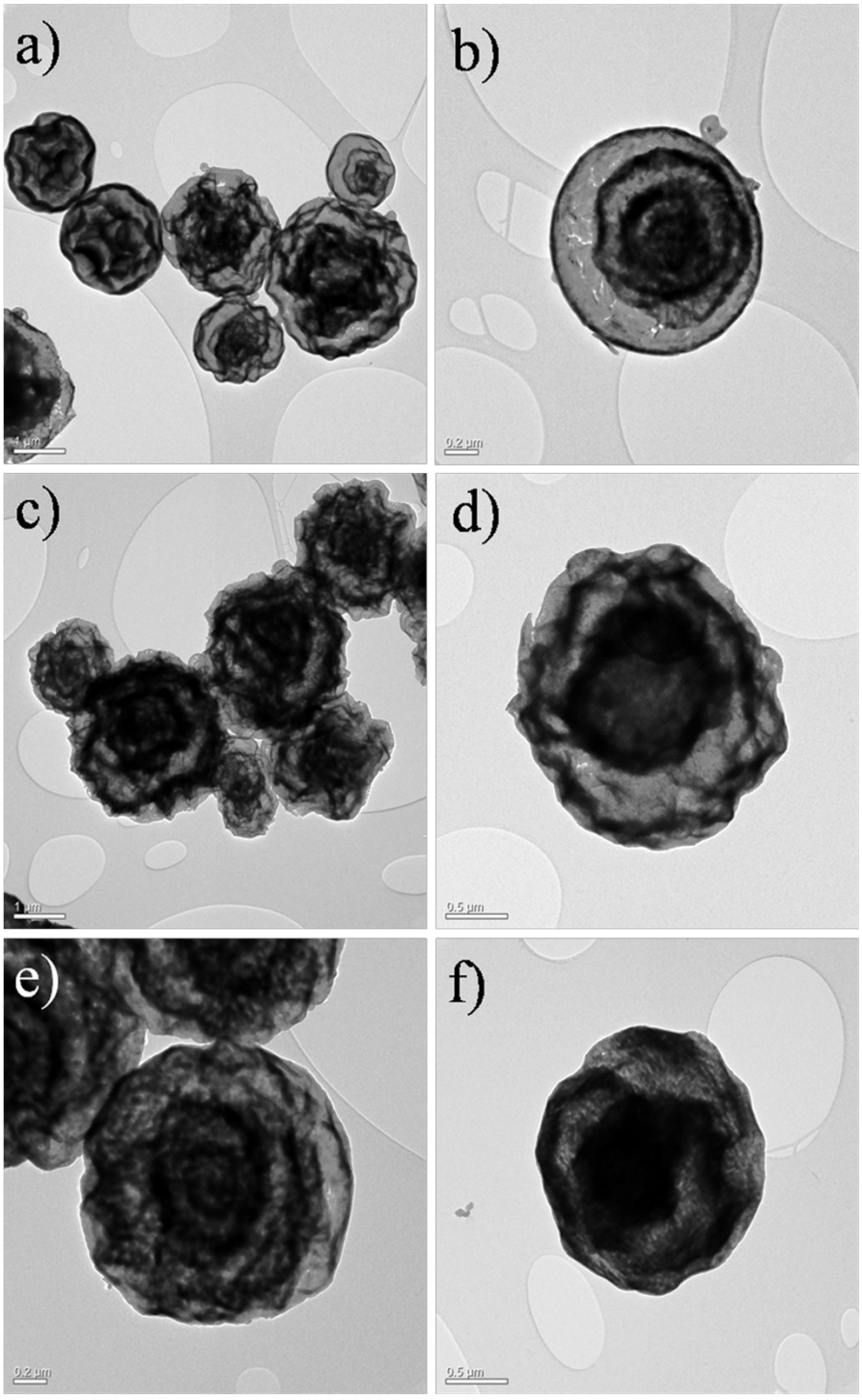

Fig. S1. TEM images of synthesized multi-shells Fe_2O_3 yolk-shell particles at 300 °C (a-b), 400 °C (c-d), and 500 °C (e-f).

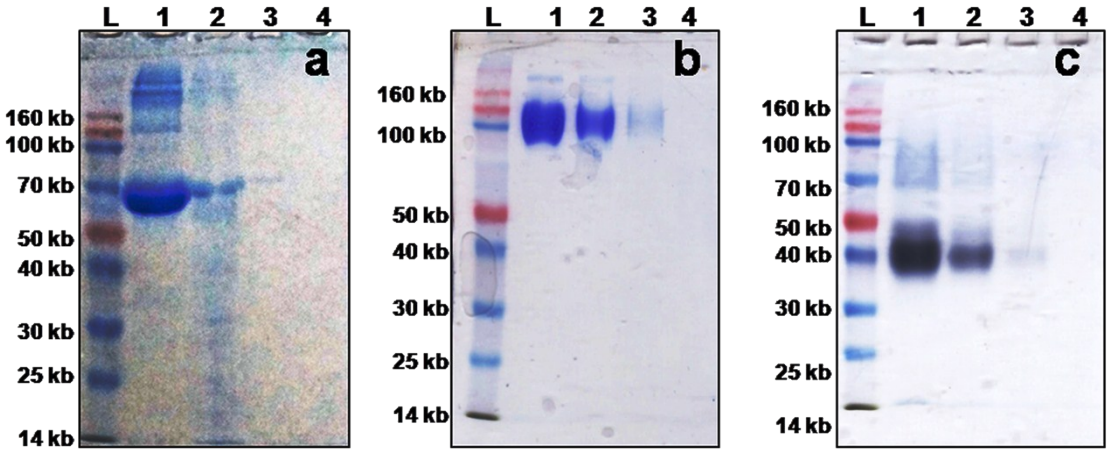

Fig. S2 SDS-PAGE analysis of Laccase (a), Glucose oxidase (b) and Horseradish peroxidase (c). L: Protein marker, Lane 1: Free enzyme, Lane 2: Supernatant after immobilization, Lane 3: 1st washing and Lane 4: 2nd washing samples.

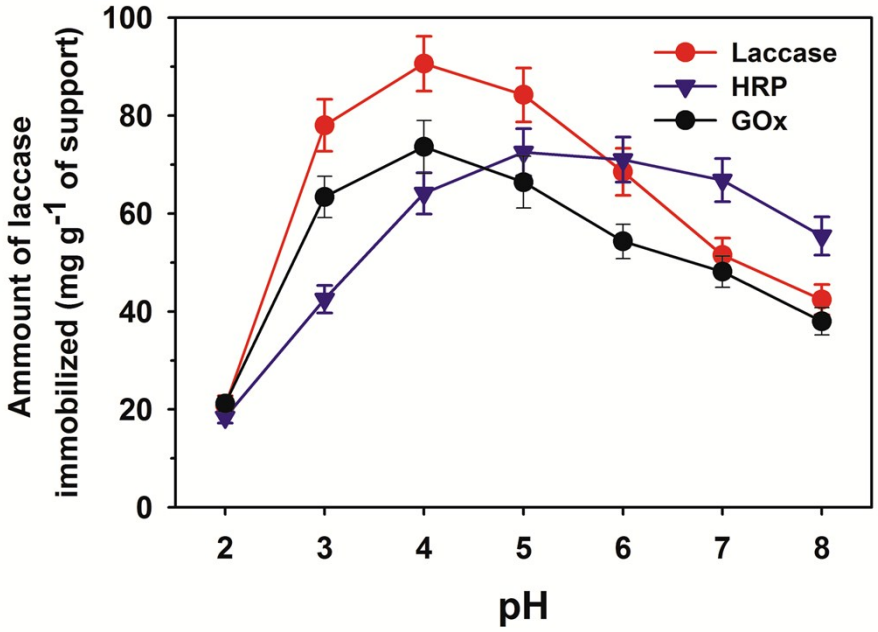

Fig. S3 Immobilization of enzymes on Fe_2O_3 yolk-shell (A300) particles at different pH values.

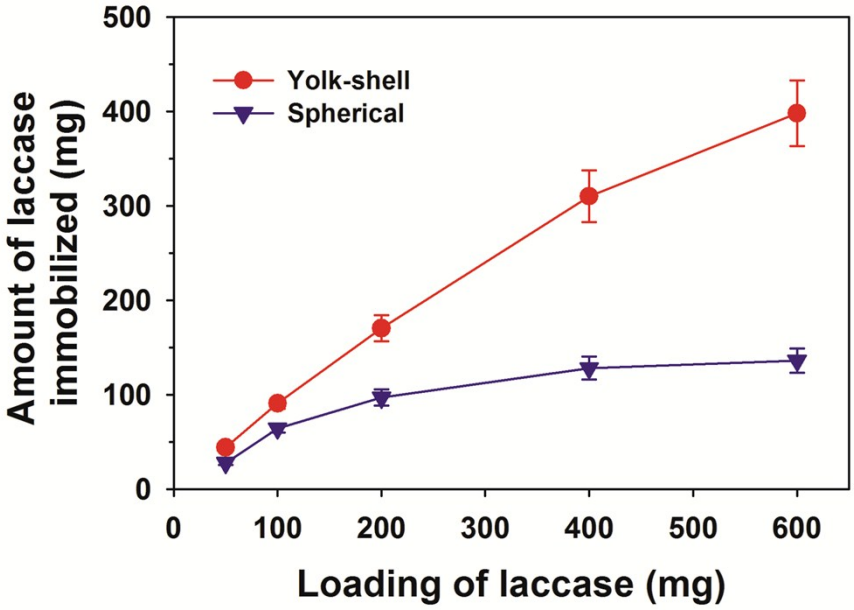

Fig. S4 Influence of Fe_2O_3 particles morphology on laccase loading.

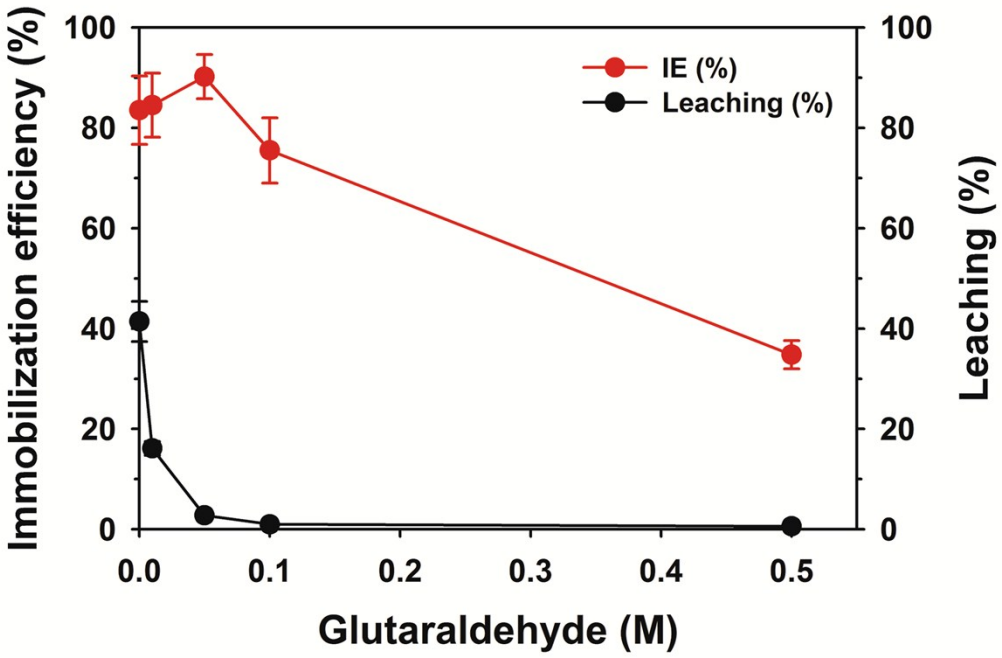

Fig. S5 Effect of glutaraldehyde concentration on the immobilization efficiency and leaching of immobilized laccase on Fe_2O_3 yolk-shell (A300) particles.

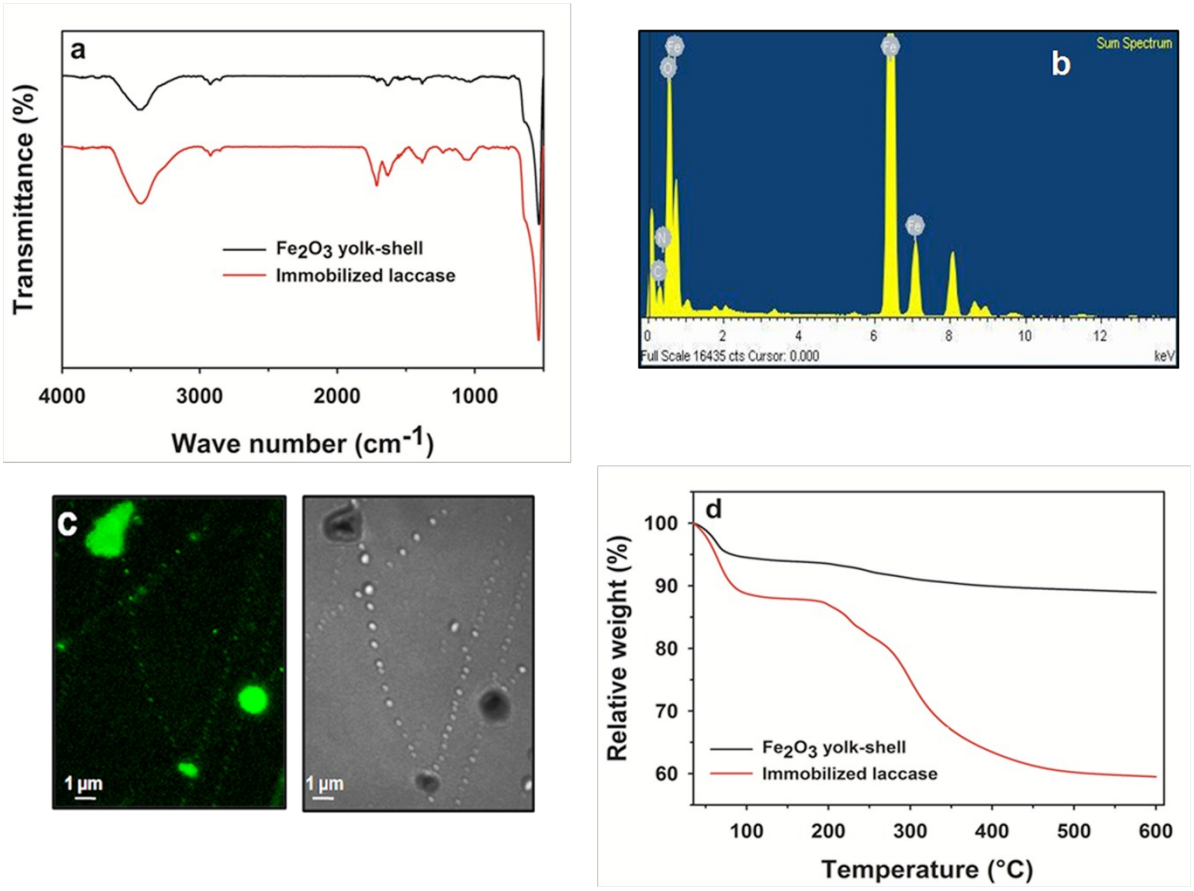

Fig. S6 Immobilized laccase analysis on Fe_2O_3 yolk-shell (A300) particles. (a) FTIR, (b) EDS, (c) Confocal microscopy in green channel and bright field, and (d) TGA.

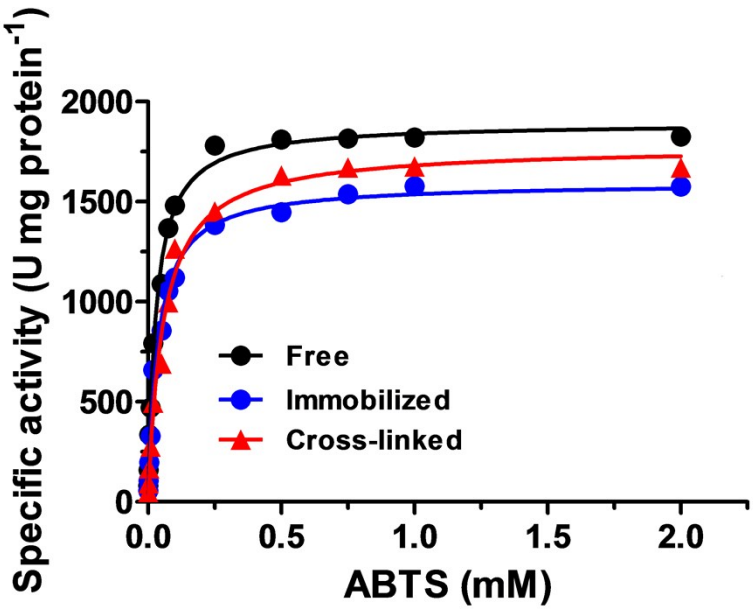

Fig. S7 Effect of ABTS concentration on the activities of the free, immobilized and cross-linked laccase. Each value represents the mean of triplicate measurements that varied from the mean by no more than 10%.

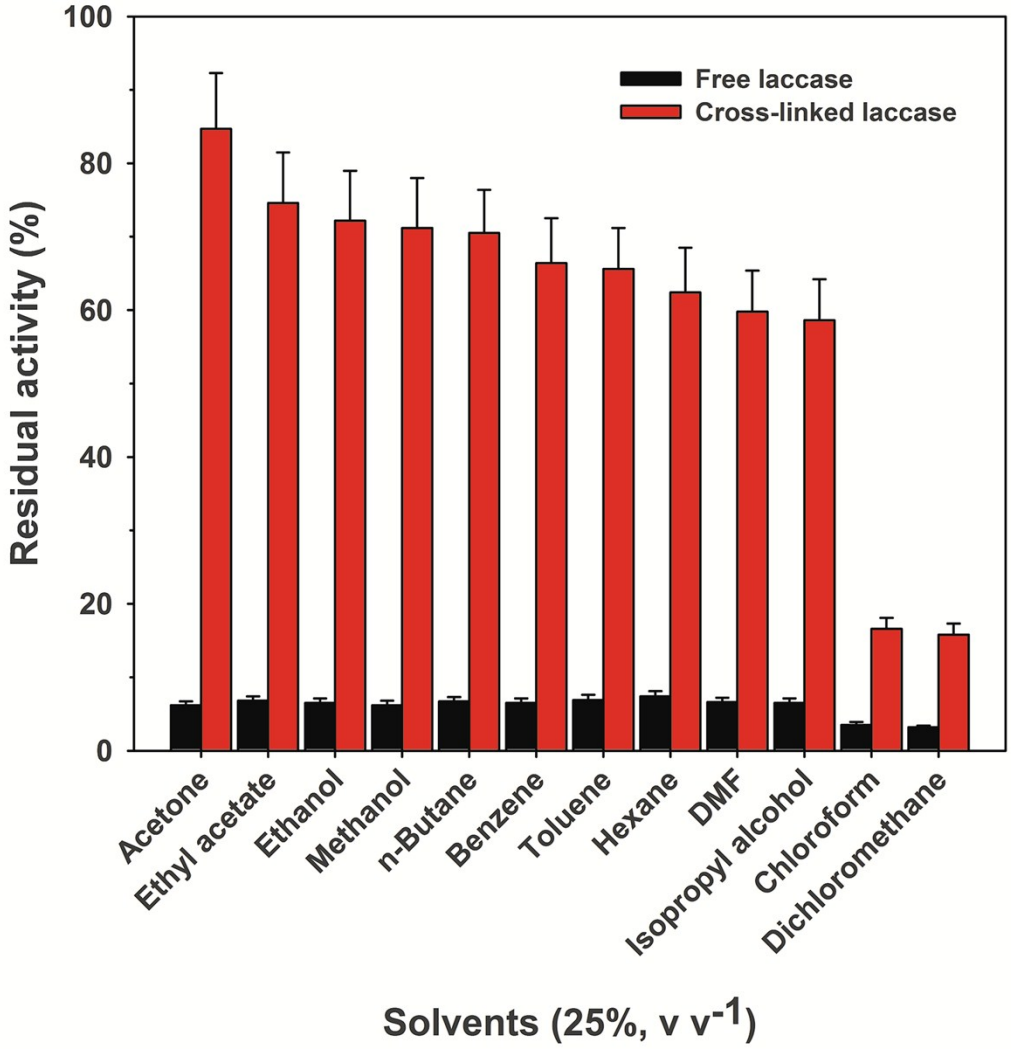

Fig. S8 Tolerance of the free and cross-linked laccase towards different solvents.

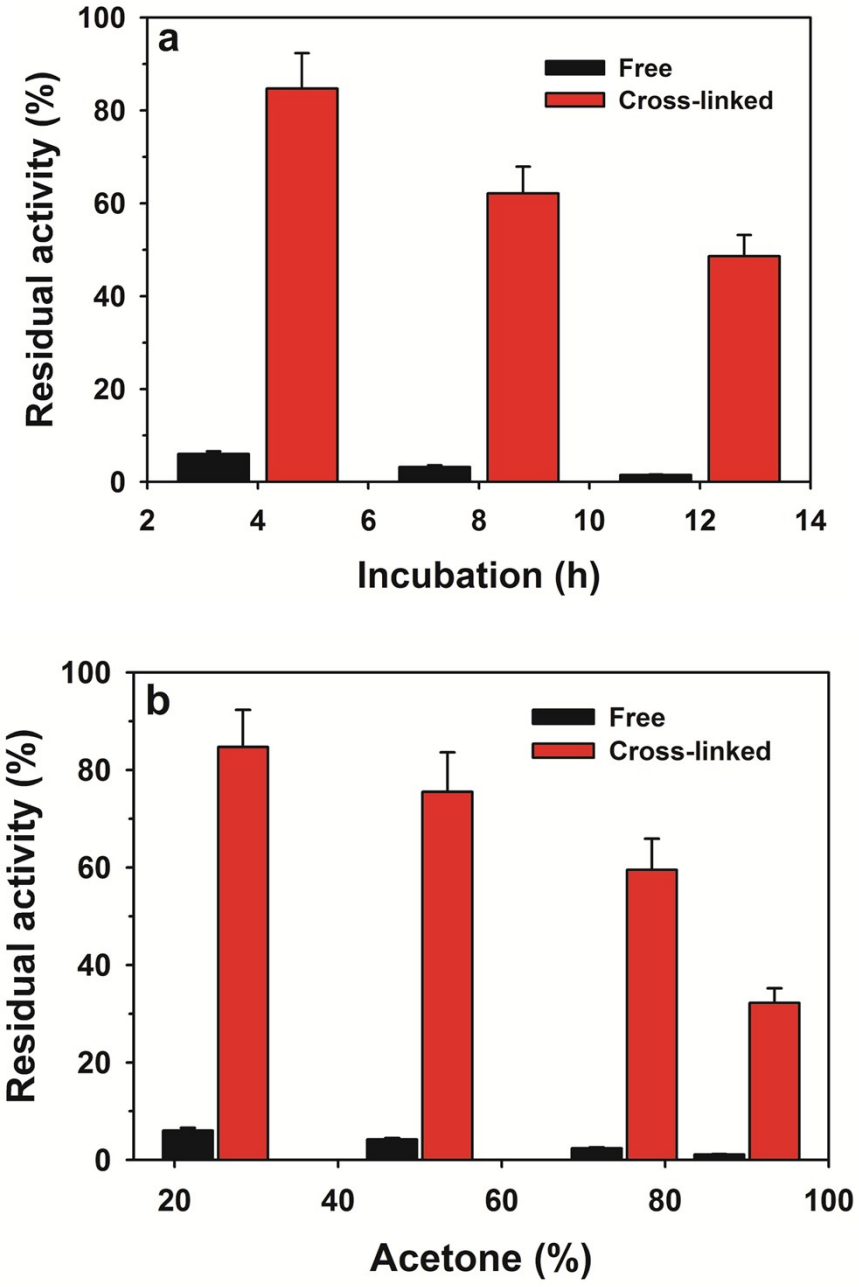

Fig. S9 Stability of the free and cross-linked laccase for acetone at different incubation periods (a) and solvent concentrations (b).

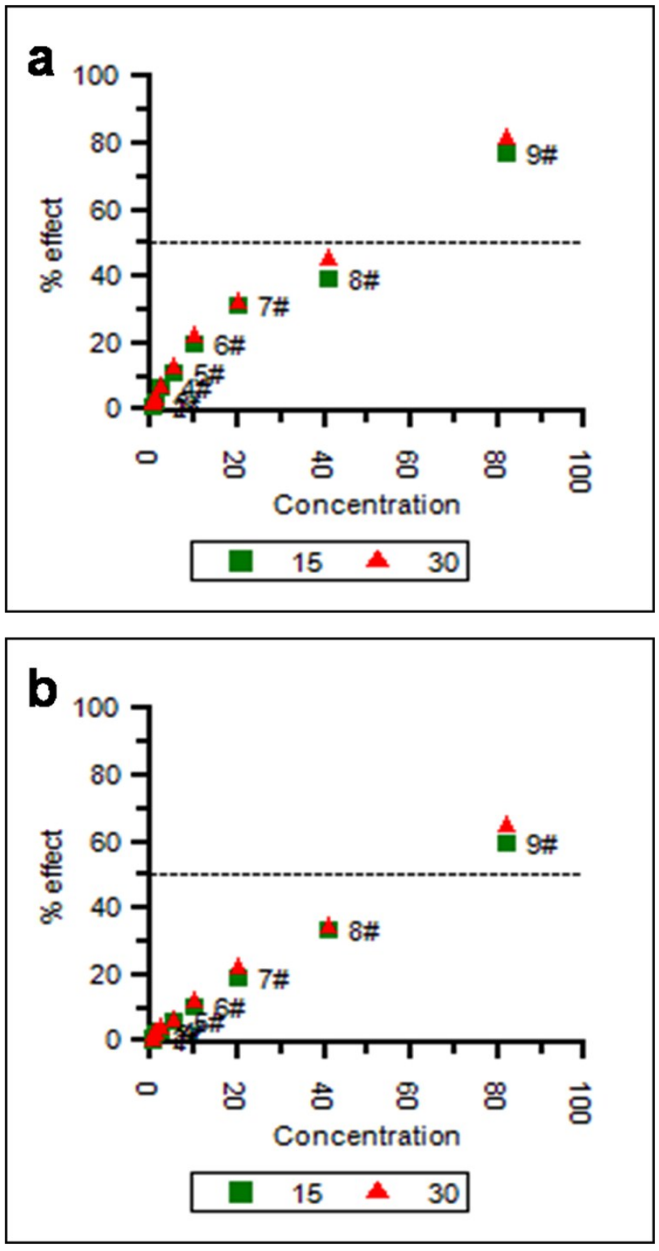

Fig. S10 Acute toxicity of Fe_2O_3 particles towards *V. fischeri*. (a) commercial spherical, and (b) synthesized yolk-shell (A300).

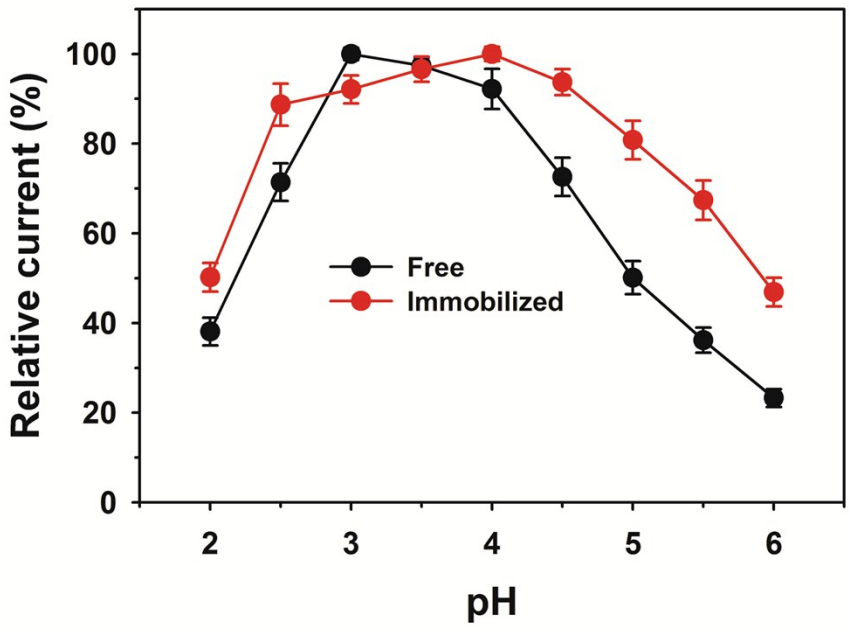

Fig. S11 Dependence of the catalytic current of free and Fe_2O_3 yolk-shell immobilized laccase coated on the glassy carbon electrode at different pH values using ABTS as a substrate (0.5 mM) at scan rate 20 mV s^{-1}.