

## Electronic Supplementary Information

### **An integrated nanocatalyst combining enzymatic and metalorganic frameworks catalysis for cascade degradation of organophosphate nerve agents**

Haibin Li,<sup>a</sup> Li Ma,<sup>a</sup> Liya Zhou,<sup>a</sup> Jing Gao,<sup>\* a</sup> Zhihong Huang,<sup>a</sup> Ying He,<sup>a</sup> and Yanjun Jiang<sup>\* a b</sup>

<sup>a</sup> School of Chemical Engineering and Technology, Hebei University of Technology,  
8 Guangrong Road, Hongqiao District, Tianjin, 300130, P. R. China

<sup>b</sup> National-Local Joint Engineering Laboratory for Energy Conservation of Chemical  
Process Integration and Resources Utilization, Hebei University of Technology,  
Tianjin 300130, China

#### **Corresponding Author**

\* E-mail: [yanjunjiang@hebut.edu.cn](mailto:yanjunjiang@hebut.edu.cn); [jgao@hebut.edu.cn](mailto:jgao@hebut.edu.cn);

Fax: 86-22-60204294; Tel: 86-22-60204945

## **Experimental section**

### **Materials**

All reagents and solvents were purchased from commercial suppliers and used without further purification. All the chemicals used for the synthesis of MIL-100(Fe) and MOF-808 in this study were purchased from Aladdin (Shanghai, China). N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC•HCl, 99 %), N-hydroxysuccinimide (NHS, 97 %), phenol, sodium hydroxide (NaOH) and fluorescein isothiocyanate (FITC) were purchased from Tianjin Chemical Reagent Company (Tianjin, China). Organophosphorus hydrolase (OPH, EC 3.1.8.1 from *Pichia pastoris*) was purchased from Beijing Schengenbiya Bioengineering Technology Co., Ltd (Beijing, China). Parathion-methyl was purchased from Dr.Ehrenstorfer GmbH (Augsburg, Germany). Paraoxon-methyl, Paraoxon-ethyl and Parathion-ethyl was purchased from shanghai sanshu biotechnology CO., LTD (Shanghai, China).

### **Characterization**

Scanning electron microscopy (SEM) images were recorded on Nova Nano SEM450 field-emission microscope at an accelerating voltage of 200 kV. Powder X-ray diffraction (XRD) patterns were recorded using a Bruker AXS D8 Discover X-Ray diffractometer with a Cu K $\alpha$  anode ( $\lambda = 0.15406$  nm) at 40 kV and 40 mA. Fourier transform infrared spectroscopy (FT-IR) spectra were measured with a Bruker VECTOR22 spectrometer. The metal contents of the samples were determined quantitatively by atomic absorption spectroscopy (AAS) on a Thermo-M6 instrument. Confocal laser scanning microscopy (CLSM) micrographs were recorded on a Leica

TCS SP5 optical microscope with excitation wavelength of 488 nm and emission wavelength of 525 nm. Before tested, OPH were marked with fluorescein isothiocyanate (FITC) and then immobilized on the MIL-100(Fe). The intermediates and end products were determined by gas chromatography-mass spectrometry (GC/MS) on a TRACE DSQ instrument.

### **Synthesis of MIL-100(Fe)**

MIL-100(Fe) was prepared following previously reported procedures with slight modifications.<sup>1</sup> Specifically, 112 mg metal iron, 280 mg trimesic acid, 0.178 mL HF, 0.084 mL HNO<sub>3</sub> and 100 mL H<sub>2</sub>O were put into a Teflon-lined stainless-steel bomb and heated up to 160°C and then kept at this temperature for 8 h. After being cooled down to room temperature, the orange solid was recovered by centrifugation and washing with DMF and water for three times, respectively. Finally, the purified product was dried at 80 °C under vacuum overnight.

### **Synthesis of OPH@MIL-100(Fe)**

OPH was immobilized on the MIL-100(Fe) by covalent bonding method. Firstly, the MIL-100(Fe) was activated by EDC/NHS mixture. Specifically, 200 mg of MIL-100(Fe) in 20 mL of phosphate buffer solution (50 mM, pH 8) were sonicated for 10 min to obtain a homogeneous dispersion. This dispersion was mixed with 10 mL of 400 mM EDC and 100 mM NHS in phosphate buffer solution (50 mM, pH 8) and shaken gently for 2 h at 25°C. Subsequently, 10 mg activated MIL-100(Fe) was stirred in 2 ml OPH solution (0.3 mg/ml) for 12 h at 25°C. Finally, the OPH@MIL-

100(Fe) was centrifuged and stored in a refrigerator at 4°C.

### **Synthesis of MOF-808**

MOF-808 was synthesized following previously reported procedures.<sup>2</sup> H<sub>3</sub>BTC (0.105 g) and ZrCl<sub>4</sub> (0.35 g) were dissolved in a solvent mixture of DMF/formic acid (45 ml/45 ml) and placed in a 200 ml flask with three necks. The flask was then transferred into a microwave oven for 30 min at 130°C. Next, the resulting products were filtered and washed with ethanol for three times and then dried at 60°C under vacuum overnight.

### **Synthesis of OPH@MOF-808**

The synthesis of OPH@MOF-808 is similar to that of OPH@MIL-100(Fe). The MOF-808 was also activated by EDC/NHS mixture. Then, 10 mg activated MIL-808 was stirred in 2 ml OPH solution (0.6 mg/ml) for 12h at 25°C. Finally, the OPH@MIL-808 was centrifuged and stored in a refrigerator at 4°C. Determined by the Bradford method, the OPH loading was 96 mg/g<sub>MOF-808</sub>.

### **Optimization of the degradation conditions for organophosphate nerve agents**

The order of the optimization is as follows: substrate concentration, pH, temperature and NaBH<sub>4</sub> concentration. Based on the preliminary experiments, 0.75 μmol/ml of the substrate concentration was chosen firstly. Then, the pH value was optimized with the substrate concentration of 0.75 μmol/ml (about 197 ppm), the temperature of 30°C and the NaBH<sub>4</sub> concentration of 30 mg/ml. The temperature was optimized with the substrate concentration of 0.75 μmol/ml, the pH value of 9.0 and

the NaBH<sub>4</sub> concentration of 30mg/ml. The NaBH<sub>4</sub> concentration was optimized with the substrate concentration of 0.75 μmol/ml, the pH value of 9.0 and the temperature of 40°C.

### **The hydrolysis activity assay of free OPH and OPH in OPH@MIL-100(Fe)**

A colorimetric assay was used to assess the hydrolysis activity of the free OPH. Typically, the parathion-methyl solution (50μl, 30 μmol/mL in acetonitrile) was added into the mixture of the free OPH (1 mg) and Tris-HCl (1.95 mL, 50 mM, pH 9.0) at 40°C. After reaction for 5min, trichloroacetic acid solution (1ml, 10% (W/W)) was added into the reaction solution to end the hydrolysis reaction. The resulting solution was determined using a spectrophotometer at 409 nm. One unit of the hydrolysis activity was defined as the amount of enzyme needed to release 1μmol of 4-NP per minute under assay conditions.

A similar method is used to measure the hydrolysis activity of OPH in OPH@MIL-100(Fe). Specifically, the parathion-methyl solution (50μl, 30 μmol/mL in acetonitrile) was added into the mixture of the OPH@MIL-100(Fe) (20 mg) and Tris-HCl (1.95 mL, 50 mM, pH 9.0) at 40°C. After reaction for 5min, trichloroacetic acid solution (1ml, 10% (W/W)) was added into the reaction solution and then the mixture was separated by centrifugation to obtain supernatant. The supernatant contained 4-NP was determined using a spectrophotometer at 409 nm. One unit of the hydrolysis activity was defined as the amount of enzyme needed to release 1μmol of 4-NP per minute under assay conditions.

### **Degradation activity assay**

A colorimetric assay was used to assess the catalytic property of the OPH@MIL-100(Fe). Typically, NaBH<sub>4</sub> (60 mg) and the substrate solution (50 μl, 30 μmol/mL in acetonitrile) was added into the mixture of the OPH@MIL-100(Fe) (20 mg) and Tris-HCl (1.95 mL, 50 mM, pH 9.0) at 40°C. After reaction for 5min, the mixture was separated by centrifugation to obtain supernatant. Degradation activity was defined as micromoles of 4-AP produced per gram of OPH@MIL-100(Fe) per minute. The conversion (%) was defined as the molar ratio between produced 4-AP and initial parathion-methyl.

### **Degradation activity assay of the three different catalysts for cascade reaction**

Degradation activity was compared when the cascade reaction catalyzed by OPH+MIL-100(Fe), OPH@MIL-100(Fe), and OPH@MOF-808+MIL-100(Fe). The same concentration of OPH and MIL-100(Fe) were used in each reaction solution. Specifically, NaBH<sub>4</sub> (600 mg) and the substrate solution (0.5 ml, 30 μmol/mL in acetonitrile) was added into the mixture of the OPH@MIL-100(Fe) (200 mg) and Tris-HCl (19.5 mL, 50 mM, pH 9.0) at 40°C. For the other two cases, OPH@MIL-100(Fe) was replaced by free OPH (10.066 mg) and MIL-100(Fe) (189.92 mg), OPH@MOF-808 (114.92 mg) and MIL-100(Fe) (189.92 mg), respectively.

### **Measurement of 4-aminophenol (4-AP)**

The measurement method of 4-AP was based on the previously reported

literature.<sup>3</sup> Its mechanism is that 4-AP and phenol can generate blue compounds in air under alkaline conditions and the compounds has maximum absorption peak at 630 nm. The relationship between the absorbance and the concentration of 4-AP obeys the Lambert-Beer law. More importantly, in this method, the influence of the substrate (organophosphate nerve agents) and the intermediate (4-NP) on the experimental results can be excluded. Specifically, 1 ml of the 4-AP containing supernatant was mixed with 1 ml of phenol solution (5 % (w/w)) and 1 ml of NaOH (0.5 % (w/w)) solution. Subsequently, the mixed solution was incubated at 60°C for 30min and then cooled to room temperature. The absorbance of the resulting light blue solution was measured at 630 nm using an ultraviolet spectrophotometer.

### **The hydrolysis activity of OPH and the hydrogenation activity of MIL-100(Fe) during multiple catalytic cycles**

For easy reuse, OPH@MOF-808 instead of free OPH was used to measure the change of the hydrolysis activity of OPH during multiple catalytic cycles. Typically, NaBH<sub>4</sub> (60 mg) and parathion-methyl solution (50 µl, 30 µmol/mL in acetonitrile) was added into the mixture of the OPH@MOF-808 (11.492 mg) and Tris-HCl (1.95 mL, 50 mM, pH 9.0) at 40°C. After reaction for 5 min, the mixture was centrifuged and the supernatant contained 4-NP was spectrophotometrically measured at 409 nm. Then, all obtained precipitation was repeatedly washed with Tris-HCl for several times to remove any residual substrate or product. After centrifugation, the OPH@MOF-808 was used in next cycle under same conditions. Hydrolysis activity

was defined as micromoles of 4-NP produced per gram of OPH@MOF-808 per minute.

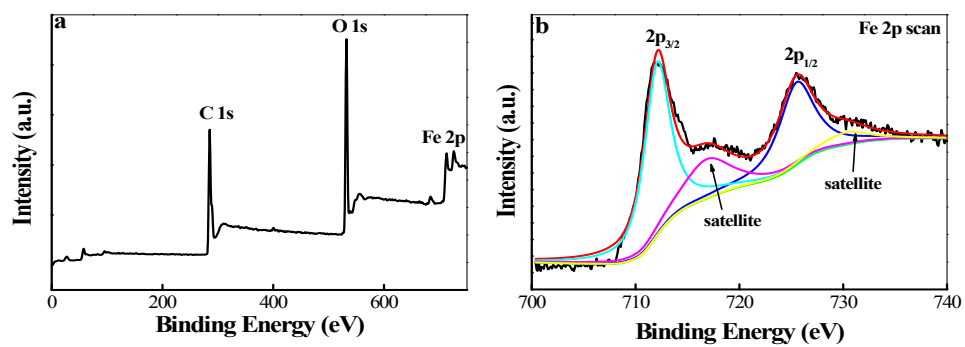
A similar method is used to measure the hydrogenation activity of the MIL-100(Fe). Specifically, NaBH<sub>4</sub> (60 mg) and the 4-NP solution (50 µl, 30 µmol/mL in acetonitrile) was added into the mixture of the MIL-100(Fe) (18.992 mg) and Tris-HCl (1.95 mL, 50 mM, pH 9.0) at 40°C. After reaction for 5 min, the mixture was filtered and the supernatant contained 4-AP was measured by the method in *Measurement of 4-aminophenol (4-AP)*. Then, all obtained precipitation was repeatedly washed with Tris-HCl for several times to remove the residual substrate or product. After centrifugation, the MIL-100(Fe) was used in next cycle under same conditions. Hydrogenation activity was defined as micromoles of 4-AP produced per gram of of MIL-100(Fe) per minute.



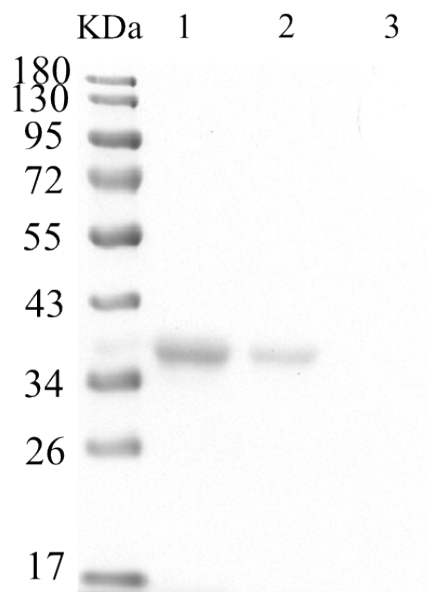
**Table S1.**

	Acute toxicity*
	LD50 Oral Rat (mg/kg)
<b>Paraoxon-methyl</b>	3.27
<b>Parathion-methyl</b>	14
<b>Paraoxon-ethyl</b>	1.8
<b>Parathion-ethyl</b>	13
<b>4-Nitrophenol (4-NP)</b>	202
<b>4-Aminophenol (4-AP)</b>	375

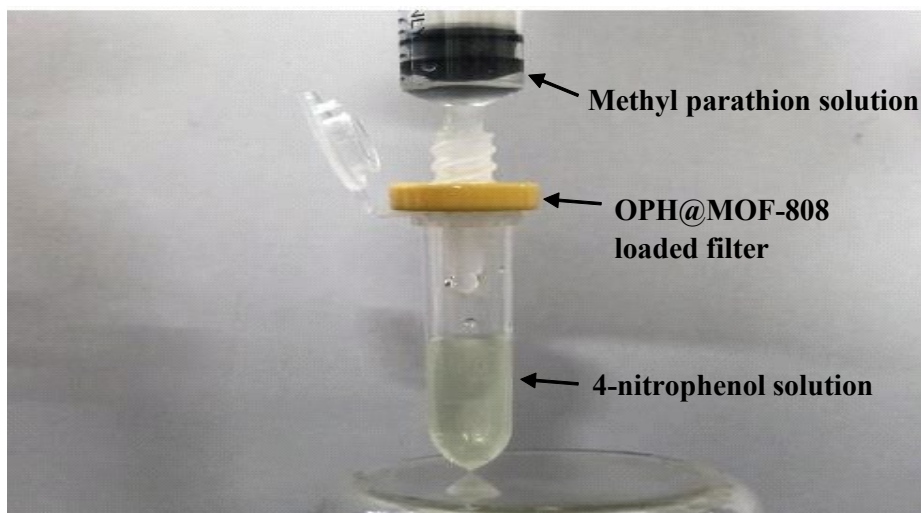
\* The toxicological information was obtained from the MSDS (www.msdsonline.com).



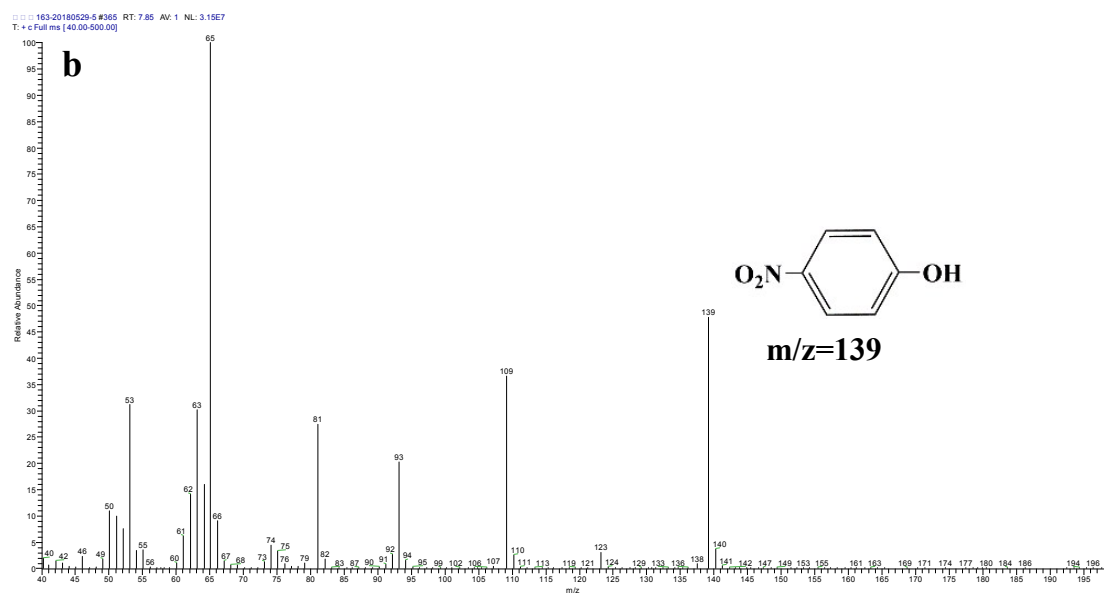
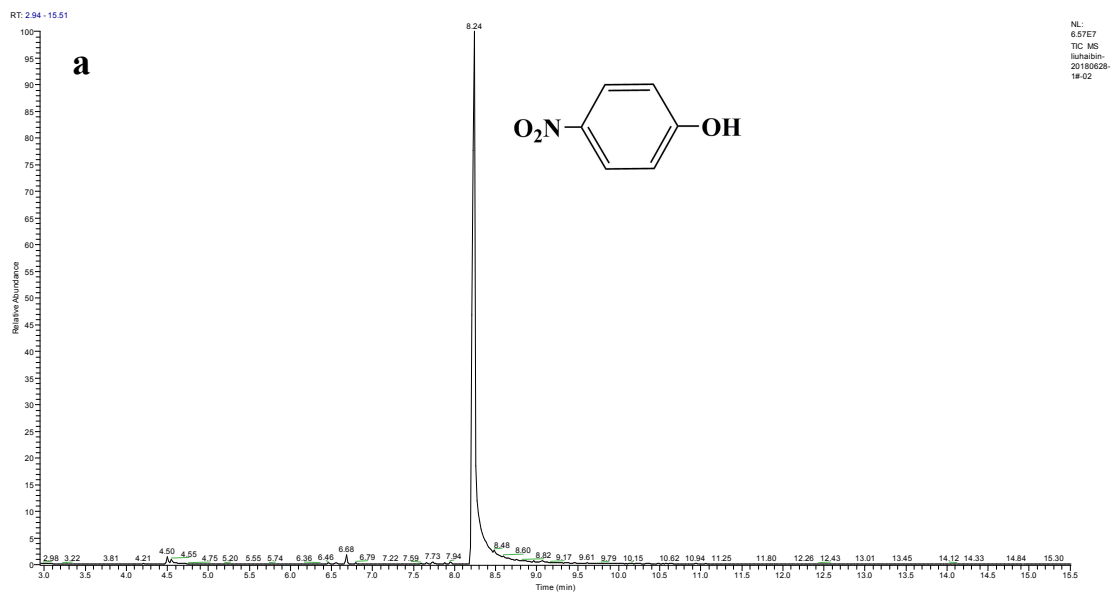
**Figure S1.** XPS spectra of MIL-100(Fe): (a) survey spectrum, (b) high resolution of Fe spectrum.



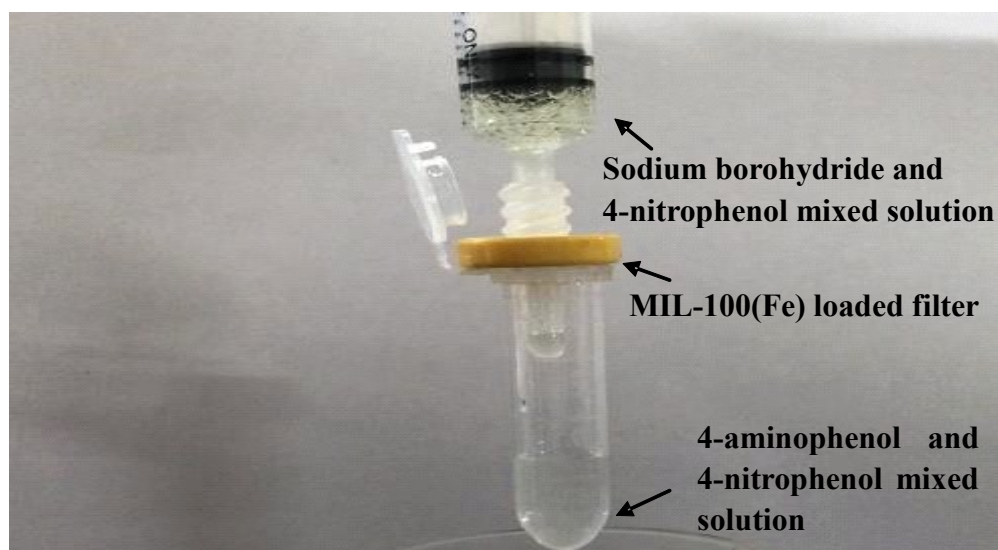
**Figure S2.** SDS-PAGE gel. Lane 1: free OPH; Lane 2: acid-treated OPH@MIL-100(Fe) solution; Lane 3: sodium dodecanoate solution that washed the OPH@MIL-100(Fe).



**Figure S3.** Schematic representation of the OPH@MOF-808-based plug-flow reactor for hydrolysis of parathion-methyl into 4-NP.

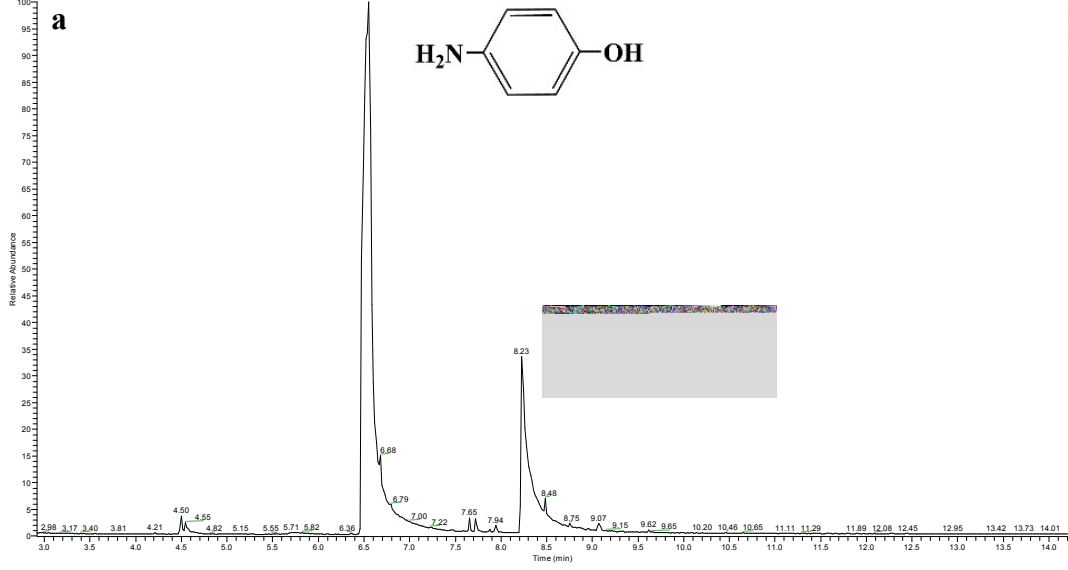


**Figure S4.** (a) GC chromatogram of the dichloromethane extract of hydrolysis product of parathion-methyl; (b) Representative mass spectrum of the peak appearing at 8.24min in GC chromatogram.



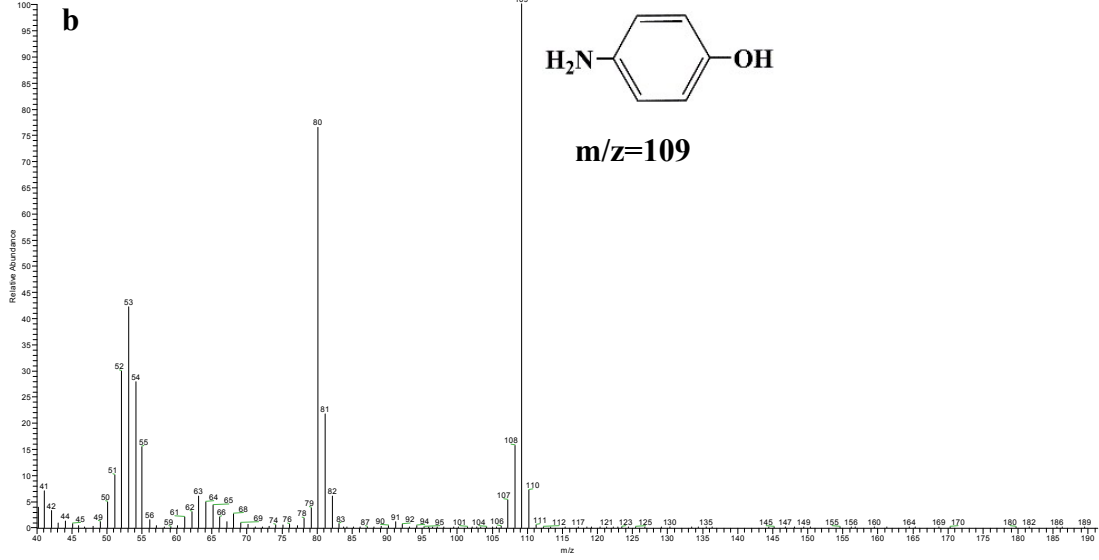
**Figure S5.** Schematic representation of the MIL-100(Fe)-based plug-flow reactor for hydrogenation of 4-NP into 4-AP in the presence of  $\text{NaBH}_4$ .

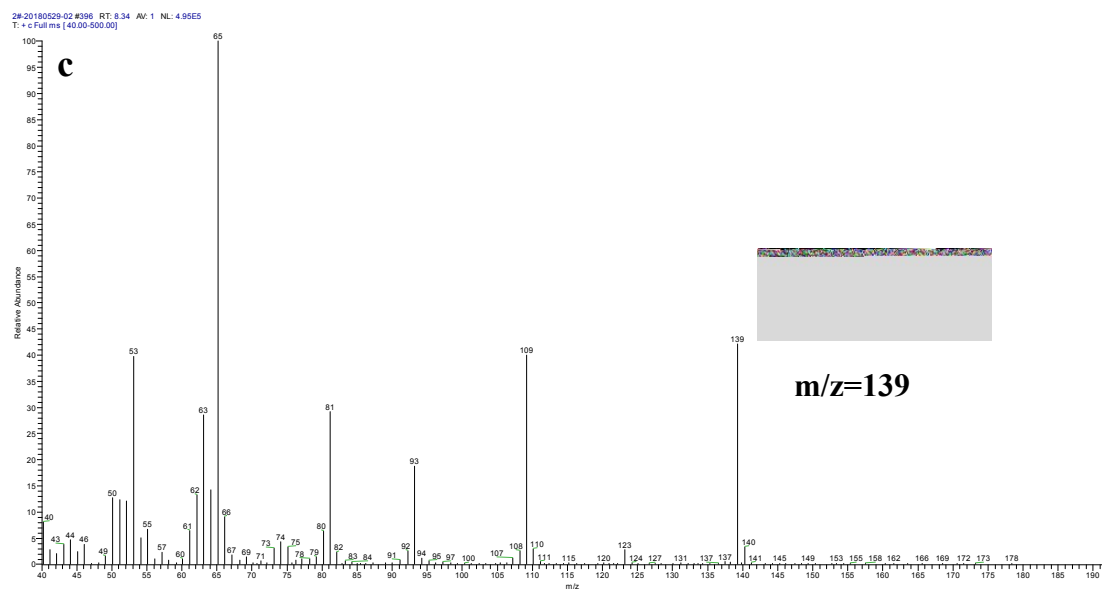
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NL:  
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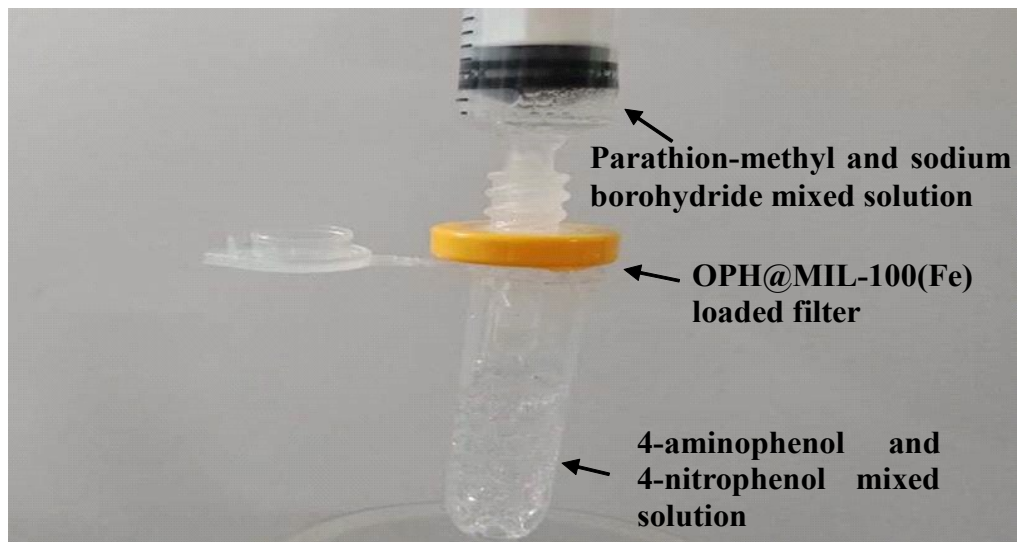
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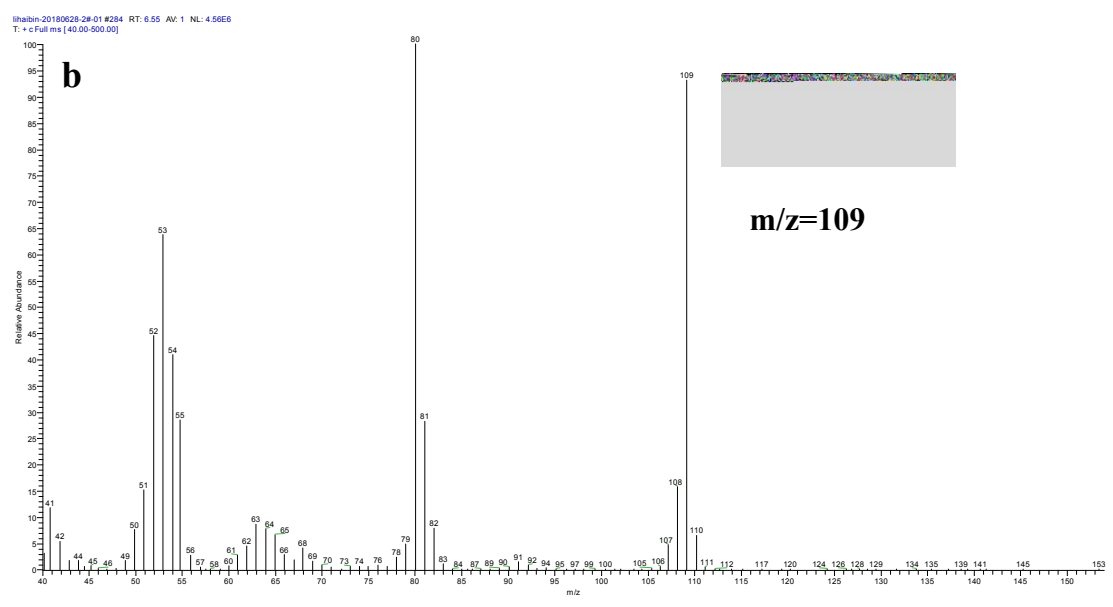
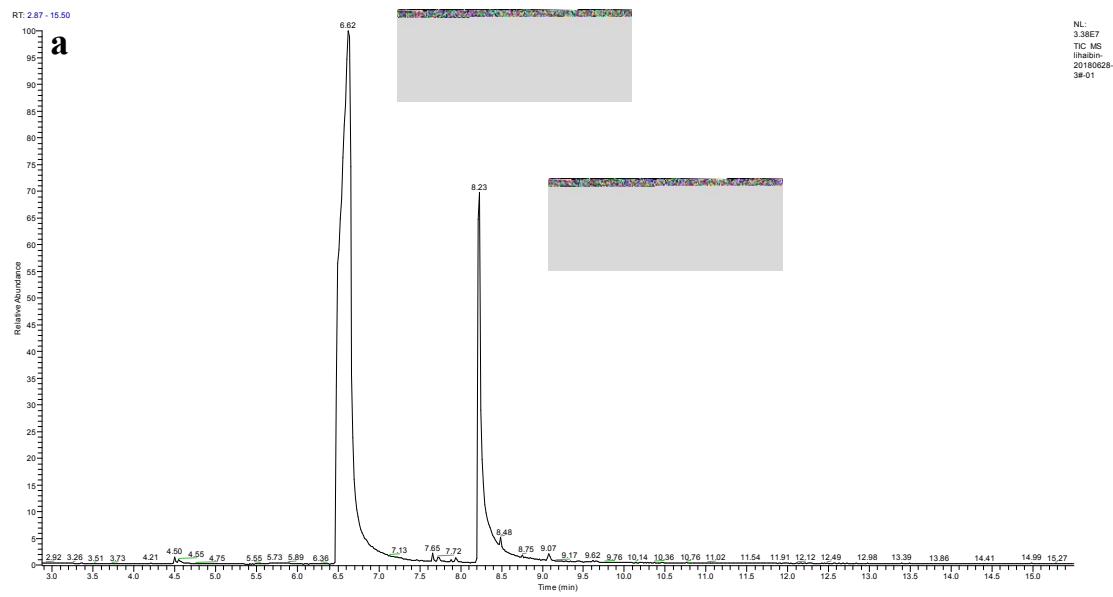


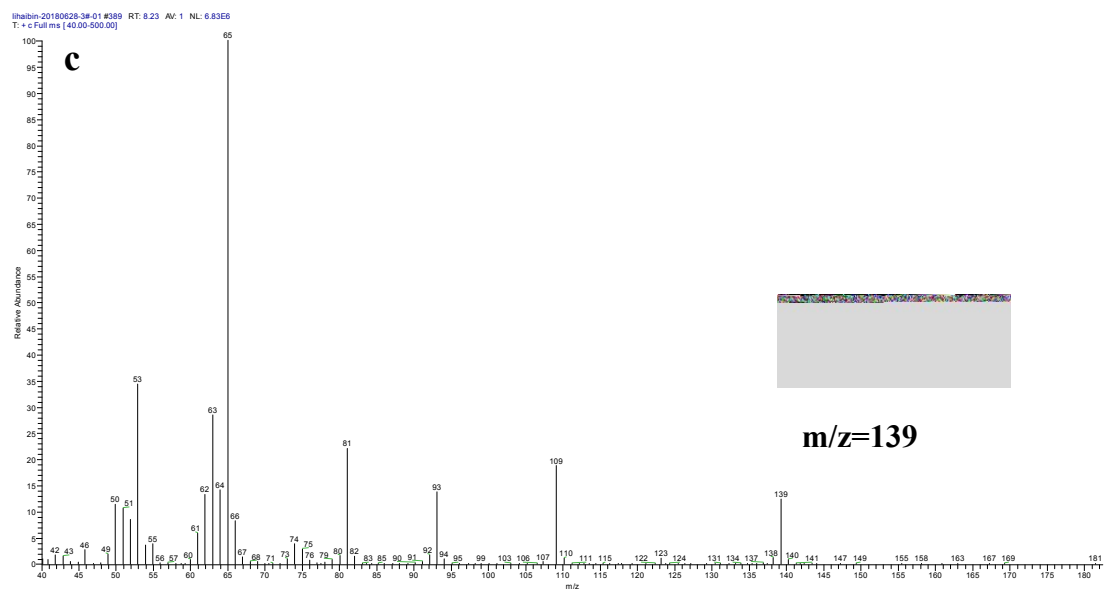
**Figure S6.** (a) GC chromatogram of the hydrogenation products of 4-NP; (b) Representative mass spectrum of the peak appearing at 6.55min in GC chromatogram; (c) Representative mass spectrum of the peak appearing at 8.23min in GC chromatogram.



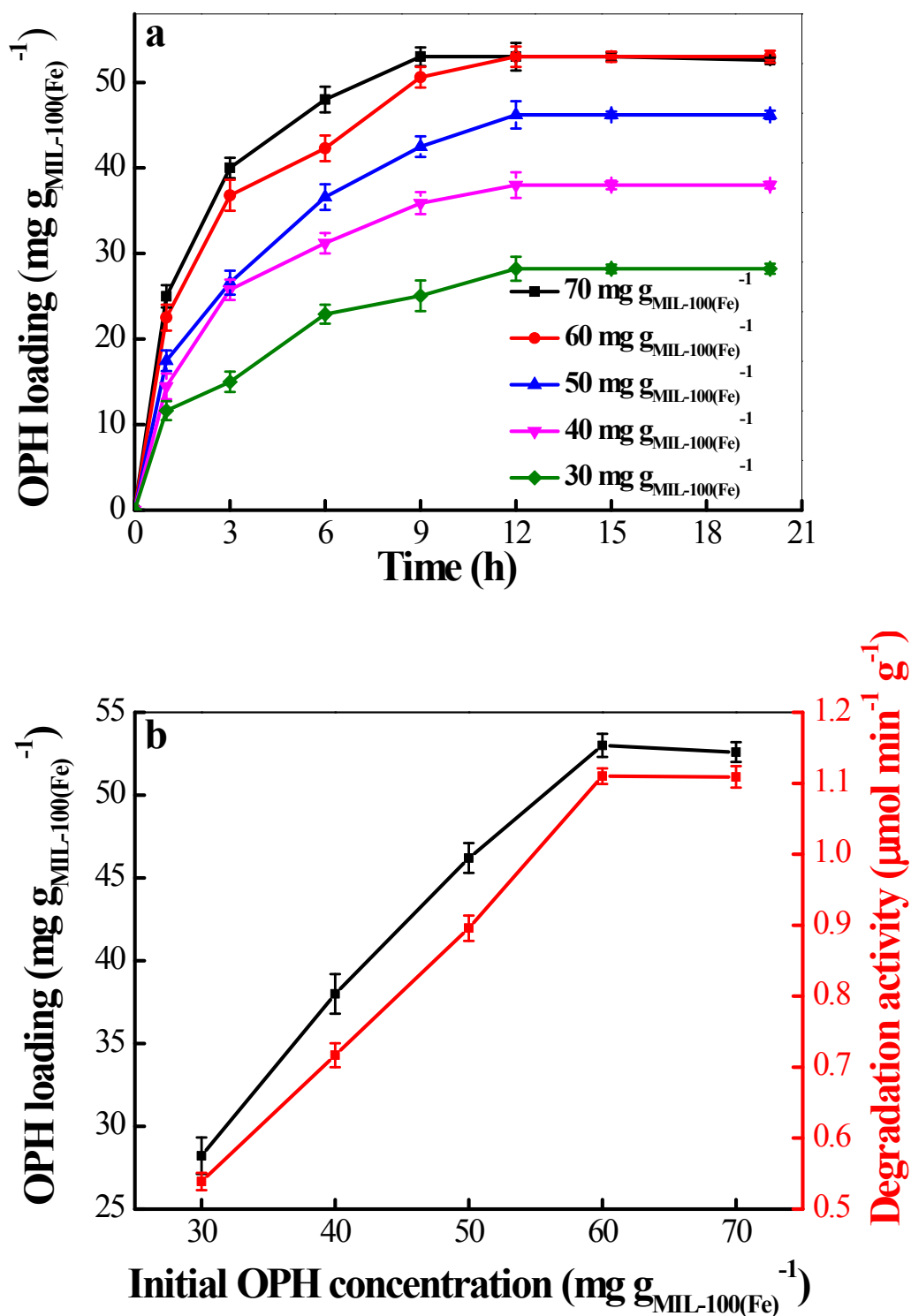


**Figure S7.** Schematic representation of the OPH@MIL-100(Fe)-based plug-flow reactor for degradation of parathion-methyl in the presence of  $\text{NaBH}_4$ .

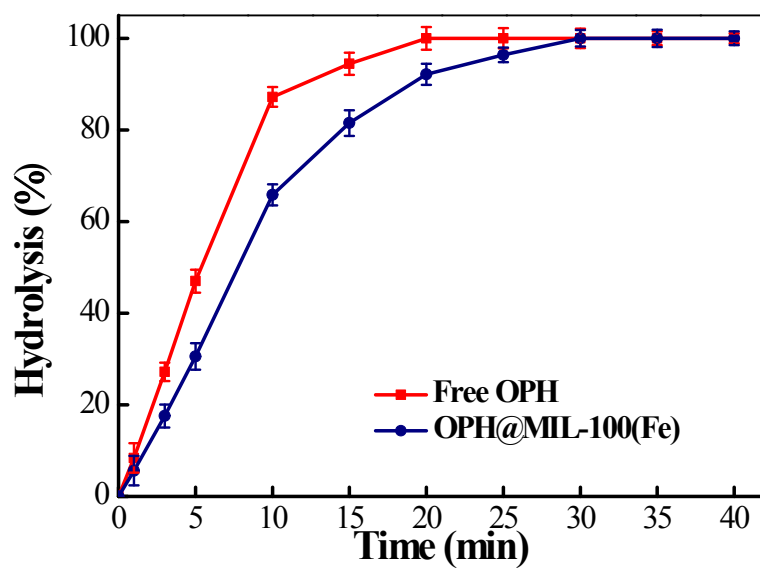




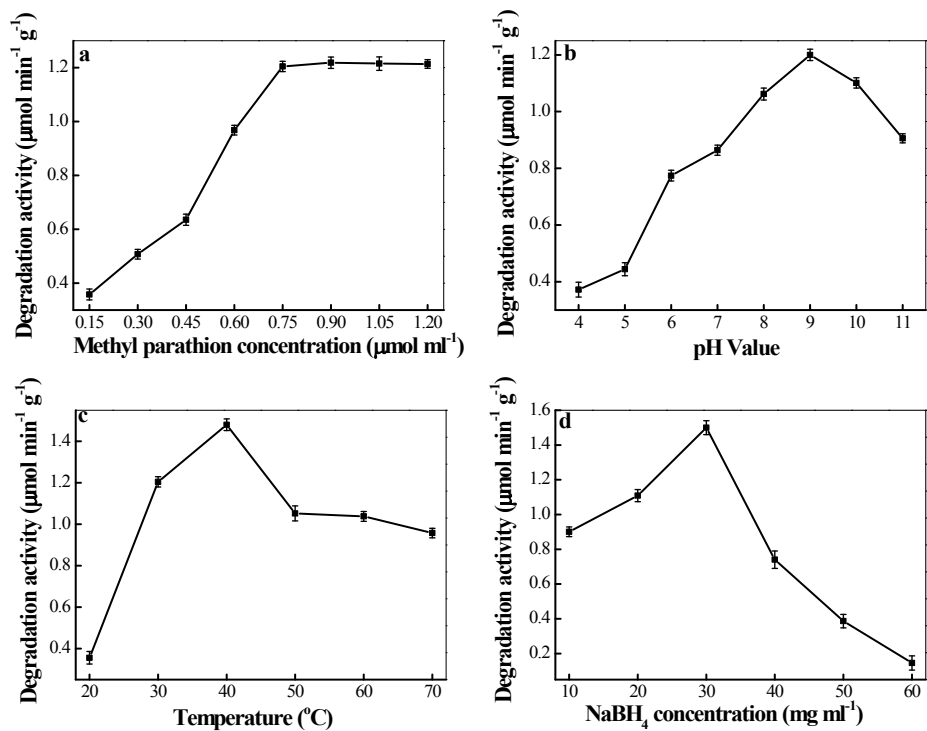
**Figure S8.** (a) GC chromatogram of the degradation product of parathion-methyl in the presence of  $\text{NaBH}_4$ ; (b) Representative mass spectrum of the peak appearing at 6.62min in GC chromatogram; (c) Representative mass spectrum of the peak appearing at 8.23min in GC chromatogram.



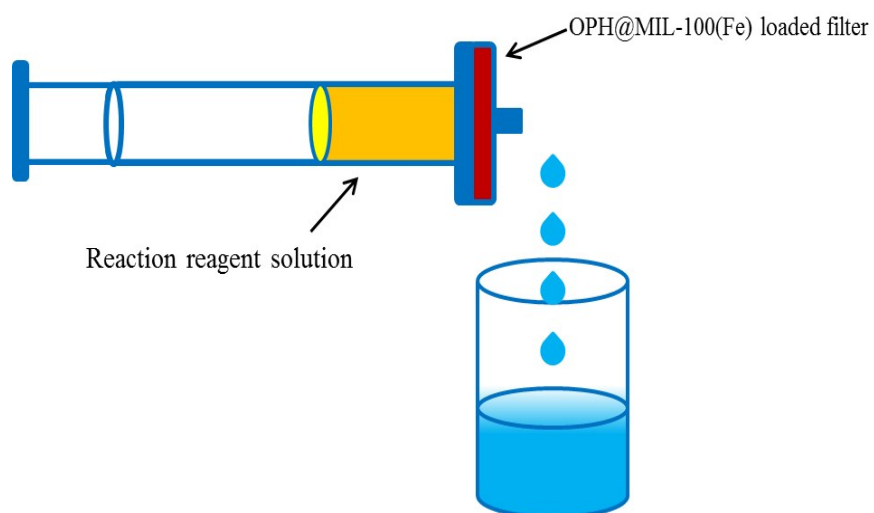
**Figure S9.** (a) OPH loading profiles with time at different initial OPH concentration; (b) Effect of initial OPH concentration on degradation activity of OPH@MIL-100(Fe) and OPH loading amount at 12h.



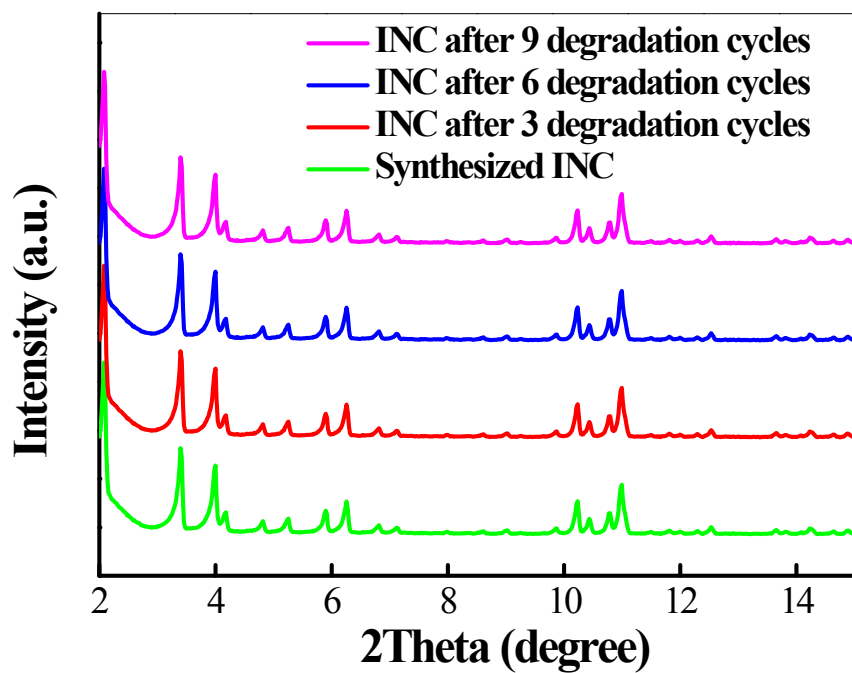
**Figure S10.** Hydrolysis profiles of parathion-methyl catalyzed by free OPH and OPH@MIL-100(Fe).



**Figure S11.** The catalytic activity of OPH@MIL-100(Fe) under different conditions of (a) parathion-methyl concentration, (b) pH value, (c) temperature and (d)  $\text{NaBH}_4$  concentration.

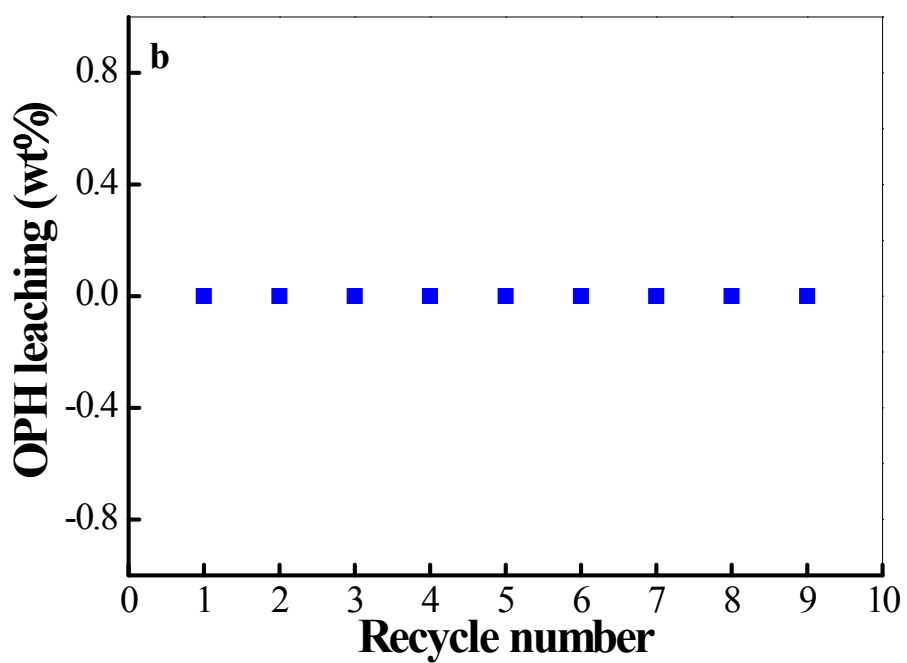
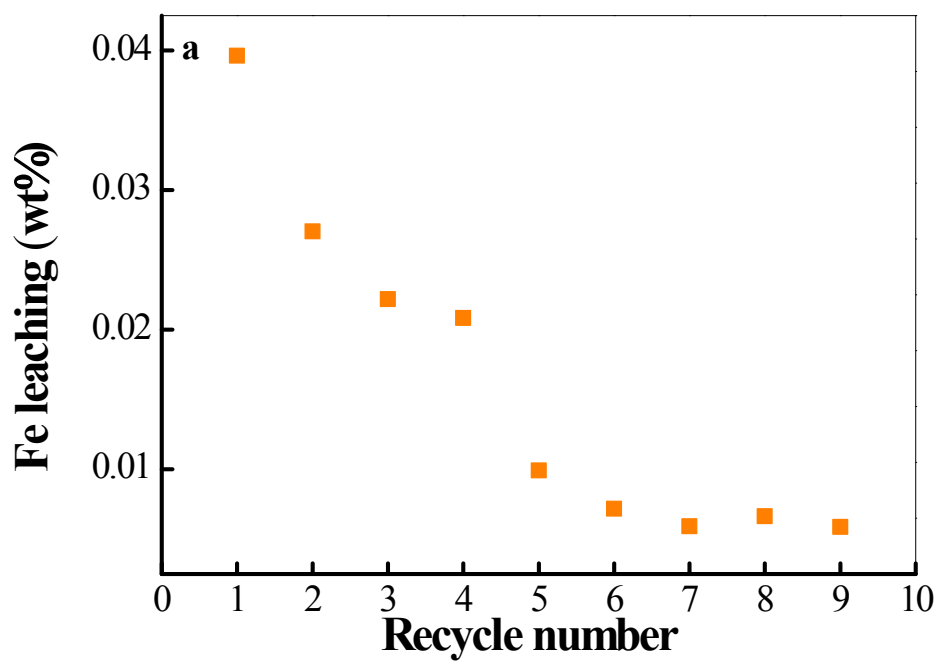


**Figure S12.** Schematic representation of the OPH@MIL-100(Fe) plug-flow reactor.

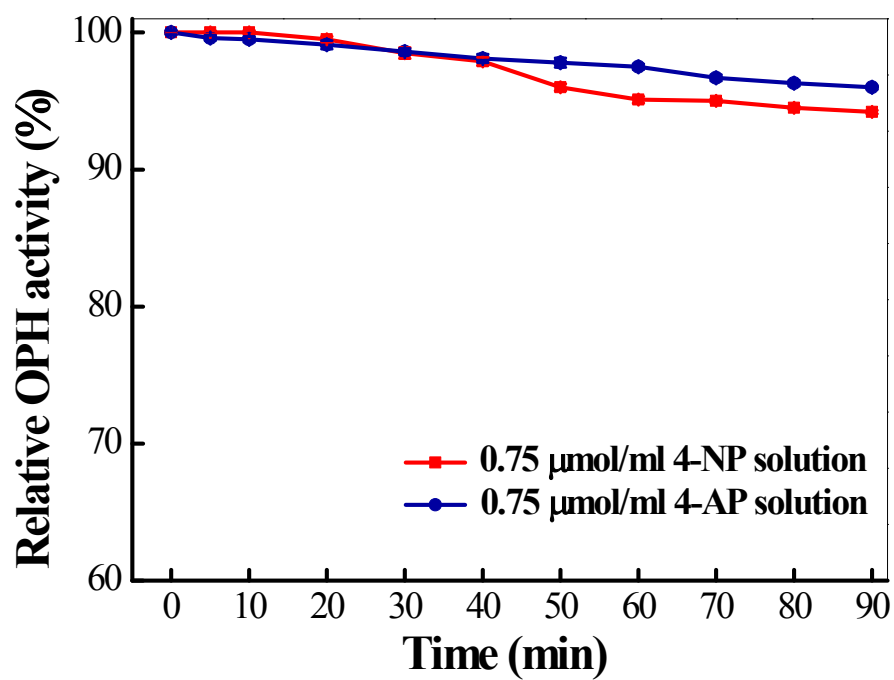


**Figure S13.** Powder X-ray diffraction patterns of OPH@MIL-100(Fe) after multiple degradation cycles.

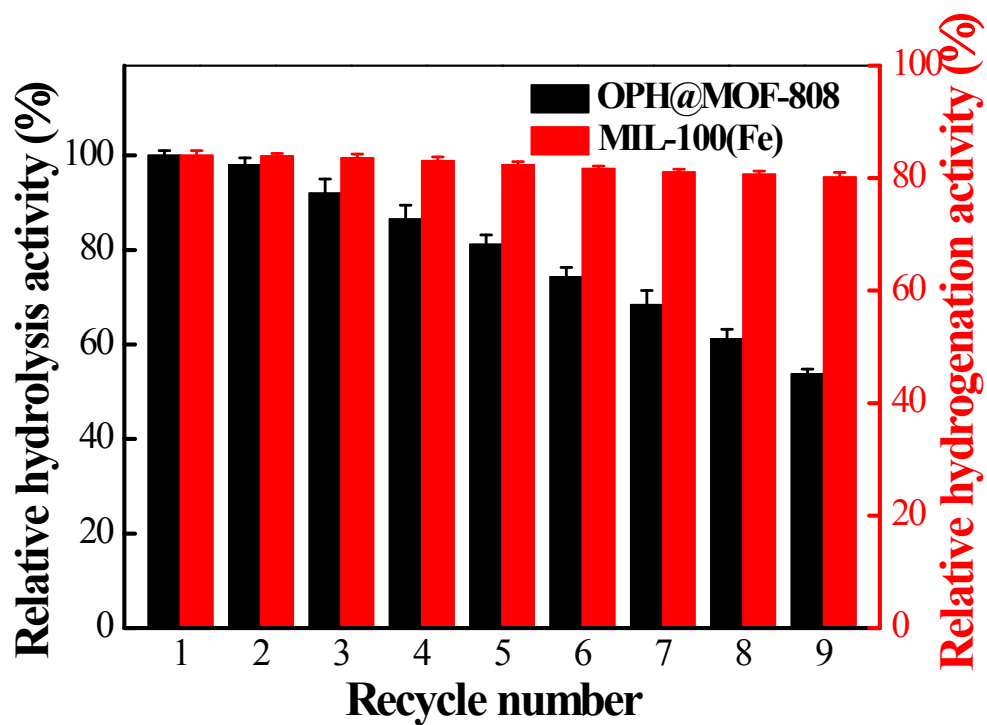




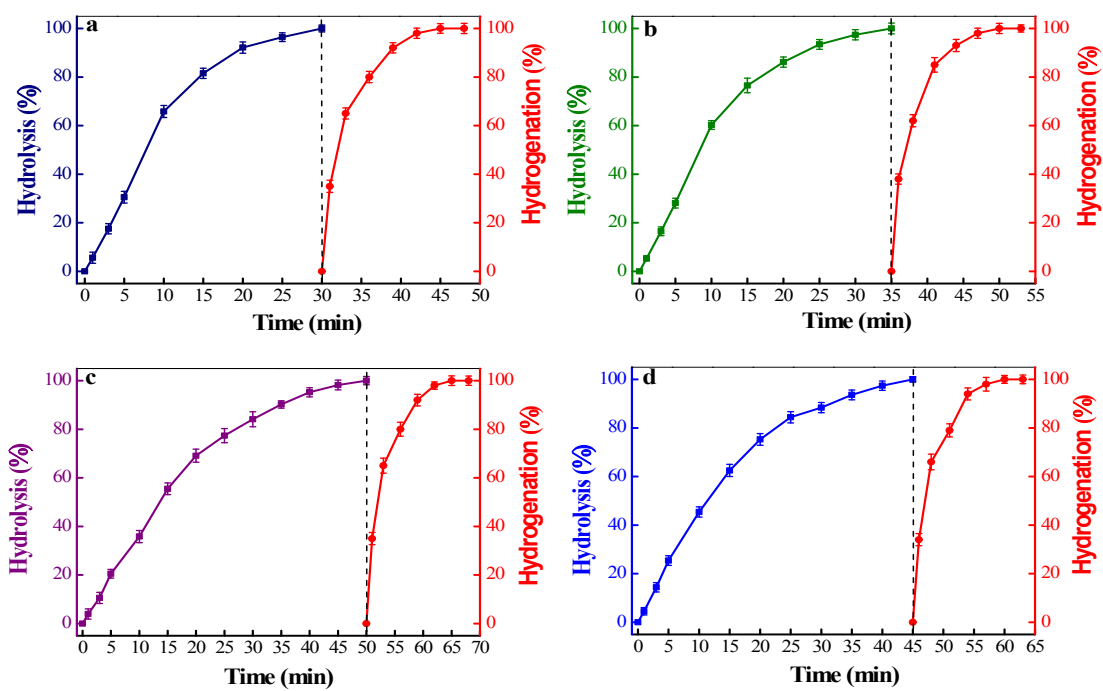
**Figure S14.** Weight percentage of (a) Fe leaching and (b) OPH leaching after multiple degradation cycles of OPH@MIL-100(Fe).



**Figure S15.** Relative activity of OPH in OPH@MIL-100(Fe) after being treated in 4-NP (red line) and 4-AP (blue line) solution for different time.



**Figure S16.** The relative hydrolysis activity of OPH and relative hydrogenation activity of MIL-100(Fe) after multiple degradation cycles.



**Figure S17.** Cascade degradation process including hydrolysis and hydrogenation under different substrates ( $\text{NaBH}_4$  was added after the hydrolytic step). (a) parathion-methyl; (b) paraoxon-methyl; (c) parathion-ethyl; (d) paraoxon-ethyl.

1. H. Patricia, S. Suzy, S. Christian, H. Do-Young, S. You-Kyong, C. Jong-San, G. Jean-Marc, M. Irene and F. Gerard, *Chem. Commun.*, 2007, **27**, 2820-2822.
2. Z.-Q. Li, J.-C. Yang, K.-W. Sui and N. Yin, *Mater. Lett.*, 2015, **160**, 412-414.
3. S.-P. Wei, G.H. Qin, L.-J. Zhang and Z.-M. Wei, *Technology & Development of Chemical Industry*, 2002, **31**, 32-33. (in chinese with english abstract)