Electronic Supplementary Information for

Simple purification and immobilization of His-tagged organophosphohydrolase from cell culture supernatant by metal organic frameworks for degradation of organophosphorus pesticides

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Expression of His-tagged OpdA and preparation of crude enzyme solution

The gene sequence of OpdA from *Agrobacterium radiobacter* P230 was obtained from NCBI (Genbank: DD088220.1), encoded by N-terminal polyhistidine tag, and expressed by pET-28a vector. The recombinant plasmid (pET-28a) was introduced into *E. coli* Rosetta (DE3). The obtained recombinant *E. coli* was inoculated into 15 mL of LB liquid medium containing kanamycin sulfate (50 μg/mL), and cultured overnight at the agitation rate of 180 rpm at 37 °C. Then, the culture solution (1 mL) was inoculated to 50 mL of new LB liquid medium containing kanamycin sulfate (50 μg/mL). When the OD_{600} value of the bacterial concentration reached 0.6-0.8, IPTG was added to induce the expression of His-tagged OpdA gene. After incubation at 30 °C for 12 h, the fermentation liquid was incubated at 15 °C for 12 h. The cells were collected by centrifugation and washed three times with pre-cooled buffer (PBS, pH 7.4). After that, cells were lysed by sonication in lysis buffer (containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 50 mM Tris-HCl (pH 8.0) and 20% glycerol as well as 150 mM NaCl) in ice bath. The crushed cells were centrifuged, and the supernatant was collected and stored at 4 °C. The supernatant was named crude enzyme solution.

The reactivity difference between His-tagged OpdA and OpdA.

100 μL of His-tagged OpdA crude enzyme solution was added to 900 μL Tris-HCl (50 mM, pH 8.0) buffer containing 5 μL of methyl parathion (10 mg/mL). The mixture was incubated at 37 °C for 5 min and filtered. The absorbance of the supernatant was measured at 410 nm. The enzyme activity was calculated based on

the yield of p-nitrophenol. After calculation, the enzyme activity was 328.3±4 $U/g_{protein}$.

100 μL of OpdA crude enzyme solution was added to 900 μL Tris-HCl (50 mM, pH 8.0) buffer containing 5 μL of methyl parathion (10 mg/mL). The other steps were the same as the above experiment and the enzyme activity was 325.1±5 $U/g_{protein}$.

Thus, the activity of His-tagged OpdA and OpdA was almost the same. The same phenomenon was observed in organophosphorus hydrolase (OPH) and His-tagged OPH[1-3], and the insertion of histidine tag did not inhibit the catalytic activity of OPH. Additionally, the amino acid sequence consistency of OpdA and OPH was more than 93%, and they had the same degradation mechanism. The results further confirmed that the insertion of histidine tag didn't cause the decrease of enzyme activity. Because the insertion of histidine tag could achieve the one-step purification and immobilization of OpdA by coordination between CUS of Fe^{3+} in MIL-88A and imidazolyl groups of histidine, His-tagged OpdA was used for subsequent experiments.

Synthesis of MIL-88A

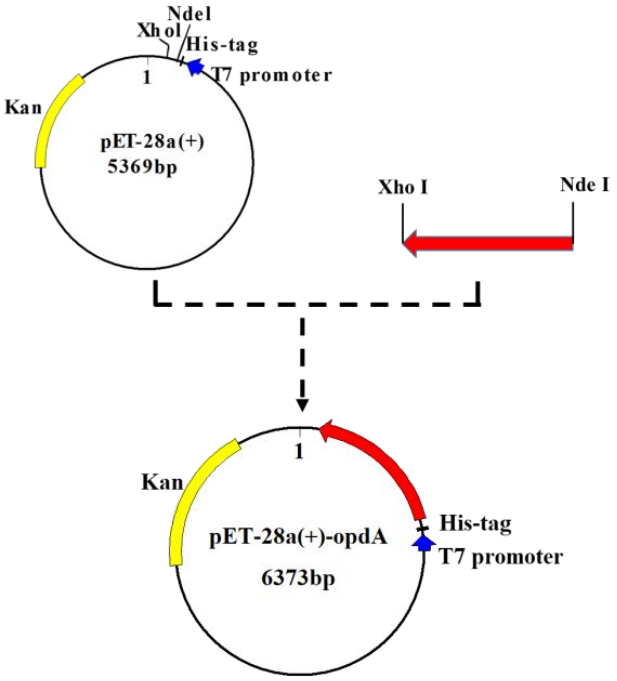
MIL-88A was prepared by hydrothermal synthesis method. Briefly, 0.542 g $FeCl_3 \cdot 6H_2O$ and 242 mg of fumaric acid were dissolved in 50 mL of ultrapure water, and then the homogeneous solution was transferred into a Teflon-lined stainless steel autoclave and reacted for 24 h at 65 °C. The reaction solution was centrifuged. The obtained MIL-88A was washed with water and ethanol repeatedly, and then dried in a vacuum oven below 60 °C for 12 h.

Calculation of protein loading

Protein loading was calculated by the ratio of the immobilized protein to the MIL-88A. The immobilized protein was calculated by the reduction of protein in crude enzyme solution before and after immobilization."

Protein loading was calculated by using the following equation:

$$\text{Protein loading} = \frac{\text{total protein content (mg)} - \text{supernatant protein content after immobilization (mg)}}{\text{the quality of MIL} - 88\text{A(g)}}$$



Scheme S1. The engineered plasmid pET-28a.

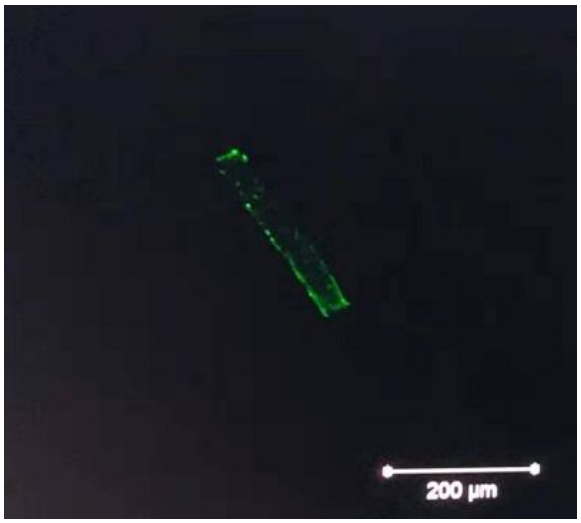


Figure S1. CLSM image of OpdA@MIL-88A.

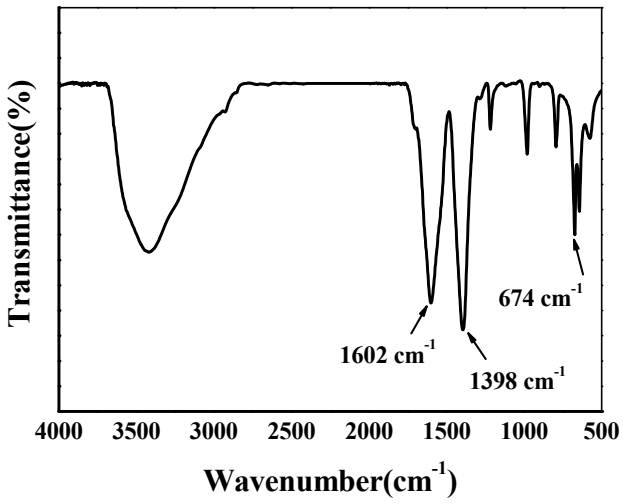


Figure S2. FTIR spectrum of MIL-88A

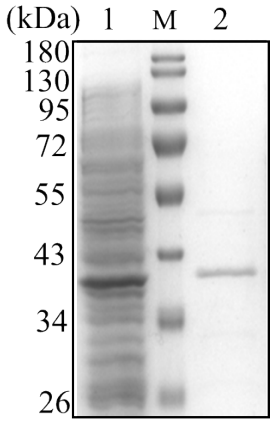


Figure S3. SDS-PAGE analysis. Lane M: protein marker; lane 1: crude cell lysates; lane 2: protein that bounded to MIL-88A

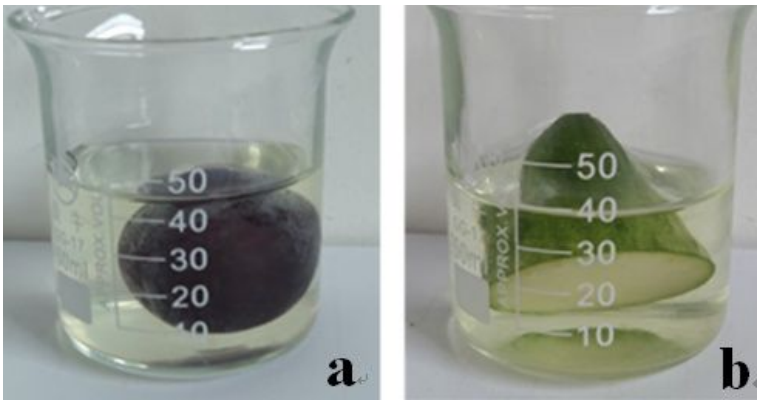


Figure S4. Degradation of 0.38 μmol methyl parathion from contaminated grapes (a, 12.02 g) and cucumbers (b, 10.03 g) in 25 mL tap water using 10 mg OpdA@MIL-88A

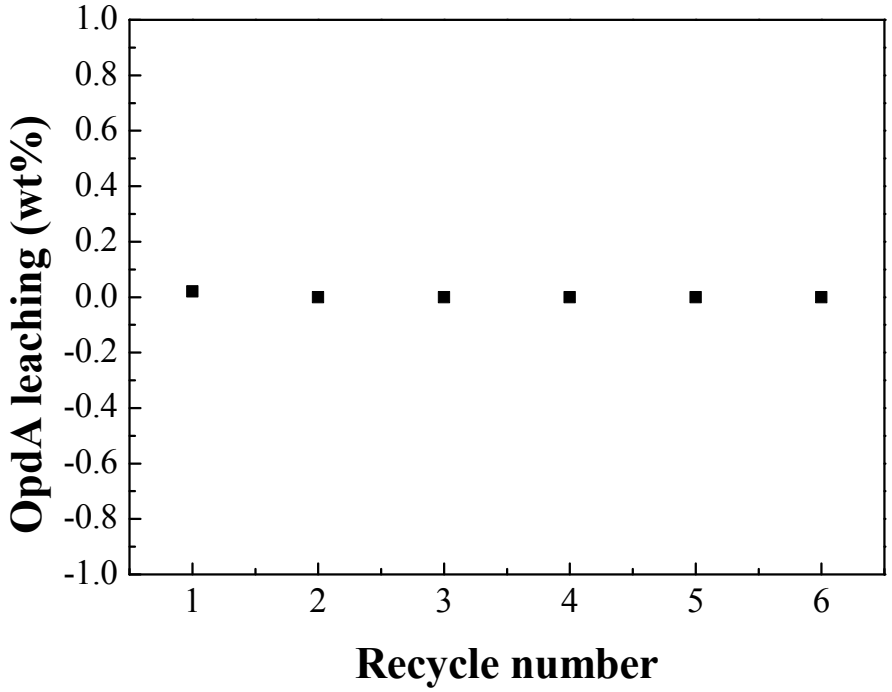


Figure S5. Weight percentage of OpdA leaching from OpdA@MIL-88A in degradation cycles

Table S1. The kinetic parameters for free OpdA and OpdA@MIL-88A

Kinetic parameters	Methyl parathion	
	Free OpdA	OpdA@MIL-88A
K_m (μM)	367.0	108.2
V_{max} (μM/min)	30.24	37.22

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