

## Electronic Supporting Information

### Identification of chlorpyrifos-degrading microorganisms in farmland soils via cultivation-independent and -dependent approaches

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### **1. MNPs synthesis**

The 1 mL of FeCl<sub>2</sub> (1.0 M) was mixed with 2 mL of FeCl<sub>3</sub> (2.0 M). Then, 25 mL of NaOH (2.0 M) was added dropwise to the mixture. After 30 min of continuous shock, the black Fe<sub>3</sub>O<sub>4</sub> composite MNPs were retrieved using a magnet and then washed with 30 mL of deionized water several times until the pH 7.0. The concentration of synthetic MNPs was 9.1 g/L.

### **2. Nucleic acid extraction**

DNA was extracted via a Power Soil DNA extraction kit (MOBIO, USA) in accordance with the manufacturer's instruction. To determine the microbial community structure, V3 and V4 regions of 16S rDNA were amplified with a primer pair of 27F (5'-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5'-TACCTTGTTACGACTT-3'). The PCR procedure was set at: 5 min at 94°C; 30 cycles of 1 min at 94°C, 1 min at 55°C, and 1.5 min at 72°C; followed by 5 min at 72°C. The PCR products were detected via 1% agarose gel electrophoresis and Nanodrop 2000, and purified by EZNA Gel Extraction Kit (Omega, USA). Sequencing libraries were generated using NEBNext®Ultra™DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) following manufacturer's instruction. The library was sequenced on an illumina Hiseq 2500 platform and 250 bp paired-end reads were generated.

### **3. The composition of culture medium**

**Enrichment media:** 5 g beef extract, 10 g peptone, 5 g NaCl, 0.5 g NaH<sub>2</sub>PO<sub>4</sub>, and 1.5 g Na<sub>2</sub>HPO<sub>4</sub> in 1L of sterilized water, with CPF concentration 20 mg/L.

**Mineral medium agar plates (MMA)** which was prepared by adding 0.5 g  $\text{KH}_2\text{PO}_4$ , 0.5 g NaCl, 0.1 g  $\text{CaCl}_2$ , 0.2 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g  $\text{MnSO}_4$ , and 1.5 g  $(\text{NH}_4)_2\text{SO}_4$  in 1 L of sterilized water, and 1.5% (w/v) agar was added.

#### **4. CPF analysis**

The CPF extraction was conducted from soil by solid phase extraction, during each extraction procedure, 0.5 g NaCl, 2 mL acetone, and a certain volume of acetonitrile (3 mL, 2 mL, and 1 mL for the 3 extractions, respectively) were added into the supernatant and sonicated for 10 min. Supernatants from the 3 successive extractions were mixed, and subsequently dried at 30°C by a rotary evaporator. The CPF standards (concentration ranges 5-200 mg/mL) were used to derive the calibration curve (Fig. S2).

The volume was then adjusted to 5 mL with acetonitrile, followed by a membrane filtration (pore size, 0.22  $\mu\text{m}$ ; nylon) into brown vials for analysis. Concentrations of CPF and its metabolites were detected GC-MS (Shimadzu, QP2010SE) with a DB-5MS capillary column (30m length, 0.25mm diameter, 0.25 $\mu\text{m}$  thickness). A total of 5  $\mu\text{L}$  sample was injected in the splitless mode with a 3-min solvent delay time. The carrier gas was  $\text{N}_2$  (99%) at a rate of 36 mL/min, the hydrogen flow rate was 6 mL/min and the air flow rate was 60 mL/min. The GC oven temperature was set at 60°C for 1 min, raised to 200°C at a rate of 25°C/min and maintained at 200°C for 5 min, and finally raised to 300°C at a rate of 30°C/min and maintained at 300°C for 2 min. Electron impact (EI) source and selected ion monitoring (SIM) mode were used to identify the CPF degradation metabolites. The ion source temperature was 230°C

and scanning range was from 45 to 450 atomic mass units (amu). For CPF metabolites, the molecular mass of each metabolite was compared with previous studies and the database of CPF metabolites (National Institute of Standards and Technology, NIST), and the chemical structure of each metabolite was determined by the pattern of fragment ions in the mass spectrum.

**Table S1 The physicochemical properties data of the soil samples**

<b>Projects</b>	<b>Concentration</b>
pH	7.5
H <sub>2</sub> O (%)	16.4
Humus (g/kg)	10.3
SOM (g/kg)	17.7
TOC (g/kg)	10.3
TN (g/kg)	1.12
Available N (mg/kg)	79.3
TP (g/kg)	1.10
Available P (mg/kg)	52.5
Available K (mg/kg)	125

**Table S2 PCR primers used for the amplication of CPF-degrading genes.**

Target	Sequence(5'-3')
<i>opd2</i>	F:5'-ATGCACGAGCACGTCTTCATCATGA-3' R:5'-TCACTGATAGCCGCCCTGCCGCT-3'
<i>opd4</i>	F:5'-TTGCGCCATCTTGAACATGC R:5'-CAGGCAGTCGCACCCTATGC
<i>opdB</i>	F:5'-CGTCGTCGGCTGGGCAGGGT-3' R:5'-GCGTGCGGCCTACCTCGTTG-3'
<i>opdC2</i>	F:5-'ATGCGTCTTTTCTCGCTGAGC-3' R:5'-TCAGCGGTCGCTACGGATCGG-3'

**Table S3** Microbial taxonomic analysis in different treatments.

Kingdom	Phylum	Class	Order	Family	Relative Abundance (%)									
					OS_0	MMI_CP F_7	MMI_C PF_21	MMI_C PF_35	OS_CPF _7	OS_CPF _21	OS_CPF _35	MFC_ CPF_7	MFC_ CPF_21	MFC_ CPF_35
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	2	30.14	12.25	12.29	9.01	6.02	3.53	36.9	65.47	33.23
Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	13.41	3.44	0.2	0.73	2.79	0.36	0.26	0.74	0.22	0.46
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillales_Incertae Sedis XII	5.82	3.67	6.53	6.16	4.84	6.27	0	2.05	3.38	1.71
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	4.35	9.4	7.84	6.36	7.2	7.82	6.97	3.86	0.76	2.21
Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	3.08	1.42	1.57	2.14	3.64	3.15	3.7	3.13	3.45	13.05
Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	5.52	4.86	5.8	5.33	7.65	8.56	5.93	2.14	0.59	0.88
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	4.04	2.24	4.07	3.84	3.18	3.75	0.01	1.09	1.93	1.11
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	0.69	1.51	0.94	2.83	1.76	0.97	2.9	2.57	0.37	3.53
Bacteria	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	3.89	2.71	3.3	4.28	2.04	2.9	5.94	0.89	0.18	0.49
Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	0	0	0	0.31	0.01	0	0.02	18.97	0.39	18.82
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	2.41	2.98	3.95	2.99	3.84	3.76	1.86	1.24	0.22	0.46

Bacteria	Proteobacteria	Gammaproteobacteria Pseudomonadales Moraxellaceae			1.89	1.12	2.14	2.18	1.67	2.05	0.02	0.67	1.02	0.54
Bacteria	Proteobacteria	Gammaproteobacteria Xanthomonadales Xanthomonadaceae			2.18	2.69	3.5	2.14	2.62	1.87	1.48	0.61	0.12	0.43
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	1.51	1.72	2.52	2.14	2.11	2.04	2.51	0.58	0.17	0.62
Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	0.41	0.17	0.45	0.81	0.3	0.29	2.66	0.06	0.03	0.08
Bacteria	Proteobacteria	Gammaproteobacteria Xanthomonadales Sinobacteraceae			1.86	1.19	1.77	2.43	1.75	1.55	2.36	0.34	0.1	0.23
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	0.24	0.59	0.29	0.62	0.39	0.43	0.3	1.39	2.06	1.32
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	1.32	0.91	1.25	1.22	0.81	1.15	2.04	0.63	0.18	0.27
Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	0.78	0.36	1.02	1.54	1.29	2.3	1.31	0.34	0.15	0.23
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Kofleriaceae	1.26	0.89	1.6	1.33	1.76	2.15	1.12	0.33	0.11	0.15
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	0.11	0.07	0.15	0.35	0.12	0.1	0.31	2.05	6.89	1.2
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	0.01	0.16	0.06	0.92	0.04	0.03	3.45	0.01	0	1.28
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_Incertae_Sedis XVIII	0	0	0	0.05	0	0	0.2	0	0	0.19
Bacteria	Planctomycetes	Phycisphaerae	Tepidisphaerales	Tepidisphaeraceae	0.12	0.1	0.13	0.32	0.21	0.17	1.48	0.02	0	0.17
Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	0.24	0.47	0.35	0.33	0.37	0.31	0.7	0.15	0.02	0.1



Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	0.11	0.17	0.09	0.1	0.06	0.11	0.25	1.09	4.5	0.59
Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	0.1	2.6	0.41	0.43	1.04	0.18	0.21	0.78	0.05	0.56
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.01	0.01	0	0.23	0	0	0.9	0	0	0.22
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiales_incertae_sedis	0.46	0.21	0.34	0.51	0.98	0.89	0.36	0.45	0.67	0.15
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	0.89	0.36	0.67	0.64	0.59	0.81	0.57	0.08	0.03	0.05
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae 1	0.23	0.19	0.24	0.19	0.21	0.3	0.14	0.97	0.21	1.58
Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae 1	0.03	0.93	0.19	0.32	0.17	0.3	0.01	0.52	0.81	2.73
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacteraceae	0.13	0.06	0.2	0.54	0.21	0.2	1.61	0.04	0.01	0.28
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	0.14	0.03	0.09	0.05	0.08	0.06	0.04	1.09	0.04	0.04
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Parachlamydiaceae	0.27	0.14	0.22	0.43	0.24	0.37	0.42	0.22	0.21	0.2
Bacteria	Actinobacteria	Actinobacteria	Gaiellales	Gaiellaceae	0.53	0.26	0.77	0.37	0.32	0.8	0.36	0.11	0.06	0.05
Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	0.36	0.69	0.53	0.37	0.15	0.16	0.5	0.08	0.01	0.08
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae	0	0	0	0.09	0	0	0.69	0	0	0.3
Bacteria	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	0.22	0.09	0.14	0.27	0.28	0.3	0.42	0.09	0.06	0.13

Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	0.22	0.2	0.22	0.2	0.21	0.31	0.4	0.19	0.14	0.18
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	0.02	0.01	0.04	0.01	0.03	0.03	0.08	0.06	0.03	0.03
Bacteria	Firmicutes	Clostridia	Clostridiales	Gracilibacteraceae	0	0	0	0.08	0	0	0.28	0	0	0.12
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	0.09	0.25	0.13	0.13	0.15	0.1	0.26	0.82	0.64	0.14
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	0	0.01	0	0.06	0	0	0.05	0	0	0.06
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_incertae_sedis	0.34	0.19	0.2	0.26	0.26	0.21	0.27	0.11	0.03	0.05
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	0.26	0.16	0.55	0.12	0.15	0.2	0.05	0.09	0.09	0.03
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Labilitrichaceae	0.21	0.13	0.49	0.32	0.46	0.54	0.1	0.1	0.03	0.01
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	0.19	0.26	0.43	0.24	0.2	0.17	0.14	0.07	0.03	0.06

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**Table S4** Taxonomic information for the OTUs in the MFC\_CPF treatment based on 16S rRNA gene sequences.

OTU ID	Kingdom	Phylum	Class	Order	Family	Genus
OTU0	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	<i>Methylobacillus</i>
OTU1	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	<i>Citrobacter</i>
OTU3	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>
OTU4	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillales_Incertae Sedis XII	<i>Exiguobacterium</i>
OTU8	Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	<i>Vogesella</i>
OTU10	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	<i>Arthrobacter</i>
OTU11	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>
OTU14	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>
OTU15	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	<i>Azoarcus</i>
OTU17	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	<i>Rhodococcus</i>
OTU20	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	<i>Azohydromonas</i>
OTU21	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	<i>Ramlibacter</i>
OTU22	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	<i>Devosia</i>
OTU26	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	<i>Phenylobacterium</i>
OTU31	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	<i>Gemmatimonas</i>
OTU33	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae 1	<i>Bacillus</i>
OTU34	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	<i>Nitrospira</i>

OTU39	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	<i>Povalibacter</i>
OTU40	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	<i>Geobacter</i>
OTU43	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	<i>Cupriavidus</i>
OTU45	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	<i>Flavitalea</i>
OTU47	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_Incertae Sedis XVIII	<i>Symbiobacterium</i>
OTU48	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	<i>Bdellovibrio</i>
OTU58	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	<i>Massilia</i>
OTU62	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae 1	<i>Paenibacillus</i>
OTU74	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacteraceae	<i>Anaeromyxobacter</i>
OTU80	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	<i>Lysobacter</i>
OTU88	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	<i>unclassified</i>
OTU89	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	<i>Bosea</i>
OTU96	Bacteria	Planctomycetes	Phycisphaerae	Tepidisphaerales	Tepidisphaeraceae	<i>Tepidisphaera</i>
OTU108	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	<i>Skermanella</i>
OTU109	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae	<i>Desulfuromonas</i>
OTU136	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioideaceae	<i>Nocardioides</i>
OTU171	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiales_incertae_sedis	<i>unclassified</i>
OTU176	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	<i>Clostridium III</i>
OTU186	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Kofleriaceae	<i>Kofleria</i>
OTU194	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	<i>Anaerospora</i>

OTU195

Bacteria

Firmicutes

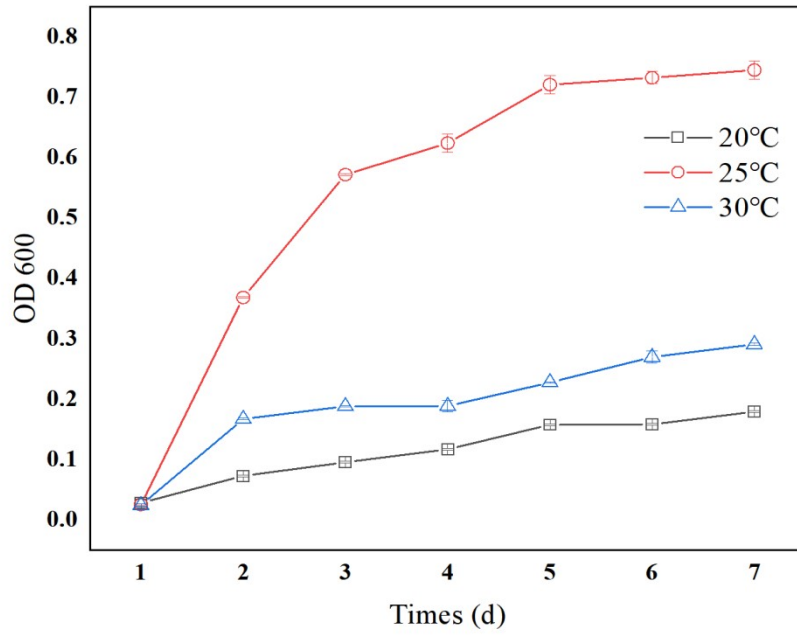
Clostridia

Clostridiales

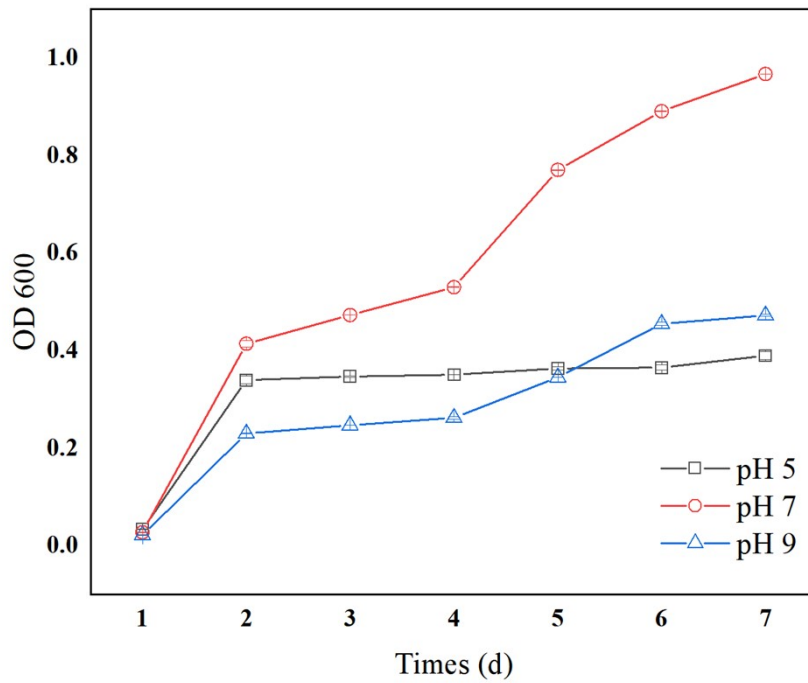
Gracilibacteraceae

*Gracilibacter*

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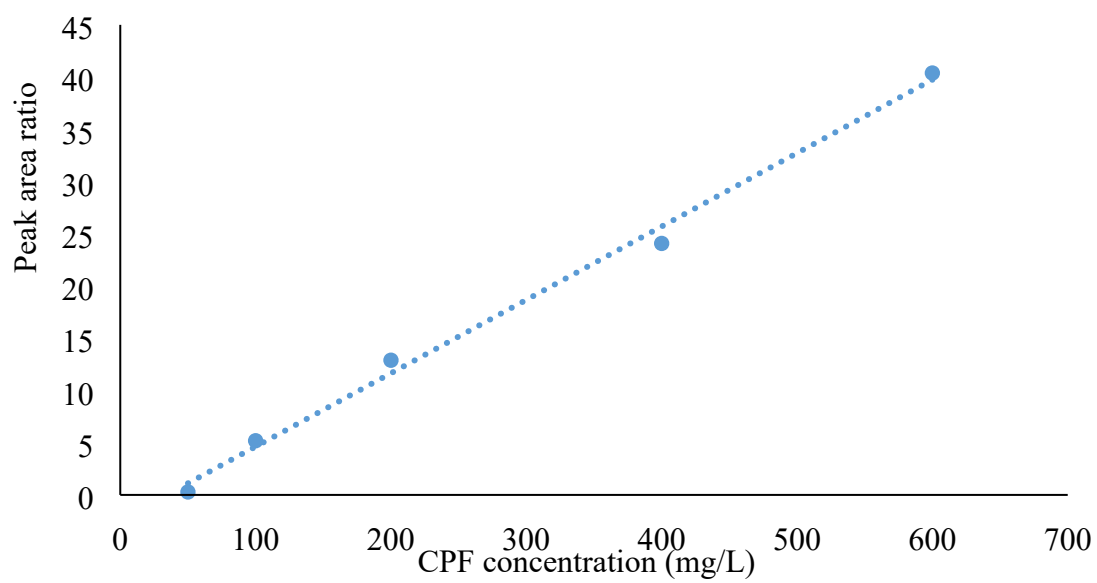


(a)



(b)

**Fig.S1** The growth curve of *Alcaligenes* L1 (a) at different temperature, (b) at different pH.



**Fig. S2** Standard curve for CPF via GC-MS.



(a)

**Fig.S3** a) *Alcaligenes* L1 on MM-agar plate; b) Degradation rate of *Alcaligenes* L1 under different pH with CPF 100 mg/L (25 °C) c)

Degradation rate of *Alcaligenes* L1 under different temperature with CPF 100 mg/L (pH 7).



(a)

(b)

**Fig.S4** The GC profiles for chlorpyrifos and its metabolites in pure culture (a) and MFC\_CPF treatment (b).