Environmental Research 236 (2023) 116619

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Pseudomonas aeruginosa based concurrent degradation of beta-cypermethrin and metabolite 3-phenoxybenzaldehyde, and its bioremediation efficacy in contaminated soils

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ARTICLE INFO

Keywords:
Beta-cypermethrin
Biodegradation
Degradation pathways
3-Phenoxybenzaldehyde
Soil remediation

ABSTRACT

Beta-cypermethrin is one of the widely used pyrethroid insecticides, and problems associated with the accumulation of its residues have aroused public attention. Thus, there is an urgent need to effectively remove the beta-cypermethrin that is present in the environment. Biodegradation is considered a cost-effective and environmentally friendly method for removing pesticide residues. However, the beta-cypermethrin-degrading microbes that are currently available are not optimal. In this study, *Pseudomonas aeruginosa* PAO1 was capable of efficiently degrading beta-cypermethrin and its major metabolite 3-phenoxybenzaldehyde in water/soil environments. Strain PAO1 could remove 91.4% of beta-cypermethrin (50 mg/L) in mineral salt medium within 120 h. At the same time, it also possesses a significant ability to metabolize 3-phenoxybenzaldehyde—a toxic intermediate of beta-cypermethrin. The Andrews equation showed that the maximum substrate utilization concentrations of beta-cypermethrin and 3-phenoxybenzaldehyde by PAO1 were 65.3558 and 49.6808 mg/L, respectively. Box–Behnken design-based response surface methodology revealed optimum conditions for the PAO1 strain-based degradation of beta-cypermethrin as temperature 30.6 °C, pH 7.7, and 0.2 g/L inoculum size. The results of soil remediation experiments showed that indigenous micro-organisms helped to promote the biodegradation of beta-cypermethrin in soil, and beta-cypermethrin half-life in non-sterilized soil was 6.84 days. The bacterium transformed beta-cypermethrin to produce five possible metabolites, including 3-phenoxybenzyl alcohol, methyl 2-(4-hydroxyphenoxy)benzoate, diisobutyl phthalate, 3,5-dimethoxyphenol, and 2,2-dimethyl-1-(4-phenoxyphenyl)propanone. Among them, methyl 2-(4-hydroxyphenoxy)benzoate and 3,5-dimethoxyphenol were first identified as the intermediate products during the beta-cypermethrin degradation. In addition, we propose a degradation pathway for beta-cypermethrin that is metabolized by strain PAO1. Beta-cypermethrin could be biotransformed firstly by hydrolysis of its carboxylester linkage, followed by cleavage of the diaryl bond and subsequent metabolism. Based on the above results, *P. aeruginosa* PAO1 could be a potent candidate for the beta-cypermethrin-contaminated environmental bioremediation.

1. Introduction

Pyrethroids are biomimetic pesticides similar to the naturally occurring pyrethrin (Cycoń and Piotrowska-Seget, 2016; Bhatt et al., 2020a; Birolli et al., 2021). They have the characteristics of broad spectrum, high efficiency, and low residue, making them one of the pesticide classes that cannot be replaced within the next several decades (Chen et al., 2011a, 2013a; Tu et al., 2016; Zhang et al., 2017). Beta-cypermethrin is an important pyrethroid pesticide (PP), which is widely applied against household and agricultural pests (Chen et al.,

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https://doi.org/10.1016/j.envres.2023.116619
Received 15 May 2023; Received in revised form 1 July 2023; Accepted 10 July 2023
Available online 21 July 2023

2013b; Yang and Ji, 2015; Tang et al., 2017). The half-life of beta-cypermethrin in soil ranges from several weeks to several months, while the residual period in plants is longer, which may reach to several years (Chen et al., 2012a; Bhatt et al., 2019; Zhan et al., 2020). Unfortunately, beta-cypermethrin residues have been frequently detected in groundwater and sludge throughout the world (Delgado-Moreno et al., 2011; Holmes et al., 2008; Huang et al., 2023). An increasing body of evidence suggests that beta-cypermethrin may have reproductive toxicity, genotoxicity, and neurotoxicity in mammals (Duan et al., 2022; Jiang et al., 2023; Lu et al., 2022). Long-term exposure to beta-cypermethrin may lead to some serious chronic diseases in humans (Fang et al., 2022; Whangchai et al., 2021; Zhao et al., 2021). In addition, beta-cypermethrin metabolites could accumulate in human and animal bodies through the food chain, and has demonstrated neurotoxicity and reproductive toxicity effects in non-target organisms (Zhao et al., 2015; Liu et al., 2023). 3-Phenoxybenzaldehyde is the main, persistent, and toxic metabolite of beta-cypermethrin (Chen et al., 2011b). 3-Phenoxybenzaldehyde exhibits more mobility as compared to beta-cypermethrin and thus it causes widespread soil contamination (Chen et al., 2012b). Based on the anti-estrogenic property, 3-phenoxybenzalde has been classified as endocrine-disrupting toxicant (Akbar et al., 2015; Zhao et al., 2021). Hence, it is necessary to develop beta-cypermethrin degradation technologies to remove these contaminants from various environmental compartments.

To date, several remediation techniques have been devised, which include oxidation, photodegradation, bacterial degradation, and adsorption (Bhatt et al., 2021a; Bilal et al., 2021; Colombo et al., 2013; Mishra et al., 2021; Li et al., 2022; Chen et al., 2023; Zhang et al., 2023). Among these, microbial degradation is an efficient and safer method for the removal of beta-cypermethrin contaminants from the environment without producing further pollution (Lin et al., 2011; Xiao et al., 2020; Zhao et al., 2019; Bhatt et al., 2022a, 2023). Several beta-cypermethrin and 3-phenoxybenzaldehyde degrading bacterial strains have been retrieved from contaminated soils including *Brevibacillus parabrevis* BCP-09, *Ochrobactrum lupini* DG-S-01, and *Bacillus subtilis* BSF01, *Streptomyces aureus* HP-S-01, *Bacillus licheniformis* B-1, *Eurotium cristatum* ET1, and *Serratia* spp. strains JCN13 and JC1 (Zhang et al., 2010; Chen et al., 2011b, 2012a; Xiao et al., 2015; Li et al., 2016; Tang et al., 2018; Hu et al., 2018; Bhatt et al., 2022b). Among them, only one bacterium (*Streptomyces aureus* (HP-S-01)) could degrade both beta-cypermethrin and 3-phenoxybenzaldehyde (Chen et al., 2012a). In a previous study, the fungal strain *Cladosporium* sp. HU was reported to effectively degrade multiple pyrethroids and 3-phenoxybenzaldehyde in liquid medium; however, it failed to degrade pyrethroids in soil (Chen et al., 2011c).

This study evaluated the biodegradation ability of beta-cypermethrin and 3-phenoxybenzaldehyde using a *Pseudomonas aeruginosa* strain, PAO1, optimize the beta-cypermethrin degradation conditions through response surface methodology (RSM), and determine the degradation kinetics of beta-cypermethrin and 3-phenoxybenzaldehyde. Furthermore, the application prospect and possible metabolic pathways of strain PAO1 for the remediation of beta-cypermethrin contaminated environments are clarified. These results highlight the bioremediation potential and advantages of *P. aeruginosa* strain PAO1 for beta-cypermethrin contaminated environments. Meanwhile, the considered strain provides a microbial resource for the concurrent beta-cypermethrin and 3-phenoxybenzaldehyde degradation.

2. Materials and methods

2.1. Media and chemicals

Beta-cypermethrin (95% purity) and its metabolite 3-phenoxybenzaldehyde (98% purity) were purchased from Jiangsu Yangnong Chemical Group Co., Ltd., China and Zhongshan Aestar Fine Chemical Inc. Ltd., China, respectively. Chromatography-grade methanol and acetonitrile were purchased from Sigma-Aldrich, USA. The remaining analytical grade solvents and chemicals were commercially obtained. Beta-cypermethrin and 3-phenoxybenzaldehyde stock solutions (10,000 mg/L) were dissolved in acetone, and stored at 4 °C in dark bottles.

LB (Luria–Bertani) medium consisted of the following components: yeast extract (5 g/L); tryptone (10 g/L); and NaCl (10 g/L). MSM (mineral salt medium) contained: $FeSO_4{\cdot}7H_2O$, 0.001 g/L; $(NH_4)_2SO_4$, 2 g/L; KH_2PO_4, 1.5 g/L; $MgSO_4{\cdot}7H_2O$, 0.2 g/L; $Na_2HPO_4{\cdot}12H_2O$, 1.5 g/L; and $CaCl_2{\cdot}2H_2O$, 0.01 g/L. The pH of both media was set at 7.2 and sterilization was carried out for 20 min at 121 °C.

2.2. Micro-organism and culture conditions

The pyrethroid-degrading strain *Pseudomonas aeruginosa* PAO1 (GeneBank accession number: KF290566) has been deposited into the China Center for Type Culture Collection (Collection Number: CCTCC M 2014630).

The stored strain (in 20% (*v*/*v*) glycerol at −80 °C) was passaged every 1–2 years. Strain PAO1 was added into 50 mL of LB medium broth, incubated at 30 °C and 200 rpm for 24 h, and then centrifugation was carried out for 5 min at 4000 rpm to harvest. Normal saline (0.9% sterile) was used to wash it twice before inoculation and again suspended in normal saline to achieve an OD_{600} value of 0.6. Then 2% of culture suspension (approximately 1.5×10^7 CFU/mL) was inoculated to assess beta-cypermethrin and 3-phenoxybenzaldehyde degradation (Chen et al., 2014).

2.3. Degradation and growth properties of strain PAO1

The frozen PAO1 strain was thawed and cultured for 24 h using LB medium. The harvesting of bacterial cells was performed as inoculum, according to the previously mentioned methods (Chen et al., 2012c, 2015). Inoculum (0.2 g/L) was added to MSM medium (50 mL) containing beta-cypermethrin (100 mg/L) and placed in rotary shaker at 30 °C and 200 rpm whereas a non-inoculated flask served as control. Culture samples were collected regularly, at 0, 6, 12, 24, 48, 72, 96, and 120 h. All experimental groups were conducted in triplicate. PAO1 growth was estimated spectrophotometrically at an OD of 600 nm. High-performance liquid chromatography (HPLC) was conducted to assess the residual amount of beta-cypermethrin. The mobile phase was composed of acetonitrile and deionized water (80:20) at a flow rate of 1.0 mL/min. The detection wavelength and injection volume were 235 nm and 10 μL, respectively (Guo et al., 2021).

2.4. Optimization of beta-cypermethrin degradation conditions

Box–Behnken design-based response surface methodology (RSM) was adopted for the optimization of crucial factors and interactive influences affecting the beta-cypermethrin degradation activity of strain PAO1 (Zhan et al., 2018; Bhatt et al., 2020b). According to the results of previous pre-experiments, inoculum size, temperature, and pH were taken as independent variables. Center point and range values of these variables, determined using the Design Expert software, are presented in Table S1. Beta-cypermethrin (100 mg/L) degradation in MSM over 5 days was a dependent variable. The analysis of data was performed using SAS statistical software (Design-Expert 12.0), and a regression model was constructed to predict optimal processing parameters, as follows:

$$Y_i = b_0 + \sum b_i X_i + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 \quad (1)$$

where Y_i represents predicted response, b_0 is the constant, X_j and X_i are variables, b_i are linear coefficients, b_{ij} are interaction coefficients, and b_{ii} are quadratic coefficients.

2.5. Degradation of beta-cypermethrin and 3-phenoxybenzaldehyde at different concentrations by strain PAO1

The degradation capability at different beta-cypermethrin and 3-phenoxybenzaldehyde concentrations was assessed by inoculating the strain PAO1 in sterilized MSM under optimal culture conditions. The experiment was repeated three times and controls were non-inoculated. Residual beta-cypermethrin and 3-phenoxybenzaldehyde concentrations were determined at 24 h intervals and the specific degradation constant (q) was calculated at various initial concentrations by using the following Andrews equation (Feng et al. 2020; Bhatt et al., 2020c).

$$q = \frac{q_{\max} S}{S + K_s + \left(S^2 / K_i\right)} \quad (2)$$

where $q_{\max}$ represents maximum specific degradation constant, K_i represents substrate inhibition coefficient, K_s represents half-saturation constant, and S represents inhibition concentration. Half-saturation constant gradient was used to calculate the q value of beta-cypermethrin or 3-phenoxybenzaldehyde concentration in MATLAB 7.8 by following a non-linear regression method (Chen et al., 2012d).

2.6. Bioremediation of beta-cypermethrin in soils

To evaluate the ability of strain PAO1 to remediate soils contaminated with beta-cypermethrin, soil samples were obtained (5–20 cm depth) from the field (Longitude: 113°35′37″; Latitude: 23°15′57″) without fertilizer or beta-cypermethrin application history, at South China Agricultural University, Guangzhou, China. The physico-chemical soil properties (g/kg of dry weight) were noted as: pH (6.9); total N (0.5); total K (18.2); total P (0.4); and organic matter (10.5). The texture of the soil samples was sandy loam (sand, 65.0%; clay, 7.0%; and silt, 28.0%). Air-drying of samples was carried out followed by sieving (2 mm) before using in bioremediation investigations.

Two hundred grams of sterile and non-sterile soil were added into Erlenmeyer flasks (500 mL) along with beta-cypermethrin solution (50 mg/kg). Then, the suspension of PAO1 (1.0×10^7 CFU/g of soil) was inoculated through drip irrigation and incubated at 30 °C. Non-inoculated non-sterile or sterile soil samples containing insecticides served as controls. The water contents of all soil samples were adjusted to a water-holding capacity of 40% with deionized sterile water (Chen et al., 2011d; Cycoń et al., 2014; Bhatt et al., 2020d). The collection of soil samples was carried out at 0, 3, 6, 9, 12, and 15th day for analyses to determine the residual concentrations of beta-cypermethrin by HPLC. The metabolites of beta-cypermethrin in soil degraded by strain PAO1 were detected by gas chromatography-mass spectrometer (GC-MS).

First-order kinetic model was followed to determine the degradation constant (k):

$$C_t = C_0 \times e^{-kt} \quad (3)$$

where C_t represents beta-cypermethrin concentration at t time, C_0 represents initial beta-cypermethrin concentration at zero time whereas k and t respectively represent degradation constant (day^{-1}) and degradation period (days).

The beta-cypermethrin half-life ($t_{1/2}$) in various soil samples was calculated using Eq. (4):

$$t_{1/2} = \ln 2 / k \quad (4)$$

where k represents degradation constant (day^{-1}).

2.7. Chemical analysis

Analyses of beta-cypermethrin filterate residues were performed using Water 2690 HPLC system (Waters 2690, Milford, MA) with a Phenomenex C_{18} reversed phase column (250 × 4.60 mm, 5 μm). Mobile phase consisted of an acetonitrile to water ratio of 90:10 at 1.0 mL/min flow rate whereas 10 μL injection volume was used. Beta-cypermethrin was detected at 235 nm.

HPLC of the samples containing 3-phenoxybenzaldehyde was carried out as well. Mobile phase consisted of a water to acetonitrile ratio of 4:6 (v/v) and adjusted to a pH of 2.4 using phosphoric acid. 20 μL samples were injected and 3-phenoxybenzaldehyde was detected at 210 nm.

Beta-cypermethrin metabolic products were identified and analyzed through high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The analytes were separated by a Phenomenex C_{18} reversed phase column (150 × 2.1 mm, 5 μm). Methanol, water, and trifluoroacetic acid (65:35:0.01) served as the mobile phase at 0.2 mL/min flow rate. The products were further established through standard MS, with electrospray-based ionization as a positive or negative polarity. Nitrogen served as the collision gas and nebulizer. The detector voltage was set at 5.5 kV. First- and second-order MS were used to detect the characteristic fragment ions.

2.8. Statistical analysis

All data represent mean values of three replicates with standard deviation. The Tukey's Honestly Significant Difference (HSD) test was used to analyze the data, and $p < 0.05$ indicated that the data were significantly different.

3. Results and discussion

3.1. Growth and degradation characteristics of strain PAO1

PAO1-based beta-cypermethrin degradation and its growth was assessed through MSM where 100 mg/L beta-cypermethrin served as the energy and carbon source (Fig. 1). During 0–12 h (initial period), PAO1 strain exhibited slow growth with a short leg phase. Then, PAO1 growth rapidly increased in the logarithmic phase (12–48 h); significant degradation of beta-cypermethrin was noted during this period. The stationary phase (48–96 h) was characterized by slow cellular growth. During this time, the degradation trend of beta-cypermethrin by strain PAO1 flattened. Finally, PAO1 growth was not observed during decline phase (>96 h), and caused an overall beta-cypermethrin biodegradation of 91.4%. Contrarily, beta-cypermethrin concentration did not change significantly in controls (non-inoculated) (Fig. 1).

Pseudomonas strains are known for their metabolic capability and environmental versatility as well as for their ability to manage bacterial

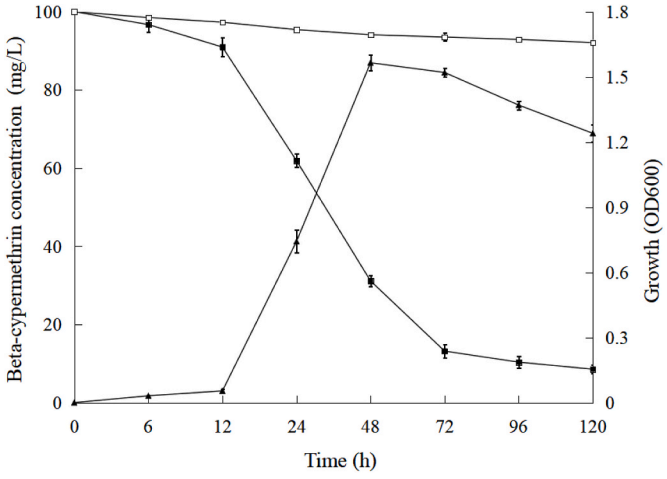


Fig. 1. Biodegradation of beta-cypermethrin (100 mg/L) during growth of strain PAO1. Symbol: □, beta-cypermethrin control; ■, beta-cypermethrin degradation by strain PAO1; ▲, growth of strain PAO1 (OD_{600}). Data represent mean values of three replicates with standard deviation.

and fungal pathogens (Yang et al., 2018; Liu et al., 2020; Wang et al., 2020; Liu et al., 2020; Zhang et al., 2021). Recently, *Pseudomonas* strains have become the main bacteria widely used in biological control (Wang et al., 2022; Lin et al., 2023). However, the potential use of *Pseudomonas* in bioremediation of pyrethroid-polluted environment has not received the attention it deserves. Although it has been proved that some *Pseudomonas* strains have the ability to degrade various organic pollutants such as carbaryl, fomesafen, D-phenothrin, among others (Zhang et al., 2011; Feng et al., 2012; Zhu et al., 2018; Xu et al., 2022), thus far, there has been no report of bioremediation of beta-cypermethrin-contaminated soil using *Pseudomonas* strains. This work provides the first evidence that *P. aeruginosa* strain PAO1 participates in efficient bioremediation of beta-cypermethrin in soil.

Degradation of beta-cypermethrin was closely associated with cell growth. As many studies have reported, due to the fact that the strain needs a certain time to adapt to the environment, the use of a pesticide as the only carbon source causes a lag phase that is followed by rapid biodegradation (Deng et al., 2015; Bhatt et al., 2021b; Birolli et al., 2022). During this study, a quick adaptation of PAO1 to the environment was noted and it briskly degraded beta-cypermethrin after an extremely short lag phase. Similarly, in our previous report, we obtained a beta-cypermethrin-degrading bacterium, *Ochrobactrum lupini* DG-S-1, which could quickly degrade beta-cypermethrin without a lag phase (Chen et al., 2011b). Moreover, strain PAO1 degraded 91.4% of beta-cypermethrin within 120 h, which is higher than that of some previously reported beta-cypermethrin degradation bacteria including *Bacillus subtilis* BSF01, *Streptomyces aureus* HP-S-01, and *Pseudomonas aeruginosa* CH7 (Zhang et al., 2011; Chen et al., 2012a; Xiao et al., 2015). Hence, our results indicated that the strain PAO1 is an excellent candidate for the bioremediation of beta-cypermethrin contaminations in the environments.

3.2. Optimization of conditions for beta-cypermethrin degradation

Box–Behnken design was adopted to assess the impact of independent variables such as pH, temperature, and inoculum size, which significantly influenced PAO1-based beta-cypermethrin biodegradation (Figure S1). Table 1 summarizes variables' design matrix with the results. SAS software was employed to analyze the data through response surface regression. Quadratic polynomial model equation was obtained, which explains the beta-cypermethrin degradation performance of strain PAO1:

$$Y_1 = 92.53333 + 1.475X_1 + 1.4625X_2 + 1.8875X_3 - 6.366667X_1^2 - 0.425X_1X_2 + 0.125X_1X_3 - 4.291667X_2^2 - 0.35X_2X_3 - 2.091667X_3^2 \quad (5)$$

where Y_1 represents predicted degradation of beta-cypermethrin whereas X_1, X_2, and X_3 respectively represent temperature, pH, and inoculum size.

ANOVA results of the fitted quadratic polynomial model are tabulated in Table 2. The model had a coefficient of determination (R^2) of 0.9748 and a lower coefficient of variation (CV = 1.4), demonstrating reliable explanation of response randomness by the applied model. The predicted values generated by the model were noted to be consistent with experimental results. Regression analysis showed significant impact ($p < 0.05$) of square and linear terms of temperature (X_1), pH (X_2), and inoculum size (X_3) on beta-cypermethrin biodegradation ability of strain PAO1.

In order to effectively and intuitively reflect the interaction effects on PAO1-based beta-cypermethrin degradation, the response surface diagram and its contour map were drawn using the SAS software. As shown in Fig. 2A, the degradation plot had a maximum theoretical value of 93.1% at the stationary point. X_1, X_2, and X_3, were noted as 0.11529, 0.14664, and 0.44237, respectively (Fig. 2B) at the theoretical maximum point. The optimum conditions for PAO1-based beta-cypermethrin degradation remained as pH 7.7, 30.6 °C, and 0.2 g/L inoculum size. Subsequently, predicted model's accuracy was tested by observing beta-cypermethrin degradation by strain PAO1 under optimum conditions. Beta-cypermethrin degradation was noted as 93% within 5 days under the optimal degradation conditions, which was similar to the predicted value (Table S2). These findings revealed that degradation conditions optimized by this model were reliable.

Fig. 3 presents the single-factor effects of temperature (X_1), pH (X_2), and inoculum size (X_3) on the degradation ability of strain PAO1 for beta-cypermethrin. Temperature and pH had obvious impact on the strain PAO1 degradation capability. The increase in inoculation amount did improve the degradation rate of beta-cypermethrin; however, when the inoculation amount increases to a certain level, continuing to increase the inoculation amount will not improve the removal of beta-cypermethrin by strain PAO1.

RSM is commonly applied to optimize microbial culture conditions, and is considered to be a very comprehensive and effective method, especially in the field of microbial pesticide degradation (Ghevariya

Table 1
Experimental matrix and results of Box-Behnken.

Run	Independent variables						Dependent variables
	Coded levels			Uncoded levels			Y_1
	X_1	X_2	X_3	Temperature (°C)	pH	Inoculum size (g/L)	Degradation efficiency (%)
1	−1	−1	0	25	6	0.2	78.6 ± 1.3n
2	−1	1	0	25	9	0.2	83.1 ± 0.3k
3	1	−1	0	35	6	0.2	81.5 ± 0.7l
4	1	1	0	35	9	0.2	84.3 ± 1.6i
5	0	−1	−1	30	6	0.1	83.4 ± 1.5j
6	0	−1	1	30	6	0.3	86.7 ± 1.1f
7	0	1	−1	30	9	0.1	86.3 ± 1.3g
8	0	1	1	30	9	0.3	88.2 ± 1.2e
9	−1	0	−1	25	7.5	0.1	79.8 ± 0.5m
10	1	0	−1	35	7.5	0.1	83.4 ± 1.0j
11	−1	0	1	25	7.5	0.3	84.5 ± 1.6h
12	1	0	1	35	7.5	0.3	88.6 ± 0.4d
13	0	0	0	30	7.5	0.2	91.7 ± 0.6c
14	0	0	0	30	7.5	0.2	92.4 ± 0.8b
15	0	0	0	30	7.5	0.2	93.5 ± 0.5a

Note: Each data in the table is the mean of triplicates with standard error. Mean values followed by the same letters in the same column are not significantly different at level $P = 0.05$ according to Duncan's multiple range test (DMRT).

Table 2
Analysis of variance (ANOVA) for the fitted quadratic polynomial model.

Source of variation	Quadratic polynomial model for degradation by strain PAO1				
	DF [a]	SS [b]	MS [c]	F value	*P* Level
X_1	1	17.405	17.405	12.29311	0.0172
X_2	1	17.11125	17.11125	12.08564	0.0177
X_3	1	28.50125	28.50125	20.13037	0.0065
X_1X_1	1	149.6656	149.6656	105.7085	0.0002
X_1X_2	1	0.7225	0.7225	0.5103	0.5070
X_1X_3	1	0.0625	0.0625	0.044144	0.8419
X_2X_2	1	68.00641	68.00641	48.03278	0.0009
X_2X_3	1	0.49	0.49	0.346086	0.5819
X_3X_3	1	16.1541	16.1541	11.40961	0.020
Model	9	274.2542	30.47269	21.52279	0.0017
Residual	5	7.079167	1.415833		
Lack of fit	3	5.4325	1.810833	2.199399	0.3277
Pure error	2	1.646667	0.823333		
Total	14	281.3333			

Note:
P Level less than 0.05 indicates the model terms are significant; P Level less than 0.001 indicates the model terms are very significant.
[a] refers to degrees of freedom;
[b] refers to sum of sequences;
[c] refers to mean square.

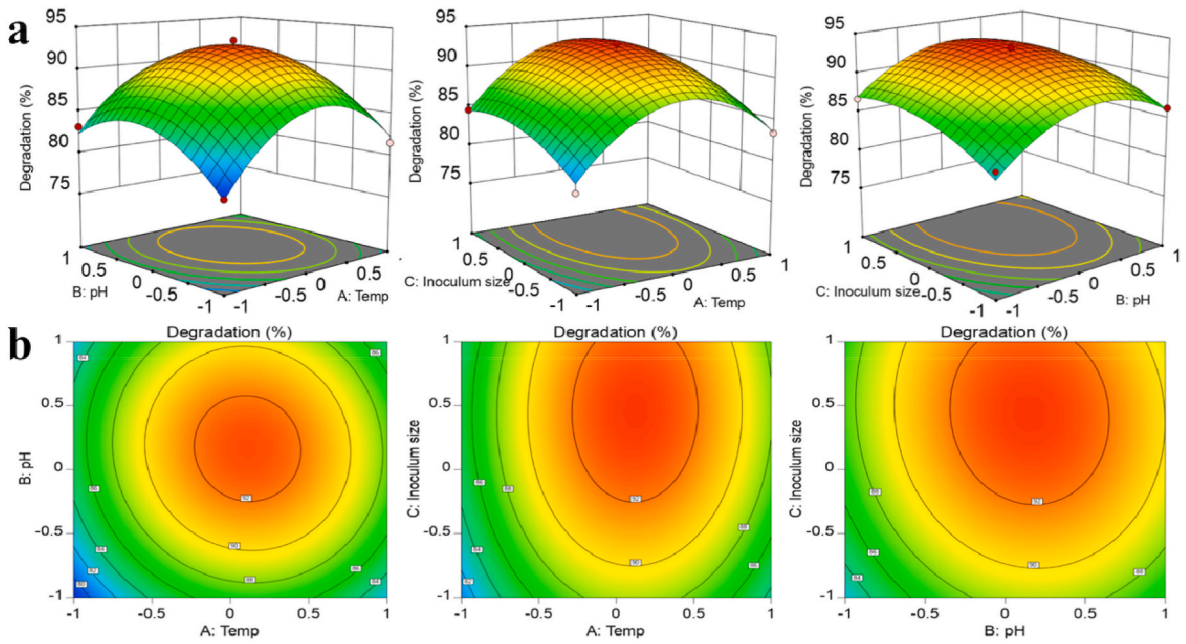


Fig. 2. Three-dimension degradation map based on the response surface model. A: The interactive effects of temperature, pH and inoculation amount on the degradation of cypermethrin in strain PAO1. B: Ring heat map of degradation efficiency under different parameters of three independent variables.

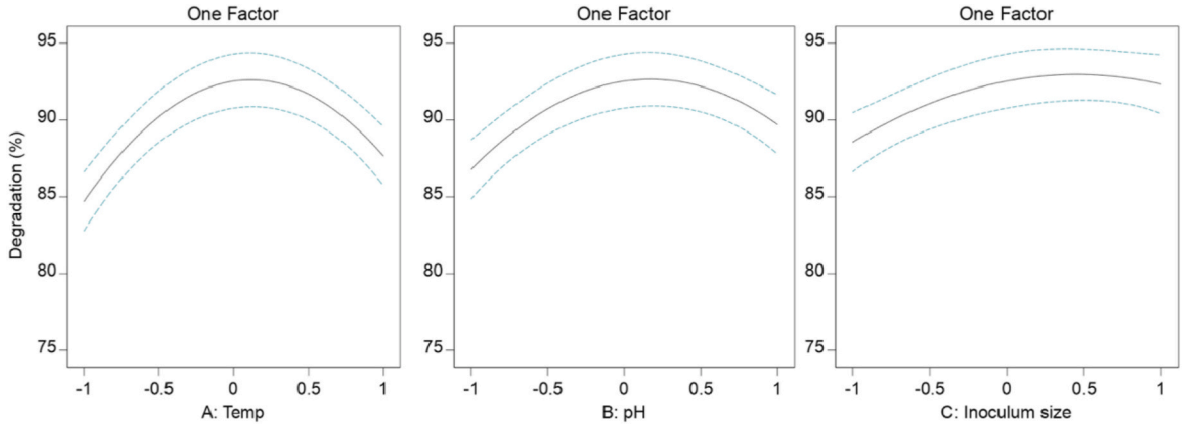


Fig. 3. Effect of a single factor on the degradation of beta-cypermethrin by strain PAO1. a: Temperature; b: pH; c: Inoculum size.

et al., 2011; Pang et al., 2023; Wu et al., 2023). RSM could reliably optimize key parameters for better biodegradation through a quadratic polynomial model (Zhao et al., 2017; Bhatt et al., 2021d). During the current investigation, Box–Behnken design-based RSM effectively improved strain PAO1-based biodegradation at pH 7.7, 30.6 °C, and 0.2 g/L inoculum size. Quadratic polynomial model was also additionally developed, which indicated a maximum degradation of 93.1% under the optimal conditions. Similar findings have been reported in *Serratia* spp. JCN13and JC1, *Acinetobacter baumannii* ZH-14, and *Pseudomonas fulva* P31 (Zhang et al., 2010; Zhan et al., 2018; Yang et al., 2018).

3.3. Degradation of beta-cypermethrin and 3-phenoxybenzaldehyde at different concentrations by strain PAO1

The degradation of beta-cypermethrin and its intermediate, 3-phenoxybenzaldehyde, at different concentrations was simultaneously carried out using strain PAO1. As shown in Fig. 4a, strain PAO1 could tolerate and degrade 800 mg/L beta-cypermethrin, and there was no obvious lag period in the degradation process. At an initial concentration of ≤200 mg/L, the degradation of beta-cypermethrin by strain PAO1 over 5 days was above 80%. However, strain PAO1 was found to degrade 73.8%, 62.9%, and 51.7% of beta-cypermethrin at 400, 600, and 800 mg/L initial concentrations after completion of the experiment, respectively. These results indicate that the degradation decreased rapidly with an increase in the concentration of the pesticide. More toxicity at high beta-cypermethrin concentration might have inhibited the micro-organism growth. In addition, beta-cypermethrin degradation by this strain was concentration-dependent.

Fig. 4b depicts the relationship between initial concentration of beta-cypermethrin and specific degradation constant of PAO1 strain according to the Andrews equation. The kinetic parameters, including substrate inhibition coefficient (K_i), half-saturation constant (K_s), and maximum specific degradation constant (q_{max})remained 168.0938 mg/L, 25.4107 mg/L, and 1.0023 d^{-1}, respectively. These values were determined through non-linear regression analysis using the Matrix Laboratory (MATLAB) software (Version 7.8). The maximum substrate utilization concentration (S_m) was determined as 65.3558 mg/L. The R^2 value remained 0.9771, depicting an alignment of degradation data with the model. Furthermore, it can be seen that the specific degradation constant of strain PAO1 had a large specific degradation ($q > 0.15$ d^{-1})

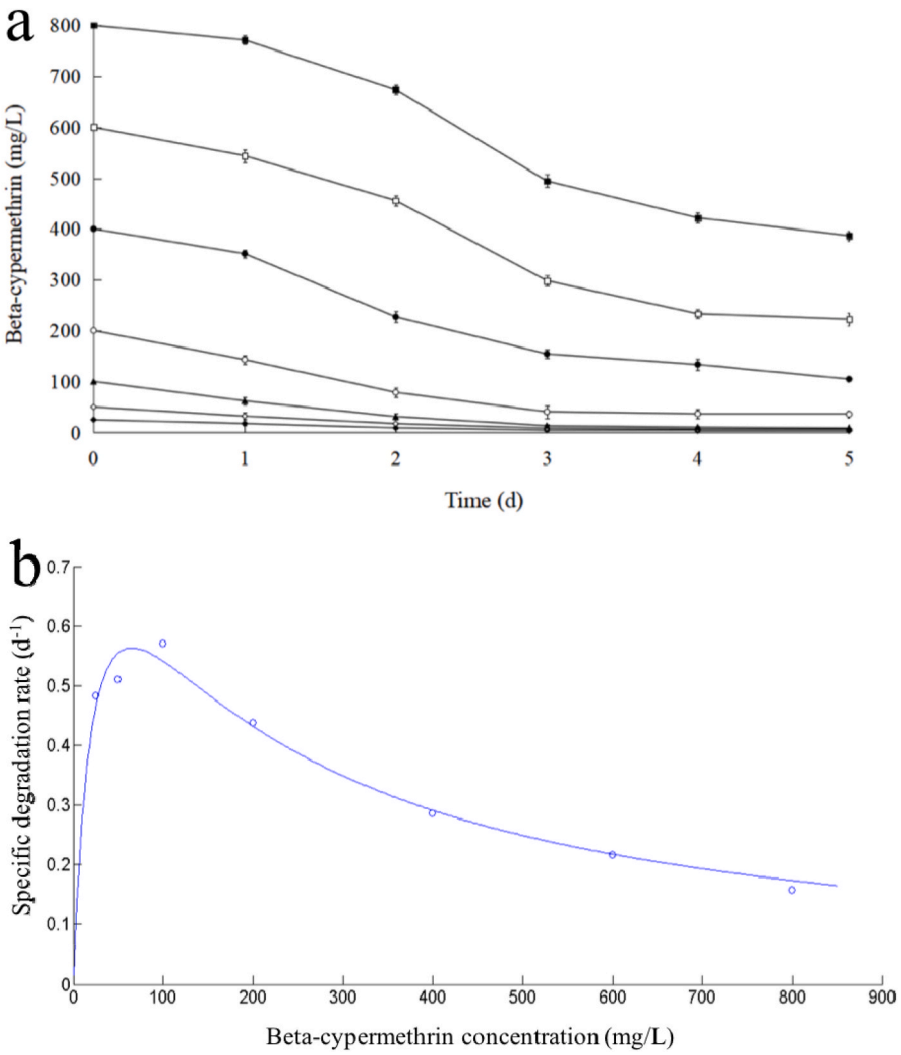


Fig. 4. Degradation kinetics of beta-cypermethrin at different initial concentrations by strain PAO1. a: Degradation of beta-cypermethrin at different concentrations; ■, 800 mg/L; □, 600 mg/L; ●, 400 mg/L; ○, 200 mg/L; ▲, 100 mg/L; ⋄, 50 mg/L; ◆, 25 mg/L b: Inhibition curve of different concentrations of beta-cypermethrin degraded by strain PAO1 by fitting the Andrews equation.

in the range of initial concentration less than 800 mg/L, indicating that strain PAO1 possesses a strong beta-cypermethrin degradation ability.

3-Phenoxybenzaldehyde is the key metabolite of beta-cypermethrin degradation (Fan et al., 2023; Xu et al., 2020). In this study, we tested the degradation ability of 3-phenoxybenzaldehyde at various initial concentrations by strain PAO1. As shown in Fig. 5a, strain PAO1 could tolerate and degrade 3-phenoxybenzaldehyde up to 500 mg/L. Similar to the degradation of beta-cypermethrin, strain PAO1 degraded more than 80% of 3-phenoxybenzaldehyde at the lower initial concentrations (<200 mg/L); however, strain PAO1 degraded only 52.6% of 3-phenoxybenzaldehyde at 500 mg/L initial concentration.

Fig. 5b demonstrates 3-phenoxybenzaldehyde degradation inhibition curve of various initial concentrations. The kinetic parameters for the 3-phenoxybenzaldehyde inhibition model, including K_i, K_s, S_m, and q_{max}, were noted as 88.8875 mg/L, 27.7675 mg/L, 49.6808 mg/L, and 1.2621 d^{-1}, respectively. The R^2 value was determined to be 0.9892 that confirms the alignment of degradation data to the model. As can be seen from Fig. 5b that at a 3-phenoxybenzaldehyde initial concentration of lower than 49.6808 mg/L, the specific degradation constant of the strain PAO1 to the 3-phenoxybenzaldehyde increased rapidly in direct proportion to the rise in initial concentration. To the contrary, at 3-phenoxybenzaldehyde initial concentration more than 49.6808 mg/L, the specific degradation constant of 3-phenoxybenzaldehyde by strain PAO1 decreased inversely to the rise in 3-phenoxybenzaldehyde initial concentrations, indicating substrate inhibition. In general, the strain PAO1 also presented a strong degradation capability for 3-phenoxybenzaldehyde.

In most cases reported to date, the degraders tended to biotransform beta-cypermethrin by hydrolysis to produce 3-phenoxybenzaldehyde, which in turn accumulated in the environment and accelerated biotransformation could not occur (Chen et al. 2011b, 2011c; Bhatt et al., 2021c; Zhao et al., 2022). In previous studies, biotransformation of beta-cypermethrin and 3-phenoxybenzaldehyde by the same strain was rarely reported (Wang et al., 2009; Chen et al., 2013a; Bhatt et al., 2020c). In this study, the particular strain was found to effectively degrade not only beta-cypermethrin but also its toxic metabolite 3-phenoxybenzaldehyde. Biotransformation of 3-phenoxybenzaldehyde by the same strain that transformed beta-cypermethrin was of vital importance because 3-phenoxybenzaldehyde is not only persistent to biotransformation but also limits the biotransformation of the beta-cypermethrin due to its antimicrobial activities.

3.4. Bioremediation of beta-cypermethrin in soils

The bioremediation of beta-cypermethrin-contaminated soil by strain PAO1 was simulated under controlled conditions in the

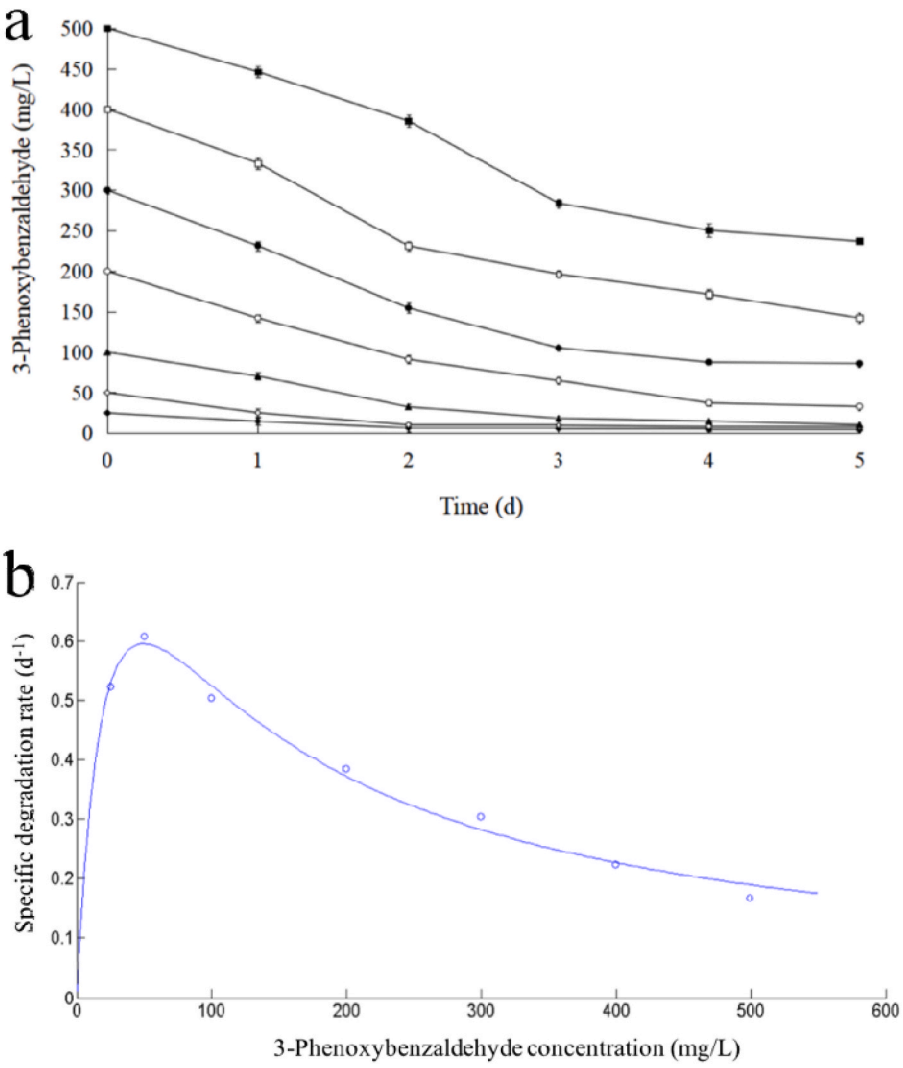


Fig. 5. Degradation kinetics of 3-phenoxybenzaldehyde at different initial concentrations by strain PAO1. a: Degradation of 3-phenoxybenzaldehyde at different concentrations; ■, 500 mg/L; □, 400 mg/L; ●, 300 mg/L; ○, 200 mg/L; ▲, 100 mg/L; ⋄, 50 mg/L; ◆, 25 mg/L b: Inhibition curve of different concentrations of 3-phenoxybenzaldehyde degraded by strain PAO1 by fitting the Andrews equation.

laboratory. The results showed that strain PAO1 could exert a highly effective removal effect on beta-cypermethrin in soil, as shown in Fig. 6. Soils with different treatments exhibited distinct degradation efficiencies, and strain PAO1 accelerated the elimination of beta-cypermethrin in soils from the third day. After 15 days of incubation at 30 °C in dark, the non-sterilized treatment supplemented with the strain PAO1 showed the highest degradation efficiency, followed by the sterilized treatment supplemented with strain PAO1, with degradation efficiencies of 84.67% and 78.4%, respectively. The lowest degradation efficiency was observed in the sterilized control group, with a natural degradation efficiency of 13.34%. The outcomes of degradation process clearly demonstrated that the treatment group added with strain PAO1 significantly reduced the presence of beta-cypermethrin residues in the soil. Additionally, the efficiency of beta-cypermethrin degradation in non-sterilized samples was significantly higher as compared to sterilized samples.

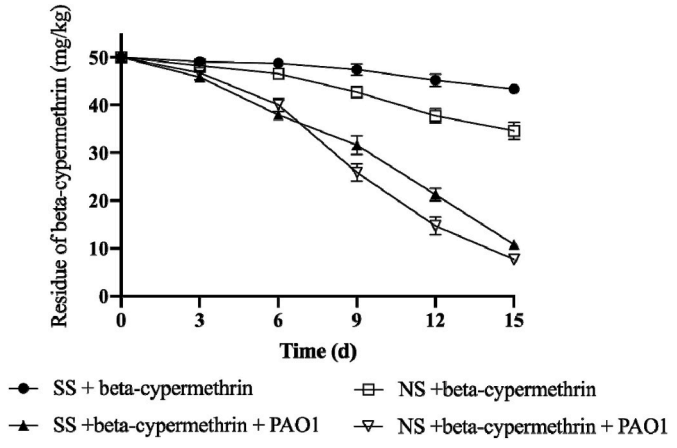


Fig. 6. Biodegradation of beta-cypermethrin in different soils by strain PAO1.

Based on the original data obtained for strain PAO1, in terms of degrading beta-cypermethrin in soil, a first-order kinetic model further analyzed the process by which strain PAO1 degrades beta-cypermethrin in the soil. The results are listed in Table 3. The correlation coefficients (R^2) for the four treatments were more than 0.8859, revealing a first-order kinetic model-based beta-cypermethrin degradation by PAO1 in soil samples. The degradation constant of the non-sterilized treatment group added with strain PAO1 was 0.1014, which was 13-fold higher than controls (sterilized), with the lowest degradation rate (0.0079 day^{-1}). More intuitively, considering the theoretical half-lives of the four treatment groups, it can be seen that the half-life of beta-

Table 3
Kinetic parameters of degradation of beta-cypermethrin in soils with strain PAO1.

Treatments	Regression equation	k	R^2	$t_{1/2}$ (d)
SS + beta-cypermethrin	$C_t = 51.9e^{-0.0079t}$	0.0079	0.9044	87.74
NS + beta-cypermethrin	$C_t = 50.6e^{-0.0228t}$	0.0228	0.9233	30.40
SS + beta-cypermethrin + PAO1	$C_t = 49.8e^{-0.0694t}$	0.0694	0.9358	9.99
NS + beta-cypermethrin + PAO1	$C_t = 50.0e^{-0.1014t}$	0.1014	0.8859	6.84

Note: SS refers to sterile soils; NS refers to nonsterile soils; C_t refers to D-cyphenothrin degradation (mg•L^{-1}); k refers to degradation constant (day^{-1}); t refers to degradation times (days); R^2 refers to correlation coefficient.

cypermethrin added with strain PAO1 was substantially shorter than controls without PAO1. The half-life of the original 87.74 days was shortened by about 9 times after the addition of strain PAO1, particularly in sterilized control group.

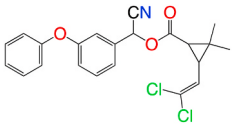
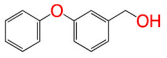
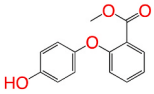
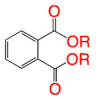
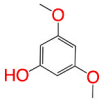
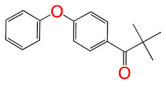
Degrading micro-organisms have been widely reported for the elimination of pyrethroids in soil (Wu et al., 2023). The research carried out by Zhao et al. (2015) has revealed that the residual amount of beta-cypermethrin in soil reduced from 22.29 mg/kg to 4.41 mg/kg after strain *Bacillus licheniformis* B-1 supplemented with surfactant Brij-35 was cultured for 22 days. The yeast *Candida pelliculosa* ZS-02 was introduced into the contaminated soil containing 50 mg/kg bifenthrin, which removed 75% of the bifenthrin within 10 days (Chen et al., 2012c). However, in this study, the half-life of the unsterilized treatment group was only 4.44-fold shorter after the addition of strain PAO1. Therefore, it can be seen that the indigenous micro-organisms contained in the non-sterilized treatment group were also involved in the biological metabolism of beta-cypermethrin. Previous studies have shown that pyrethroids can affect the community structure of soil indigenous micro-organisms when they enter the soil environment (Braganca et al., 2019). However, the fate of beta-cypermethrin in soil is closely related to the indigenous micro-organisms. Qi and Wei (2017) found that *Pseudomonas*, *Hyphomicrobium*, *Dokdonella*, and *Methylolis* were enriched in the soil of a cotton planting area which had been exposed to beta-cypermethrin for a long time, through high-throughput sequencing. In their study, 89.84% of beta-cypermethrin could be eliminated within 4 days using the microbial broth enriched from the soil sample.

3.5. Possible degradation pathways of beta-cypermethrin metabolized by strain PAO1

The extraction of samples, collected at various intervals during cypermethrin biodegradation, was carried out. Based on HPLC-MS/MS analysis, we found two distinct peaks at retention times (RTs) of 19.734 and 19.923 min, where the main part of the two peaks overlapped, and the secondary mass spectrometry showed consistent mass-to-charge ratio (m/z) of these two peaks, which was 415.07 (Figure S2). Further comparison with similar compounds in the library database of the National Institute of Standards and Technology (NIST, USA) was carried out. Based on RTs and ion fragment characteristics, the two peaks found at 19.734 and 19.923 min were identified as isomers of beta-cypermethrin. The amount of beta-cypermethrin decreased gradually with the extension of degradation time, and five intermediate products were detected in different periods. Information on all intermediates is listed in Table 4. According to similar methods, the above five intermediates were identified as 3-phenoxybenzyl alcohol, methyl 2-(4-hydroxyphenoxy)benzoate, diisobutyl phthalate, 3,5-dimethoxyphenol, and 2,2-dimethyl-1-(4-phenoxyphenyl)propanone.

Based on the detection time of the five intermediate metabolites under different culture times, combined with the reported biological metabolic pathway of beta-cypermethrin, we proposed a possible

Table 4
The metabolites of beta-cypermethrin degraded by strain PAO1.

Code	RTs (min)	m/z	Compound structures	Names
1	19.734/ 19.923	415.07		Beta-cypermethrin
2	19.098	200.08		3-Phenoxybenzyl alcohol
3	16.050	244.07		Methyl 2-(4-hydroxyphenoxy) benzoate
4	9.773	278.15		Diisobutyl phthalate
5	9.311	154.06		3,5-Dimethoxyphenol
6	18.391	254.13		2,2-Dimethyl-1-(4-phenoxyphenyl) propanone

Note: RTs refers to retention time; m/z refers to mass-to-charge ratio.

metabolic pathway of beta-cypermethrin degradation by strain PAO1 (Fig. 7): first, beta-cypermethrin is decomposed into permethric acid and α-hydroxy-3-phenoxy-benzeneacetonitrile through carboxylate bond cleavage under the catalysis of strain PAO1 hydrolytic esterase. Previous studies have shown that permethric acid and α-hydroxy-3-phenoxy-benzeneacetonitrile are not environmentally stable (Huang et al., 2020), and the former is further hydrolyzed into permethric alcohol within a short time. The latter is further hydrolyzed and oxidized to form 3-phenoxybenzyl alcohol or 3-phenoxybenzoic acid (3-PBA). 3-PBA has a higher half-life and toxicity than beta-cypermethrin (Dalsager et al., 2019), and is deoxygenated and methylated under special conditions to generate 3-phenoxybenzaldehyde and less-toxic 2,2-dimethyl-1-(4-phenoxyphenyl)propanone. In this study, we found that 3-PBA was hydroxylated to form methyl-2- (4-hydroxyphenoxy) benzoate, a previously unrevealed intermediate pyrethroid metabolite.

Subsequently, the macromolecular methyl-2-(4-hydroxyphenoxy) benzoate was further cleaved to small molecular diisobutyl phthalate and 3,5-dimethoxyphenol by strain PAO1. Diisobutyl phthalate is easily hydrolyzed and ring-opened, and converted to pyrocatechol and muconic acid. Phenol, pyrocatechol, and muconic acid are common small-molecule compounds in the downstream pyrethroid metabolites, which are eventually mineralized into water and carbon dioxide after ring cleavage.

To date, there have been many reports on the biotransformation pathways of pyrethroids (Zhai et al., 2012; Xiao et al., 2020; Huang et al., 2022; Wu et al., 2021; He et al., 2022; Lu et al., 2023). In particular, α-hydroxy-3-phenoxy-benzeneacetonitrile, 3-PBA, and 3-phenoxybenzenealdehyde are representative intermediate metabolites of type II pyrethroids (Birolli et al., 2016; Palmer-Brown et al., 2019; Bhatt et al., 2021e). Compared with α-hydroxy-3-phenoxy-benzeneacetonitrile, the current research further elaborated the metabolism of 3-PBA and 3-phenoxybenzaldehyde. Although 3-PBA and 3-phenoxybenzaldehyde are more toxic than beta-cypermethrin, there have been few reports on the simultaneous degradation of the parent compound and the intermediate 3-PBA or 3-phenoxybenzaldehyde. In this study, strain PAO1 not only efficiently removed 91.4% of beta-cypermethrin (50 mg/L) within 5 days, but also showed excellent metabolic ability under high concentrations of 3-phenoxybenzaldehyde.

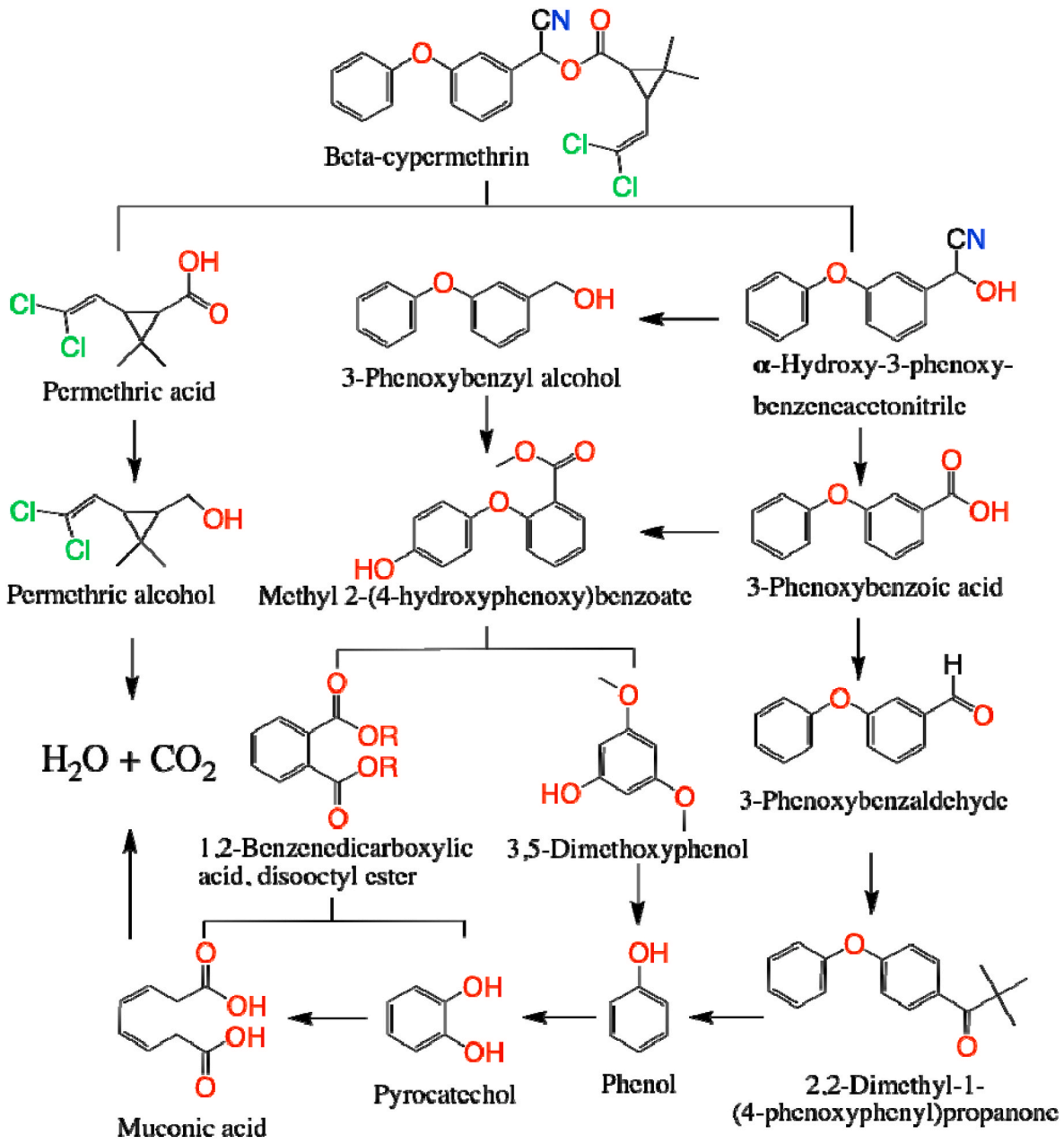


Fig. 7. Proposed pathways for metabolizing beta-cypermethrin by strain PAO1.

A 3-PBA degrading strain, *Aspergillus oryzae* M4, obtained by Zhu et al. (2016) from soy sauce fermentation, demonstrated that 3-PBA can be interconverted with 3-phenoxybenzyl alcohol and finally decomposed into protocatechuic acid, 3-hydroxy-5-phenoxy benzoic acid, gallic acid, and phenol. For *Brevibacillus parabrevis* BCP-09 isolated from activated sludge, 3-phenoxybenzaldehyde and 3-PBA were detected as the main beta-cypermethrin intermediates using an HPLC-MS/MS system (Tang et al., 2018).

4. Conclusions

In this study, *P. aeruginosa* PAO1 not only can rapidly remove beta-cypermethrin, but it also showed efficient degradation ability for its toxic intermediate metabolite, 3-phenoxybenzaldehyde. Soil remediation experiments illustrated that strain PAO1 exhibited a higher beta-cypermethrin degradation efficiency in the non-sterilized soil samples as compared to sterilized soil samples, revealing that the indigenous micro-organisms of non-sterilized soil effectively promoted the metabolic activity of strain PAO1. Ultimately, a degradation pathway of beta-cypermethrin metabolized by the strain PAO1 was suggested, in which methyl 2-(4-hydroxyphenoxy)benzoate and 3,5-dimethoxyphenol were first identified as the intermediate products during the beta-cypermethrin degradation. These intermediates were then further decomposed into non-toxic, small molecular compounds. *P. aeruginosa* PAO harbors the metabolic pathways for complete biodegradation of beta-cypermethrin, which is of great importance in beta-cypermethrin biogeocycle. Our results demonstrated that strain PAO1 is proficient in biotransformation and could be explored for developing a strategy for the remediation of beta-cypermethrin-polluted environment. However, future work such as its interaction with environment, physio-biochemical and genetic aspects, are still needed before the application of this bacterium in the field-scale bioremediation.

Credit author statement

Wen-Juan Chen, Wenping Zhang: Writing – original draft. Qiqi Lei, Shao-Fang Chen, Yaohua Huang, Kalpana Bhatt: Writing- Reviewing and Editing. Lisheng Liao, Xiaofan Zhou: Conceptualization, Investigation, Supervision, Writing-Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This study was financially supported by grants from the Key Realm R&D Program of Guangdong Province, China (Grant No. 2020B0202090001) and Guangdong Basic and Applied Basic Research Foundation of natural Science Foundation, China (Grant No. 2022A1515010564).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.116619.

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