Electronic supplementary information

Biomineralization of orange peroxidase within metal organic framework (OPP–MOF) for dye degradation

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Materials and methods

SDS- PAGE

Non-reducing SDS PAGE of the OPP was done as per procedure given by Laemelli [1]. Briefly, enzyme sample (20µl) was mixed with sample buffer (60µl) and then it was loaded in each well. Separating gel (12%) and stacking gel (4%) was used to get proper band separation. Mini Protein Cell II from Bio-Rad Laboratories, USA was used for performing the electrophoresis. Electrophoresis was conducted at 120mV. Coomassie brilliant blue staining was done for visualization of protein bands.

Confocal scanning laser microscopy

Confocal scanning laser microscopy was used to investigate the presence of mixture of OPP tagged with fluorescein isothiocyanate (FITC) within the ZIF-8 MOF. The tagging was carried out by mixing enzymes solution (10 mM, pH 9.5 carbonate buffer) and FITC (8 mg, dissolved in DMSO). The solution was incubated for 4 h at 250 rpm at ambient temperature in dark condition. Further, free FITC was removed via dialysis against DI water and subsequent used for synthesis of fluorescently labelled OPP-MOF composite. Laser scanning confocal microscope images were taken on Leica, Germany (DMi8 microscope and SP8 scanner).

Kinetics of thermal deactivation

OPP–MOF and free form of peroxidase were incubated at 40, 50 and 60°C in phosphate buffer (pH 7.4 of 10 mM). The samples were withdrawn every ten min for 60 min, chilled quickly and then assayed for residual enzymatic activity as mentioned above. A semi-log plot of percent residual activity vs. time was plotted from which the inactivation rate constant (k_d) was calculated as the slope, and $t_{1/2}$, the time required for the activity to decrease to half its original activity was calculated as $0.693/k_d$. Further, deactivation energy (E_d) of the free and immobilized OPP was calculated from Arrhenius plot.

Michaelis–Menten kinetic parameters

Kinetic parameters of free form and OPP were determined using different guicol (substrate) concentrations in the range of 5-120 mM in buffer pH 7.4 at optimum condition. K_m, V_{max} values of free and immobilized enzyme were calculated from non-linear regression fitting of the initial reaction rates corresponding to different substrate concentrations by Graph Pad Prism software.

Reusability studies

Reusability of OPP–MOF was examined by oxidizing guicol in batch operation mode at optimal conditions. After each cycle, OPP–MOF were separated and washed with sodium phosphate buffer (10 mM, pH 7.4) and then suspended again in a guicol solution to measure enzyme activity. The activity after each cycle was determined in terms of residual activity by taking the enzyme activity of the first cycle as 100%.

Storage stability studies

Storage stabilities of the free and OPP–MOF were evaluated till 18 days by incubating them at room temperature in sodium phosphate buffer (10 mM, pH 7.4) separately. After every three days, free and immobilized OPP were taken out and then assayed for residual activity as mentioned in enzyme activity assay section. The activity was determined in terms of residual activity by considering initial activity as 100%.

Figure S1a

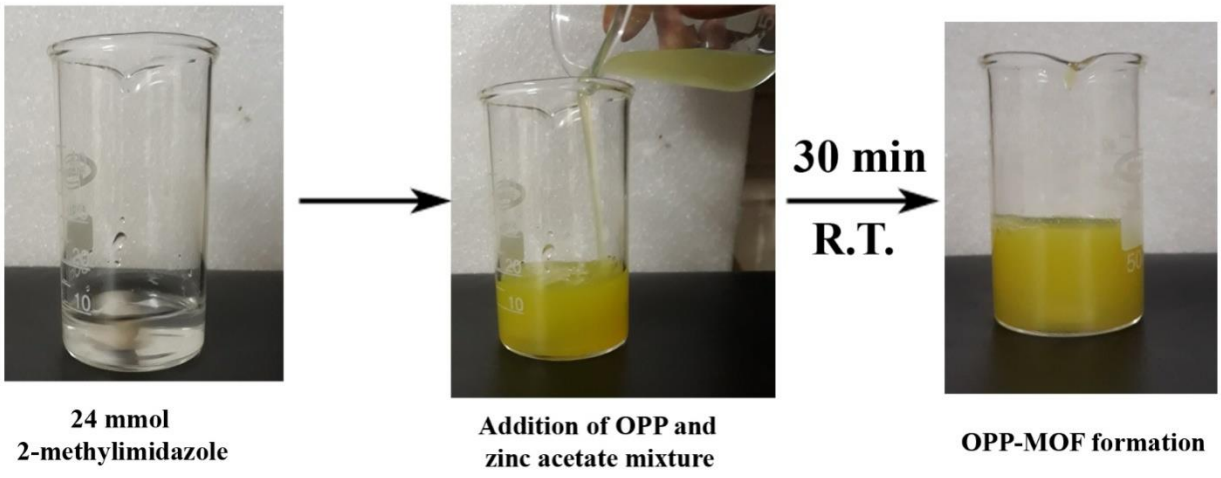

Figure S1b

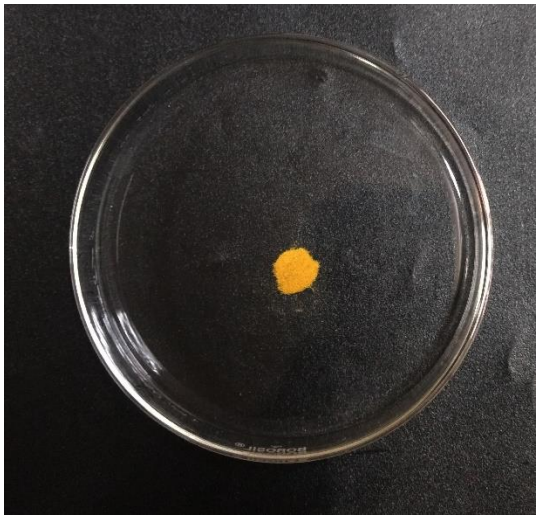

Figure S1c

Figure S1 Photographs of (a) orange peels for extraction of peroxidase (OPP), (b) synthesis of OPP–MOF as a function of time till 30 min and synthesized OPP–MOF in powder form (c) under optimized conditions.

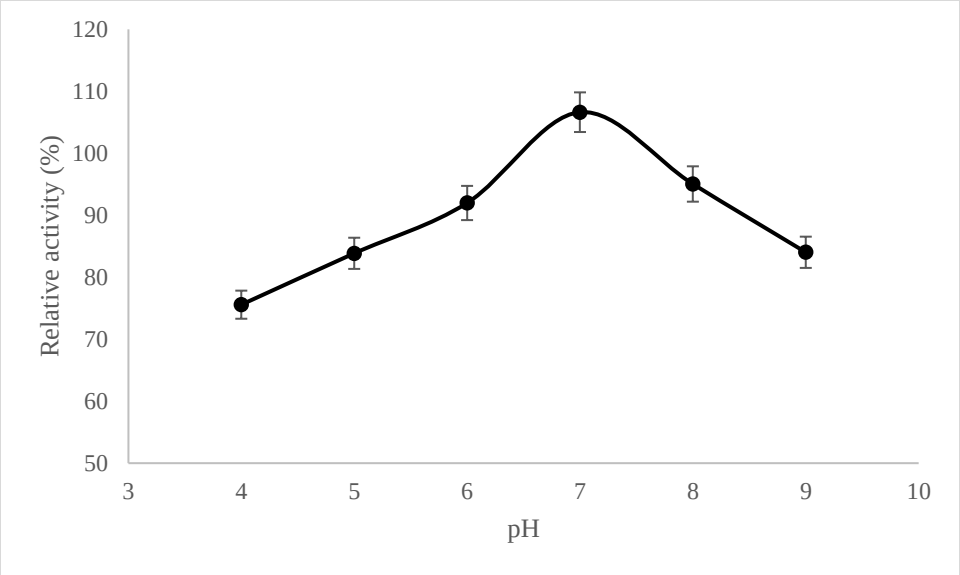

Figure S2 Effect of pH of extraction buffer on activity of OPP. The 100% activity recovery corresponds to 15U/mL OPP activity. The measurements were performed in triplicate and the error bar represents the percentage error.

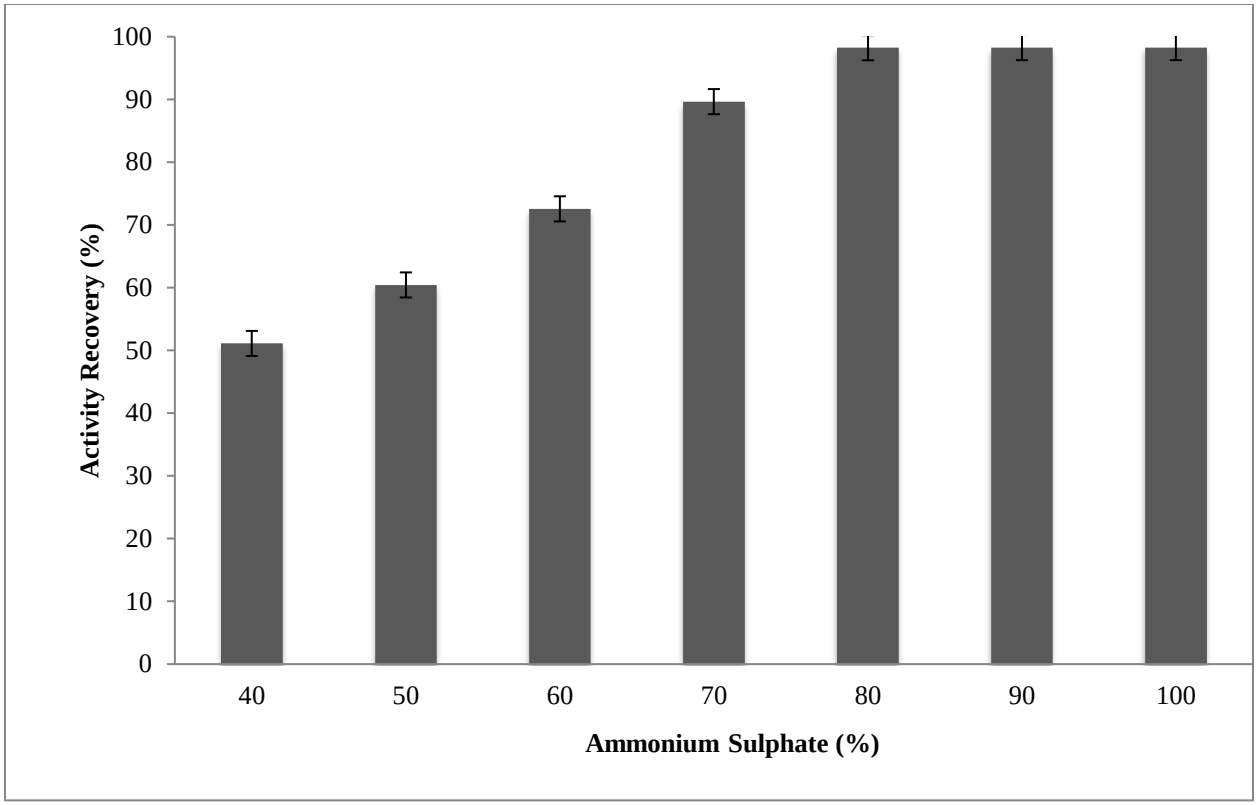

Figure S3 Effect of ammonium sulphate on OPP activity recovery.

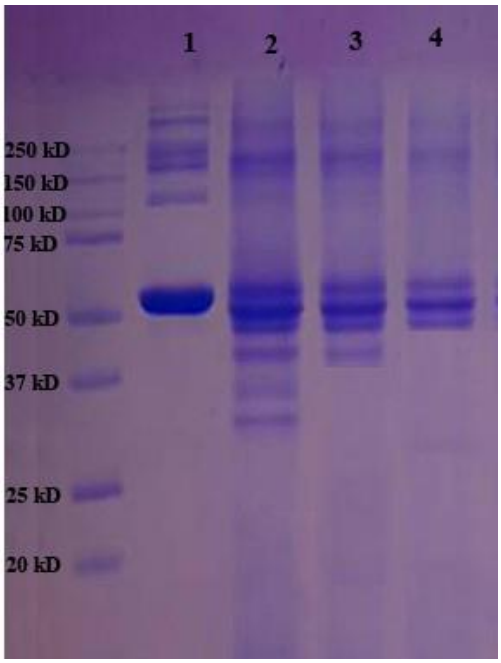

Figure S4 SDS PAGE: Lane 1- BSA (control), Lane 2- crude OPP (without partial purification by ammonium sulphate), After partially purified enzyme by different saturation of ammonium sulphate: Lane 3- 100% saturation of ammonium sulphate, Lane 4- 80% saturation of ammonium sulphate.

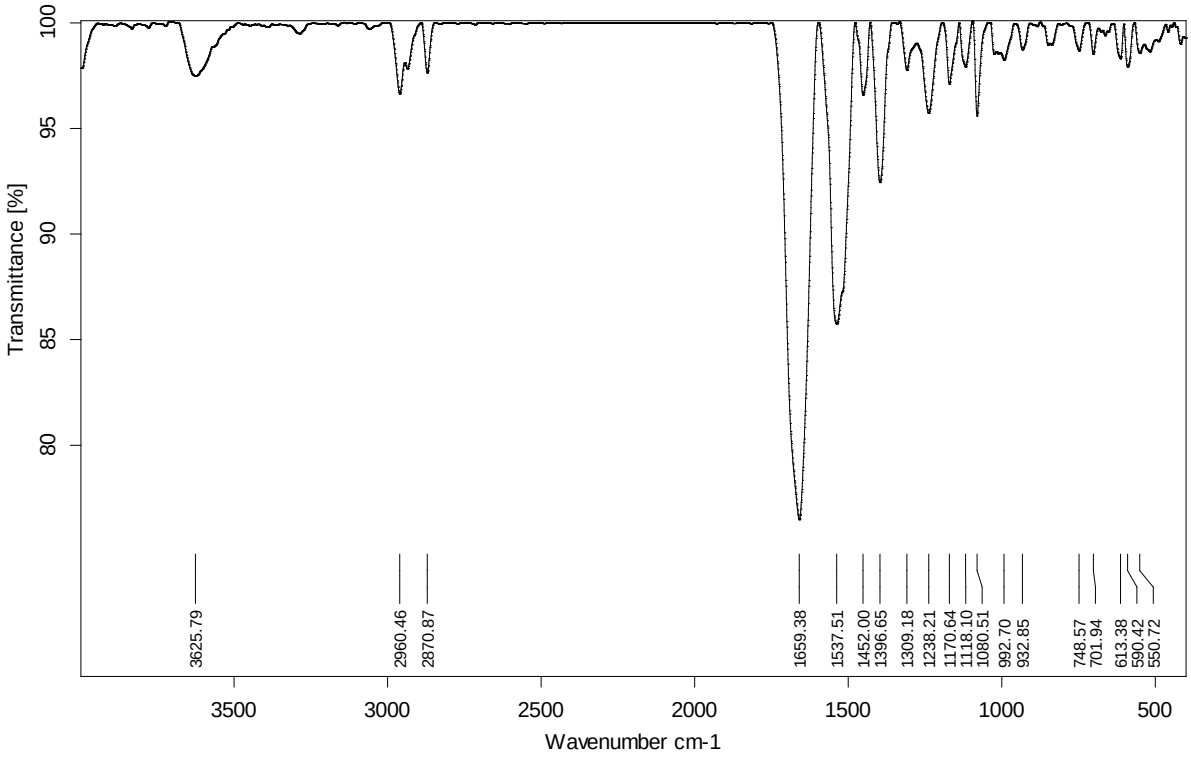

Figure S5 FT-IR spectra of extracted OPP.

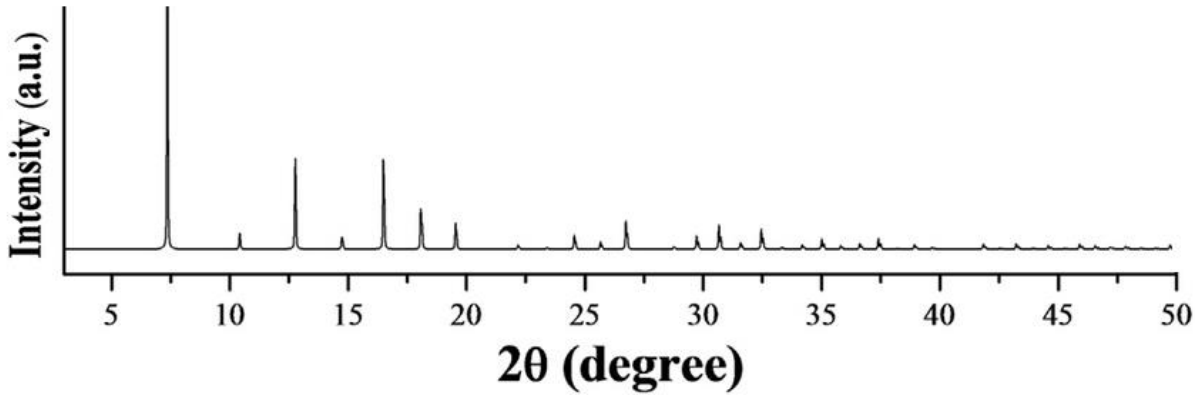

Figure S6 XRD patterns of simulated pure ZIF-8 (without enzyme)

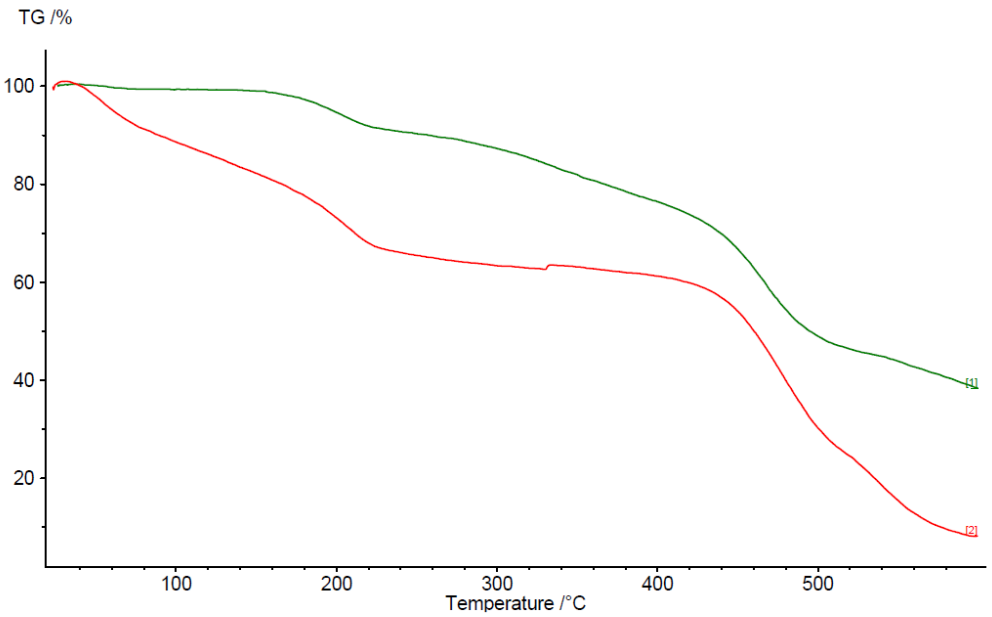

Figure S7 TGA curves of OPP–MOF (red) and pure MOF (green) (without OPP, ZIF-8) in air.

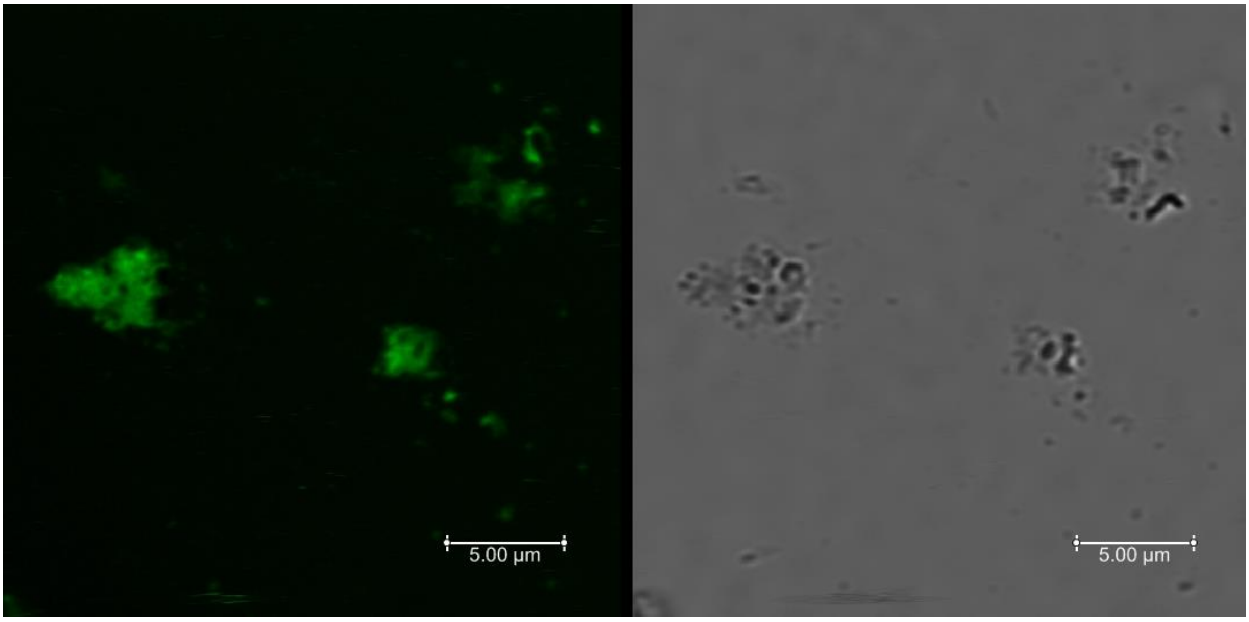

Figure S8 Confocal scanning laser microscopy image of FITC-tagged OPP molecules within the ZIF-8 MOF.

[1] U. LAEMMLI, Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4, Nature 227 (1970), 680–685