Supporting Information

Facile One-Pot immobilization of a novel thermostable carboxylesterase from *Geobacillus uzenensis* for continuous pesticide degradation in a packed bed column reactor

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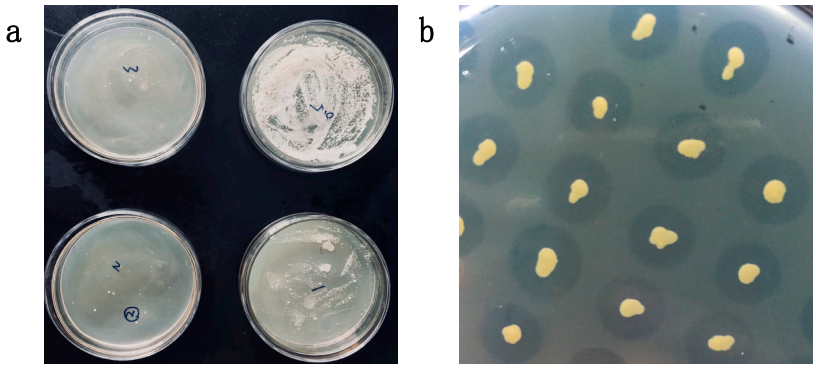


Figure S1 Transformation and screening of recombinant *P. pastoris*.

(a)The target gene-integrated *Pichia pastoris* transformants were screened using YPD plates containing different G418 concentrations (0.5-4 mg/mL). (b)Screening of strains showing a hydrolyzed circle on a BMMY plate containing tributyrin showed that the recombinant *P. pastoris* expressed a carboxylesterase.

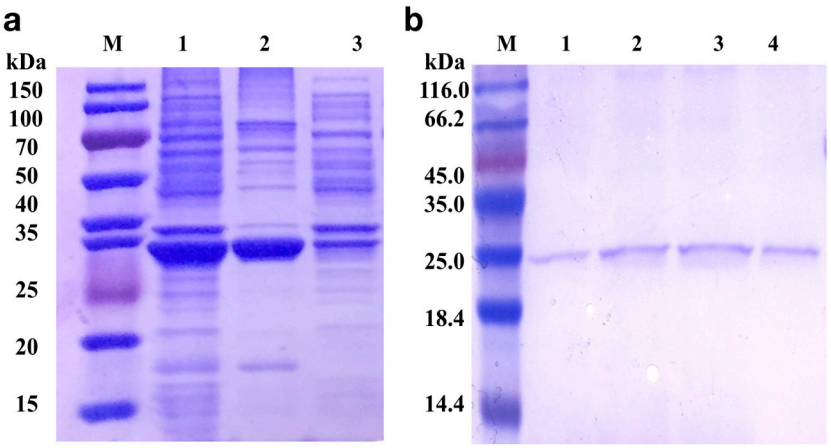


Figure S2 Expression and SDS-PAGE analysis of Est_{M160K} in *E. coli* and *Pichia pastoris*. (a) The SDS-PAGE of the recombinant protein and protein purification Est_{M160K} in *E. coli*. Lane M: Protein molecular weight marker. Lane 1-3: cell lysate, precipitation and supernatant of *E. coli* BL21 cells harbouring Est_{M160K}. (b) SDS-PAGE analysis of the recombinant protein Est_{M160K} after induction of expression for 4 days with methanol. Lane M: Protein molecular weight marker. Lane 1-4: Fermentation supernatant of *Pichia pastoris* KM71 (1-4 days).

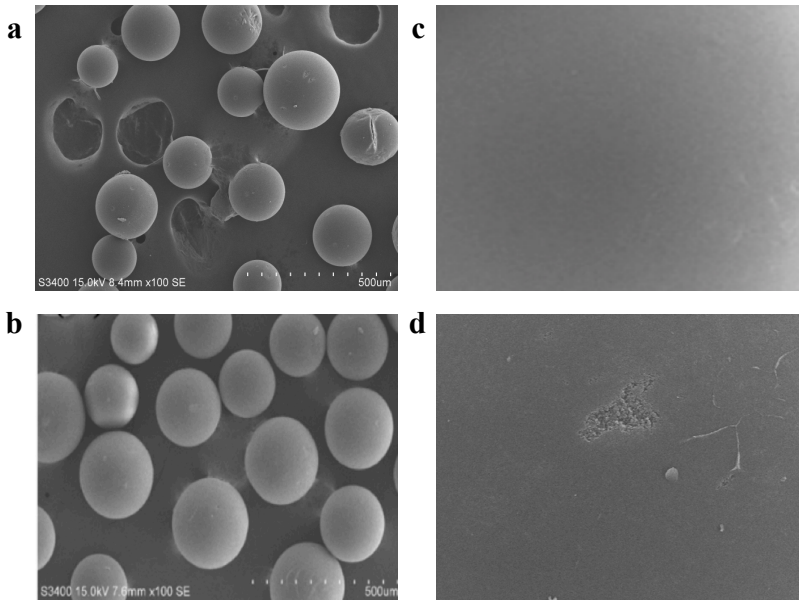


Figure S3 SEM of the surface of epoxy resin lx-105s before (a, b: Magnification ×100;) and after (c, d: Magnification ×500) immobilization of Est_{M160K}.

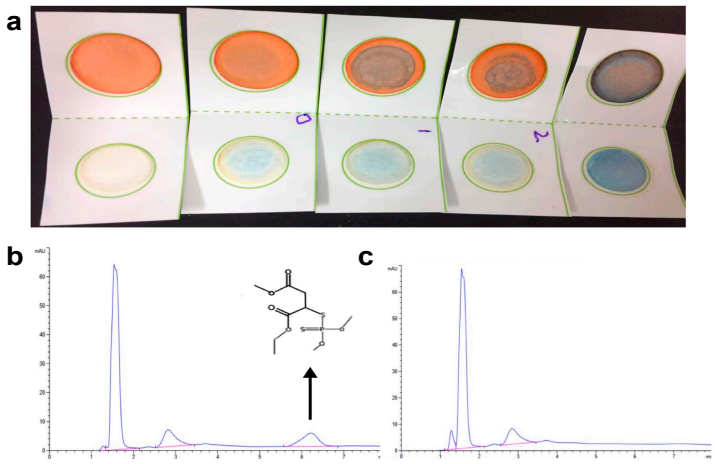


Figure S4. Degradation of malathion by immobilized lx- Est_{M160K}. (a) The content of malathion was measured using malathion residue test strips. From left to right: malathion standard, reaction result of 10 mg/L malathion, reaction result of 20 mg/L malathion and reaction result of 50 mg/L malathion, blank group (without malathion). (b)(c) Determination of degradation of malathion by HPLC.

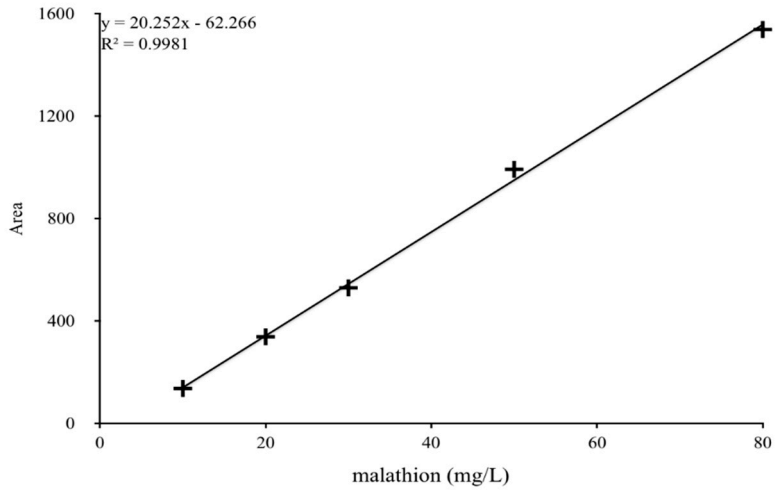


Figure S5. The standard curve of malathion.

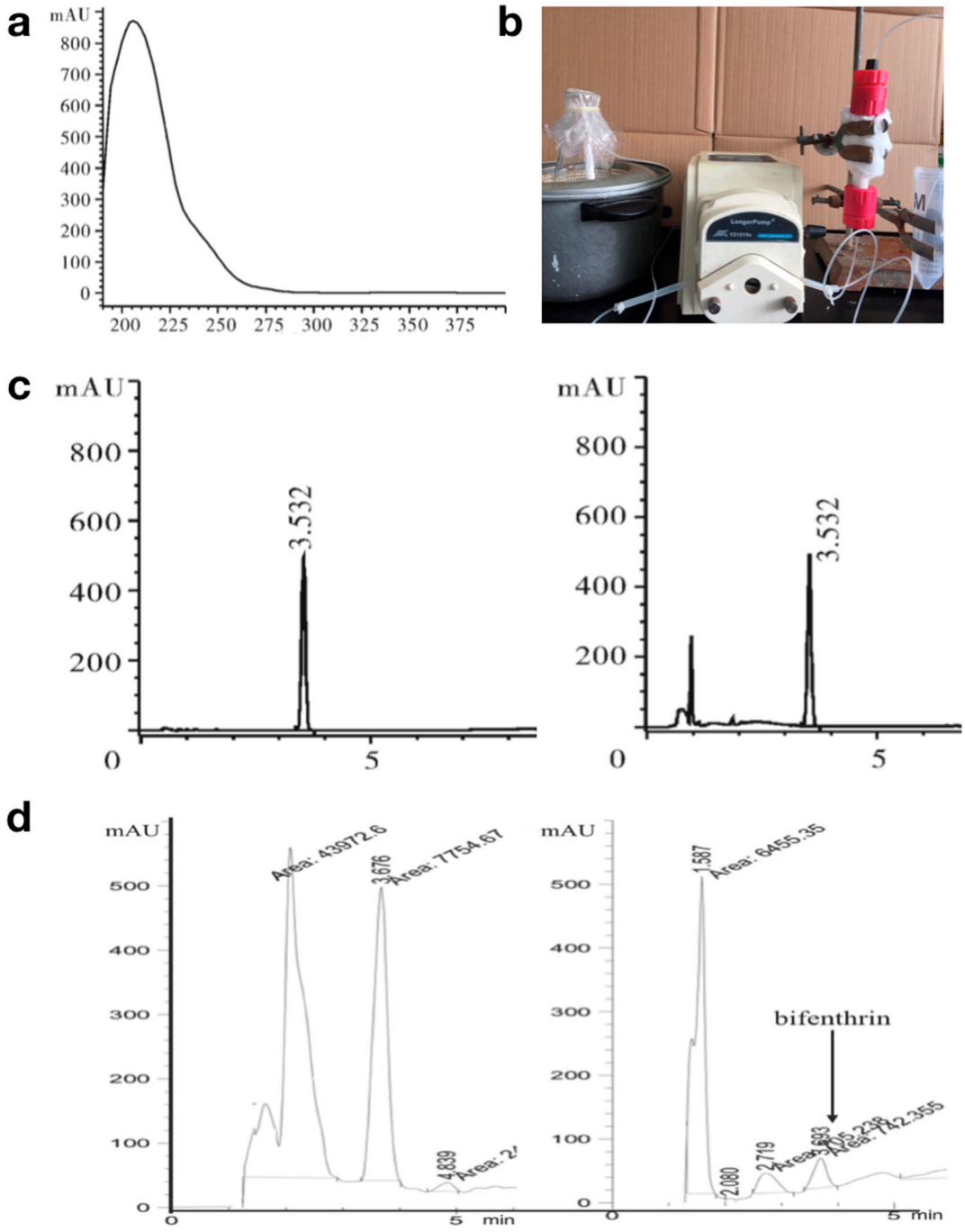
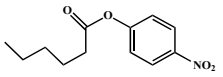
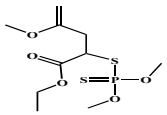
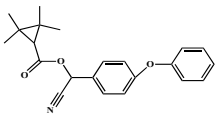
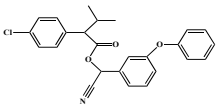
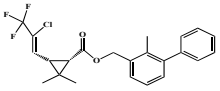


Figure S6. Detection wavelength of bifenthrin, physical picture of degradation reactor and HPLC detection results. (a) UV-Vis absorption spectrum of bifenthrin. (b)Physical picture of the packed-bed bioreactor for the biodegradation of bifenthrin. (c) HPLC detection of samples before and after adding bifenthrin standard. (d) HPLC chart before and after degradation of bifenthrin wastewater by lx- Est_{M160K}.

Table S1 Substrate specificity of lx-Est$_{M160K}$

Substrate		Specific activity (%)
p-nitrophenol hexanoate		100.00 ±3.29
malathion		57.98±4.96
fenpropathrin		56.02±3.64
fenvalerate		38.89 ±4.08
bifenthrin		208.89 ±4.64

Results are the average of triplicate measurements ± standard deviation. The relative activity was expressed as percentage of the rate observed with p-nitrophenol hexanoate.

Table S2 Sequences of primers used in this study

Primer	**Sequence (5' -> 3')**
Est$_{741/M}$-U	CGCGGATCCATGAAAATTGTTCCGCC
Est$_{741/M}$ -D	CGCAAGCTTTTACCAATCTAACGATT
Est$_{741/M}$ -F	CGCGAATTCATGAAAATTGTTCCGCC
Est$_{741/M}$ -R	CGCGCGGCCGCTTACCAATCTAACGA
Est$_{M160K}$-tF	ATTGTGTCGATGTGCGCGCCGATG
Est$_{M160K}$-tR	CGCACATCGACACAATCCCTTCTAT

Table S3 Experimental results of accuracy of bifenthrin analysis method

	actual dosage (mg)	theoretical dosage (mg)	recovery rate (%)
bifenthrin	4.92	4.52	91.87
	4.88	4.23	86.68
	4.86	4.34	89.30
	4.78	4.32	90.38
	4.75	4.17	87.79

Actual dosage: a certain amount of bifenthrin standard sample is added to the known content of 20% emulsifiable concentrate bifenthrin suspension sample; Theoretical dosage: bifenthrin standard sample. Samples are processed in the same way.