Enhanced Plant-microbe Remediation of PCBs in Soil using Enzyme Modification Technique Combined with Molecular Docking and Molecular Dynamics

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Abstract: The study on the enhanced mechanisms of the enzymes involved in plant absorption, plant degradation, and microbial mineralization in the remediation of soils contaminated with polychlorinated biphenyls (PCBs) is of great significance for the application of plant-microbe combined remediation technique in PCB-contaminated soils. The present study first used a combination of molecular docking and molecular dynamics methods to calculate the effects of the plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme on the PCBs in the soil environment. A multifunctional plant degradation enzyme was constructed with three functional roles of absorption, degradation, and mineralization using amino acid sequence recombination and site-directed mutagenesis to modify the template of plant degradation enzyme. Finally, using the Taguchi experimental design-assisted molecular dynamics simulation method, the suitable external environmental conditions of plant-microbe combined remediation of the PCB-contaminated soil were determined. In total, six multifunctional plant degradation enzymes were designed, which exhibited a significantly improved efficiency of PCB degradation. In comparison to the complex of plant absorption enzyme, plant degradation enzyme, and microorganism mineralization enzyme (6QIM-3GZX-1B85), the six multifunctional plant degradation enzymes exhibited significantly higher efficiency (2.10–2.38 times) in degrading the PCBs, with a maximum of 2.69 times under suitable external environmental conditions.

Keywords: Plant-Microbe Combined Remediation; Polychlorinated Biphenyls; Soil; Amino Acid Sequence Recombination; Site-Directed Mutagenesis; Molecular Dynamics
1 Introduction

Polychlorinated biphenyls (PCBs) have 209 congeners based on the different positions and number of chlorine atom substitutions. Previously, PCBs were used widely as dielectrics and lubricants in capacitors and transformers and also as additives in paints or plastics due to their highly stable physical and chemical properties, good electrical insulation, high heat resistance, and low flammability [1]. However, PCBs exhibit environmental persistence, high biological toxicity, high bioconcentration, and long-range migration, because of which they are now listed among the 12 environmental persistent organic pollutants (POPs) to be controlled under the Stockholm Convention [2-3]. The period between the 1920s and 1970s is considered the golden age of PCB production and application. Since the 1980s, countries worldwide have gradually banned the production and application of commercial mixtures containing PCBs. Nonetheless, large quantities of environmentally persistent commercial mixtures of PCBs continue to exist in the electrical systems, land, and landfills [4]. Besides the global pollution caused by PCBs, the incineration of chlorinated compounds and the use of chlorinated chemicals continues to introduce PCBs into the soil environment. Humans would be exposed to soil environments containing these contaminants for decades and even centuries to come [5].

The estimated cumulative global production of commercial PCBs is $1.3 \times 10^6$ tons, out of which, approximately 440–92,000 tons of PCBs are being released into the environmental media. Since PCBs have low volatility and low water solubility, the soil environment becomes their largest sink, accounting for approximately 93.1% of the PCBs existing in the environment [6]. Numerous studies have demonstrated that PCBs in contaminated soils have reached levels that could severely affect human health [7-8]. The concentrations of PCBs are observed to be in the range of 0.0626–97 ng·g$^{-1}$, with an average of 5.41 ng·g$^{-1}$ in the 191 topsoil sites investigated worldwide; PCBs have even been detected in the soil samples from the Antarctic regions [9-10]. The evaluation of 7 typical PCBs in the surface soil samples collected from highly industrialized areas around the Yellow Sea and the Bohai Sea revealed residues of PCBs in a concentration of up to 385.67 ng·g$^{-1}$ dw, with a mean value of 19.89 ng·g$^{-1}$ dw [11]. The 23 sediment samples collected from the soil sediments in the Antarctic Ross Sea and the Drake Passage revealed the presence of 51 PCBs, with the concentrations of individual and total PCBs belonging to the same species varying significantly with the sampling sites and exhibiting the maximum concentrations
of 49.75 ng·g⁻¹ and 246.86 ng·g⁻¹, respectively [12]. The data for the urban soils of the different regions of China collected between 2004 and 2018, which included the PCBs detection data, reveals that the average concentration of PCBs in these soil samples was 4984 mg/kg and that 64% of the study areas had the total carcinogenic risk values for PCBs above the individual lifetime acceptable risk level of 10⁻⁴ mg/kg. Meanwhile, the non-carcinogenic risk values exceeded the target risk level (10⁻¹ mg/kg) in 53% of the areas, and via ingestion, inhalation, and dermal exposure routes, the lifetime carcinogenic and non-carcinogenic risks of PCBs in the urban soils of China have exceeded the safe levels in most cases [8]. Therefore, it is clear that the soil is contaminated with a variety of PCBs and the residual concentration of the PCBs in the contaminated soils is considerably high, which is harmful to human health as well as for the ecological environment in the long-term perspective. Therefore, identifying an efficient and secondary contamination-free method for the remediation of PCB-contaminated soils is of great significance.

The traditional remediation approach for soil pollution include physical, chemical, and biological methods. It has been reported that PCBs can be adsorbed by adding activated carbon to the soil, and the fixation and treatment of PCBs in polluted soil can be completed [13]. However, physical methods cannot degrade PCBs completely, so they need to be combined with other treatment methods. This method has the disadvantage of easy to produce secondary pollutants. It has been shown that the combined use of H₂O₂ and KMnO₄ as chemical oxidants had a certain effect on the remediation of PCBs contaminated soil [14]. The chemicals used may cause damage to the health of the soil, and the cost is relatively high. It has been reported that the concentration of PCBs decreased by 53.1% after a combination treatment with ZY1, a strain isolated from the rhizobia of Astragalus rhizogenic, which exerted a synergistic effect and promoted the extraction and degradation of PCBs [15]. Compared with the physical method and chemical method, biological method is widely used in soil remediation of organic pollutants because of its simple operation, low cost, and no secondary pollution. Plant-microbe remediation technology is a highly efficient biological treatment method [16].

In order to improve the efficiency of bioremediation, genetically modified plants and microorganisms are applied in the bioremediation of PCBs contaminated soil. For instance, the isolation of cytochrome P450 monooxygenase genes and their functional expression in
recombinant enzyme enabled efficient oxidation of cyclohexane to cyclohexanol [17]. Biphenyl dioxygenase was modified to enhance its ability to degrade contaminants in the plant along with enabling its secretion from the root system into the soil environment to promote contaminant degradation in the soil as well [18]. The main processes involved in the plant-microbe combined remediation of organic soil contamination are plant absorption, plant degradation, and microbial mineralization [19]. The water channel enzyme in the plant roots mediates the transport of PCBs within the plant and consequently influences the ability of the plant to absorb PCBs [20]. Plants secrete the enzyme biphenyl dioxygenase, which is associated with the degradation of PCBs, and the ability of this biphenyl dioxygenase to degrade PCBs influences the effectiveness of plant-based remediation of PCB-contaminated soil [18]. The mineralization of the PCBs by plant rhizosphere is one of the approaches of plant-microbe combined remediation of PCB-contaminated soil. The microorganisms in the plant rhizosphere include both fungi and bacteria. A typical species of the white-rot fungus, viz., *Phanerochaete chrysosporium*, was investigated, and it was observed that its secreted lignin peroxidase that mainly mineralized the organic pollutants [21]. Since the plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme are involved directly in soil remediation, if we modify an enzyme with three functions of absorption, degradation and mineralization. When multifunctional enzyme is applied to the plant-microbe combined remediation technology, the remediation ability of the technology will be greatly improved.

Enzyme modification allows the identification of the key amino acid residues in the plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme that could be functionally relevant to the action of these enzymes on PCBs. Further, precisely designing the mutation sites in these amino acids helps in altering these specific residues in the target enzyme to improve their functional properties. For instance, biphenyl dioxygenase was modified to enhance its ability to degrade contaminants in the plant along with enabling its secretion from the root system into the soil environment to promote contaminant degradation in the soil as well [18]. Zhang et al. (2010) studied the effects of amino acid residues at the Cel6A active site of *Thermobifida fusca* cellulase, and mutants modified with the amino acid residues were found to exhibit improved hydrolytic activity towards carboxymethyl cellulose [22]. To promote the biodegradation of aromatic hydrocarbons in contaminated soils, the key acid residues of
naphthalene dioxygenase (NDO) was modified [23]. However, there are few reports on the application of enzyme modification to reconstitute the key amino acid fragments of plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme with the aim of obtaining a multifunctional enzyme with the combined ability of absorption, degradation, and mineralization to facilitate the remediation of PCB-contaminated soils. It is reported that the physicochemical properties of the soil are a major factor affecting the plants–microorganisms combined remediation of soil contaminants [24]. In this context, the present study identified the internal modification scheme of these three enzymes and their external environmental conditions to enhance their ability to realize plant-microbe remediation.

The present paper describes the study of the ability of plants to absorb, degrade, and mineralize the PCBs in the soil environment by using a combination of molecular docking and molecular dynamics methods. Furthermore, the enzyme identified to have the strongest efficiency against PCBs was selected as the template for generating the modified enzyme with a combination of the three functions roles of absorption, degradation, and mineralization. The multifunctional enzymes for enhanced PCB-contaminated soil remediation were constructed under consistent external environmental stimulation conditions. The suitable external environmental stimulation conditions for enhanced plant–microbial combined remediation of PCB-contaminated soil were determined by evaluating different external environmental stimulation conditions of plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme. In addition, the preferential and applicability analysis of the modified enzymes for the degradation of different PCBs was conducted to provide a basis for the enhanced plant-microbe combined remediation of PCB- contaminated soils. The findings would provide a research perspective for enhancing enzyme repair ability to enhance the plant-microbe combined remediation of PCB-contaminated soils indirectly.

2 Materials and Methods

2.1 Characterization of CBs binding to plant-microbe enzyme using molecular docking

The present study investigated the seven PCB contaminants (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB-180) present commonly in soil, among which PCB-118 is a member of the class of dioxin-like polychlorinated biphenyls [25]. Three representative functional enzymes (water channel enzyme (6QIM), biphenyl dioxygenase (3GZX) and lignin peroxidase
(1B85)) were selected as target enzymes to analyze the ability of plant absorption, plant degradation and microbial mineralization of PCBs. To find a modification method to improve the ability of functional enzymes to degrade PCBs. The structures of the water channel enzyme (6QIM), biphenyl dioxygenase (3GZX), and lignin peroxidase (1B85) were obtained from the Protein Data Bank (http://www1.rcsb.org).

The seven PCBs (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180) as the ligands and the three enzyme receptors (plant absorption enzyme: 6QIM, plant degradation enzyme: 3GZX, microbial mineralization enzyme: 1B85) were loaded into the Discovery Studio 2020 software. The three selected enzymes were defined as the corresponding three enzyme receptors using the LibDock module in the Discovery Studio software, and the ‘Find Sites from Receptor Cavities’ tool under the Define module was used for identifying the possible binding sites in these three enzyme receptors. Each of the seven PCB ligands was docked to the three enzyme receptors and integrated into the protein binding cavity the enzymes, generating a total of 21 ligand-receptor complexes.

Since the actual soil environment contains a variety of PCB pollutants, it was decided to investigate the effect of a complex of all the PCBs on the binding of the plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme to it by docking the 7 PCB ligands with each of the three enzyme receptors sequentially and then integrating them together as a whole into the respective binding cavities of the three enzymes, generating a total of 3 complexes.

2.2 Multifunctional enzyme modifications using amino acid sequence recombination and site-directed mutagenesis

The sequences of the amino acid residues of plant absorption enzyme (6QIM, PDB ID: 32363405), plant degradation enzyme (3GZX, PDB ID: 295789303), and microbial mineralization enzyme (1B85, PDB ID: 4558109) were retrieved from the website of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/protein). The active regions directly influencing the interaction of the PCBs with the three enzymes were identified, and the corresponding key amino acid residue sequences of the active regions were retrieved. The key amino acid residues influencing the absorption, degradation, and mineralization of PCBs were subjected to recombination via rational design. Based on sequence alignment, we know the
corresponding positions of the key amino acid residues of two functional enzymes in the third functional enzyme. The site-directed mutagenesis module under Discovery Studio 2020 software can perform rational design in functional enzymes [26]. Therefore, the corresponding positions of the third functional enzyme were replaced by the key amino acid residues of the other two functional enzymes. The purpose is to keep the function of the third enzyme unchanged and to combine the functions of the other two enzymes. The insertion of key amino acid residues corresponding to the active regions of two enzymes into the third enzyme exerts a significant effect on PCBs, and therefore, this approach was used for designing and constructing a multifunctional enzyme with a combination of the three functional roles of plant absorption, plant degradation, and microbial mineralization.

According to the similar phase solubility principle, PCBs are hydrophobic molecules, which bind stably to the hydrophobic region formed by hydrophobic amino acids residues [27-28]. Therefore, an attempt was made to replace hydrophilic molecules with hydrophobic amino acids residues (the hydrophobicity of the amino acid residues in descending order: Val > Ile > Leu > Cys) by the site-directed mutagenesis method [29]. The key amino acid residues in the active region of the multifunctional enzyme were mutated to produce the multifunctional enzyme. The structural reliability of the designed multifunctional enzyme was evaluated using the Ramachandran conformational map in the online evaluation server PROCHECK (https://saves.mbi.ucla.edu/). When the percentage of the amino acid residues in the core area + allowable area + maximum allowable areas was greater than 95%, it was considered that the model meets the quality requirements [30-31].

Furthermore, CHARMM force field was applied to the designed multifunctional enzyme using the “Apply Forcefield” module in the Discovery Studio 2020 software. In addition, the affinity of the multifunctional enzyme toward the mutated amino acids and the thermal stability of the enzyme structure were evaluated using the “Calculate Mutation Energy (Binding)” and “Calculate Mutation Energy (Stability)” modules, respectively, in the same software. The thermal stability mutation energy of an enzyme is an important parameter determining its, that is, the enzyme’s ability to maintain its functional activity under higher temperature conditions. The heat resistance of an enzyme is a necessity for its application in complex real-world external environments [32]. The principle for calculating the affinity of the multifunctional enzyme toward
the mutated amino acids and the thermal stability of the multifunctional enzyme structure is similar, as both are determined by the mutation energy of the enzyme structure. The only difference is that the mutation energy representing affinity is the difference in the binding free energy of the structure prior to and after mutation, while the mutation energy representing thermal stability is calculated as the difference in the folding free energy of the structure prior to and after mutation. The mutation energy \( \Delta G_{\text{mut}} \) representing the thermal stability of an enzyme is calculated using the following equation [33-35]:

\[
\Delta G_{\text{mut}} = \Delta G_{\text{folding (mutant)}} - \Delta G_{\text{folding (primitive enzyme)}}
\]

\( \Delta G_{\text{folding}} = \Delta G_{\text{folded}} - \Delta G_{\text{unfolded}} \) (2)

\( \Delta G_{\text{mut}} \) represents the difference between the free energy of the mutated enzyme \( \Delta G_{\text{folding (mutant)}} \) and the primitive enzyme \( \Delta G_{\text{folding (primitive enzyme)}} \). The folding free energy, \( \Delta G_{\text{folding}} \), is defined as the difference in the free energy between the folded \( \Delta G_{\text{folded}} \) and unfolded \( \Delta G_{\text{unfolded}} \) states of the protein. The unfolded state of the enzyme was modeled as a relaxed peptide in an extended conformation, with the mutated residue in the center, to account for the van der Waals and short-range electrostatic interactions between the mutated residue and the remaining part of the protein [33-35].

2.3 Enhancement of the plant-microbe combined remediation of PCB-contaminated soil

2.3.1 Characterization of the key enzymes influencing the plant-microbe combined remediation using enzyme-enzyme docking

In order to separately determine the conditions that would promote the simultaneous absorption, degradation, and mineralization of the PCBs in the plant-microbes combined remediation of soil in different external environment conditions, enzyme-enzyme docking was performed in the “ZDOCK” module in the Discovery Studio 2020 software. One of the three enzymes was randomly selected as the target enzyme, and its structure was loaded into the Discovery Studio 2020 software as the receptor, while the other two enzymes were designated as ligands 1 and 2 using the “ZDOCK” module. The “RMSD Cutoff” was set to a cluster radius of 6.0 Å, the “Interface Cutoff” was set to 9.0 Å, and the “ZRank” was set to false. Subsequently, the docked 6QIM-3GZX-1B85 enzyme receptor was saved as ZDock.pdb. Finally, molecular docking was performed to generate a composite between the 7 PCBs and ZDock.pdb.
2.3.2 Determination of the external environment conditions to promote the plant-microbe combined remediation using Taguchi experimental design

In this analysis, the following five different external environmental conditions influencing the degradation of PCBs by plants and microorganisms in the soil environment were used as factors for generating the Taguchi experimental design table: pH, temperature (°C), organic matter content (nitrogen and phosphorus dosing ratio), oxygen promoter concentration (mg/L), and catalyst concentration (mg/L). The two levels of external environmental conditions were selected as described below.

As the pH of soil varies widely, different pH levels influence the efficiency of PCB biodegradation. The biodegradation of PCBs is better at the pH range of 6–8, and the degradation decreases when the pH drops below 5 or is above 9 [36]. Therefore, the physically lower (pH = 5) and higher (pH = 9) pH values were selected as the two levels of the external environmental factor pH.

Temperature is one factor that directly influences the biodegradation of aromatic hydrocarbon pollutants. The optimum temperature range for the biodegradation of PCBs is 22–40 °C, although certain cold-tolerant bacteria are capable of degrading the PCBs pollutants even at low temperatures in the range of 3–20 °C [37]. Therefore, the physically lower (15 °C) and higher (35 °C) values of temperature were selected as the two levels of the external environmental factor temperature.

Since organic matter content has a huge impact on the rate of biodegradation of pollutants, this can only be improved if the nutrient requirements of the microbial metabolism are fulfilled. The best degradation of pollutants by bacteria is achieved at the appropriate nitrogen to phosphorus ratio of 10:1 [38]. Therefore, the lower (1:1) and higher (10:1) ratio values were selected as the two levels of the external environmental factor of organic matter content.

Sufficient levels of oxygen are required for the oxidation process with which the PCBs are degraded. Therefore, Oxygen promoter concentration is an important external environmental factor. H\textsubscript{2}O\textsubscript{2} concentration of 50 mg/L promotes the degradation of PCBs, while the concentrations above 300 mg/L result in slower degradation [39]. Therefore, the lower (50 mg/L) and higher (350 mg/L) physical values of H\textsubscript{2}O\textsubscript{2} concentrations were selected as the two levels of the factor of oxygen promoter concentration.
A catalyst enhances the activity of the degradation enzymes. The concentration of resveratrol, a catalyst, at 150 mg/L promotes the degradation of pollutants by the degradation enzymes, while at 200 mg/L, the degradation slows down [19]. Therefore, in the present study, the lower (150 mg/L) and higher (200 mg/L) values of resveratrol concentration were selected as the two levels of the factor catalyst.

2.3.3 Suitable external conditions for promoting plant-microbe combined remediation of PCB-contaminated soil

The Taguchi experimental design method is a particular-orthogonal method of experimentation [40]. This method could analyze many variables using a relatively small number of experiments. The molecular dynamics could simulate the interaction between ligands and receptors under different environment. The Taguchi experimental design was used to determine the different combinations of external environmental stimulation, and the molecular dynamics was used to calculate the interaction between ligands and receptors under different external conditions. The molecular dynamics method assisted by the Taguchi experimental design was applied to determine the suitable level and combination of external environmental conditions to promote plant-microorganism combined remediation of PCBs-contaminated soil.

The above-stated five factors along with their two levels were used as the standard-setting of the external environmental conditions for plant-microbe combined remediation of PCB-contaminated soil to generate the Taguchi experimental design table covering 32 different sets of external environmental conditions using the “Taguchi design method” module in the Minitab software (Table 1). The molecular dynamics simulations involving the docking of the 7 PCBs together as a whole with the plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85) were performed separately.

The magnitude of the binding energy of the PCBs for the plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85) represents the strength of binding of the PCB pollutants to these three enzymes [19]. In the present study, ten complexes (three complexes of the seven PCBs together as a whole docked with plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85); one complex of the seven PCBs together as a whole docked with the 6QIM-3GZX-1B85 enzyme receptor; and six complexes of the seven PCBs together as a whole docked with the designed
multifunctional plant degradation enzyme) were subjected to molecular dynamics simulations performed in the Gromacs software using the Dell PowerEdge R7525 server. Each complex was individually placed in a periodic 12 cube with a side length of 10 nm. Gromacs96 43a1 force field was applied for molecular beam reduction, and Na\textsuperscript{+} ions were added to neutralize the system charge. The PCBs and the enzyme complexes were established as a group and subjected to energy minimization simulations using the steepest gradient method, with the number of simulation steps set at 50,000, the simulation time set at 100 ps for both heat and pressure baths, and the size of the pressure bath set at a constant standard atmospheric pressure of 1 bar. The duration of the molecular dynamics’ simulation was set to 200 ps for each different-level group. The change in the binding energy of the PCBs to the enzyme complexes under different external environmental stimulation conditions was calculated by the simulation. Finally, the binding energy data generated by the molecular dynamics’ simulation was combined with the Taguchi experimental design method to analyze the enhanced plant-microbe combined remediation ability under different external environmental stimulation conditions.

3 Results and Discussion

3.1 Analysis of the ability of typical PCB molecules to interact with the plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme

3.1.1 Binding capacity of the individual molecules of typical PCBs for the plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme

Each of the 7 PCB ligands (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180) was docked with the receptors of plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85), generating a total of 21 complexes. The binding energies of these 21 complexes were calculated based on the molecular dynamics, and the remediation ability of the three enzymes for PCB-contaminated soil was evaluated.

The y-axis numbers represent the binding energy between PCBs and enzymes. The results revealed that as the number of chlorine atoms in the PCBs increased, the binding of the PCBs to plant absorption enzyme (6QIM) generally demonstrated an increasing trend, where the binding of heptachlorobiphenyl (PCB-180) to plant absorption enzyme was lowest. The binding of the PCBs (PCB-28, PCB-101, PCB-118, PCB-153) to plant mineralization enzyme (1B85) demonstrated an increasing trend. The average binding energies of the other three molecules (PCB-52, PCB-138,
PCB-180) are lower than those of the four molecules, but the trend is increasing. On the contrary, the binding of the PCBs to plant degradation enzyme (3GZX) generally demonstrated a decreasing trend, with the binding of pentachlorobiphenyl (PCB-118) to the plant degradation enzyme being the lowest (Figure 1).

In summary, although the binding of the plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85) to PCBs was inconsistent, the binding energy calculation results revealed that the three enzymes had the ability to degrade these PCBs.

### 3.1.2 Binding capacity of the typical PCBs molecules together as a whole to plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme

The docking of the seven PCBs as a whole with the plant degradation enzyme (3GZX) and the microbial mineralization enzyme (1B85) demonstrated synergistic effects (the sum of the docking of the seven PCBs individually with the plant degradation enzyme and the microbial mineralization enzyme was 641.802 kJ/mol and 314.784 kJ/mol, respectively), while the binding of the seven PCBs as a whole with the plant absorption enzyme (6QIM) demonstrated antagonistic effects (the sum of the docking of the seven PCBs individually with the plant absorption enzyme was 622.894 kJ/mol). It is suggested that the effect of molecular interaction on enzyme cannot be ignored.

Molecular docking of the seven PCBs together as a whole with plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85) generated a total of 3 complexes. The binding energies of the three complexes were calculated using the molecular dynamics simulation. Further, the effects of the PCBs on the plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme were analyzed (Table 2).

In summary, the binding energy of the combined action of the seven PCBs as a whole on the plant degradation enzyme (3GZX) was the largest. It was also 2.57 times and 1.21 times higher than the binding energy of the combined action of the seven PCBs as a whole on the plant absorption enzyme (6QIM) and the microbial mineralization enzyme (1B85), respectively. This, indicated that the plant degradation enzyme (3GZX) is more suitable for application in the remediation of PCB-contaminated soils.

### 3.2 Modification and evaluation of the multifunctional plant degradation enzymes for
**enhanced degradation of the PCBs**

Considering that the plant degradation enzyme (3GZX) was more suitable for the remediation of soil pollution under the combined action of PCBs, this enzyme was selected as the template for enzyme modification to construct a multifunctional plant degradation enzyme with a combination of the three functional roles of absorption, degradation, and mineralization, to achieve the objective of strengthening the combined plant–microorganism remediation of PCB-contaminated soil.

The active regions within the PCBs responsible for the interaction of these PCBs with the plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85) were identified using the NCBI database, and the amino acid residues in these active regions were retrieved. The main active sites identified within the PCBs for the plant absorption enzyme (6QIM) interaction were sites 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, with the key amino acid residues of Ala, Val, Ile, Glu, Phe, Thr, etc. The main active sites identified within the PCBs for the plant degradation enzyme (3GZX) interaction were sites 226, 227, 230, 231, 233, 234, 239, 319, 321, 331, 376, and 382, with the key amino acid residues of Gln, Phe, Asp, Met, His, Ala, etc. The main active sites identified within the PCBs for the microbial mineralization enzyme (1B85) interaction were sites 50, 53, and 54, with the key amino acid residues of Glu, His, and Tyr. The key amino acid residues corresponding to the active regions within the PCBs for interaction with the plant absorption enzyme (6QIM) and microbial mineralization enzyme (1B85) were introduced at the corresponding positions of the plant degradation enzyme (3GZX). The key amino acid residues of the microbial mineralization enzyme (1B85) had fewer sites and overlapped with certain sites of the plant absorption enzyme (6QIM). Therefore, for the enzyme modification, the overlapping amino acid residue sites were preferentially introduced into the key amino acid residues within the microbial mineralization enzyme (1B85) (Figure 2).

In order to promote the binding between the PCBs and the multifunctional plant degradation enzyme, the key amino acid residues in the active region of the multifunctional plant degradation enzyme were mutated into common hydrophobic amino acid residues. The calculations revealed that the seven PCBs docked significantly to the multifunctional plant degradation enzymes after the targeted mutation of the amino acid residues at sites 230, 234, and 319 of the multifunctional
plant degradation enzymes into Val or Ile residues. Accordingly, six multifunctional plant degradation enzymes (3GZX-1, 3GZX-2, 3GZX-3, 3GZX-4, 3GZX-5, and 3GZX-6) were designed, and the binding energies of the seven PCBs as a whole interacting with these six multifunctional plant degradation enzymes were 719.592 kJ/mol, 716.383 kJ/mol, 698.524 kJ/mol, 692.853 kJ/mol, 687.546 kJ/mol, and 669.489 kJ/mol, respectively (Table 3). In comparison to the binding energy of the complexes of the seven PCBs as a whole docked with 6QIM-3GZX-1B85 (302.290 kJ/mol), the respective binding energies increased by 2.38 times, 2.37 times, 2.31 times, 2.29 times, 2.27 times, and 2.10 times, respectively, indicating that the designed multifunctional plant degradation enzymes were significantly effective in degrading the PCBs.

The Ramachandran conformational map obtained using the online server of PDBsum (http://www.ebi.ac.uk) was used for evaluating the structural quality of the enzymes (Figure 3). It was revealed that all the amino acid residues in the six multifunctional plant degradation enzymes were located in the “allowed” region. The sum of the percentage amounts of the bases located in the core, allowed, and generously allowed regions within the six multifunctional plant degradation enzymes was 99.8%, which met the quality rationality requirement of greater than 95%.

Evaluation of the affinity and the thermal stability of the six multifunctional plant degradation enzyme structures (Table 4). When the mutation energy was between −0.5 kcal/mol and +0.5 kcal/mol, the mutation had no effect on the affinity between the multifunctional plant degradation enzyme and the mutant amino acid or on the thermal stability of the multifunctional enzyme. When the mutation energy was less than −0.5 kcal/mol, the mutation enhanced the affinity between the multifunctional plant degradation enzyme and the mutant amino acid, and also the thermal stability of the multifunctional enzyme. As presented in Table 4, the affinity mutation energy and the thermal stability mutation energy of the six multifunctional plant degradation enzymes were less than −0.5 kcal/mol, indicating that the corresponding mutant amino acids had enhanced the affinity for the multifunctional plant degradation enzymes and improved the structural stability of the six multifunctional enzymes [41].

In summary, six multifunctional plant degradation enzymes were designed, which exhibited a significantly improved efficiency of PCB degradation. In comparison to the complex of plant absorption enzyme, plant degradation enzyme, and microorganism mineralization enzyme (6QIM-3GZX-1B85), the six multifunctional plant degradation enzymes exhibited significantly
higher efficiency (2.10-2.38 times) in degrading the PCBs. The results showed that the structures of the six multifunctional plant degrading enzymes meet the requirements. The six multifunctional plant degradation enzymes exhibited improved affinity toward the mutated amino acids; in addition, the thermal stability of the enzyme structures was also improved.

3.3 Enhanced plant-microbe combined remediation of PCB-contaminated soil under suitable external environmental conditions

3.3.1 Analysis of the suitable external conditions to promote the plant-microbe enzyme remediation of PCB-contaminated soil

The efficiency of the PCBs for the plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85) is influenced by different external environmental conditions (Table 5).

It was observed that the suitable conditions for improving the binding effect of the 7 PCBs were different (Table 6) for plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85). The order of significance for the external environmental conditions promoting the interaction of the PCBs with the plant absorption enzyme (6QIM) and their optimum levels was as follows: oxygen concentration (350 mg/L), N:P ratio (1:1), pH (5), temperature (35 °C), and catalyst concentration (150 mg/L). The order of significance for the external environmental conditions promoting the interaction of the PCBs with the plant degradation enzyme (3GZX) and their optimum levels was as follows: temperature (15 °C), pH (9), catalyst concentration (200 mg/L), N:P ratio (1:1), and oxygen concentration (350 mg/L). The order of significance for the external environmental conditions promoting the interaction of the PCBs with the microbial mineralization enzyme (1B85) and their optimum levels was as follows: oxygen concentration (50 mg/L), N:P ratio (10:1), pH (5), temperature (35 °C), and catalyst concentration (200 mg/L).

In summary, owing to the interspecies variability of the three enzymes, it may be impossible to achieve the ideal improvement in the efficiency of plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85) in degrading the PCBs under the same suitable external environmental conditions. Therefore, if the multifunctional enzyme can perform the function of three enzymes under the same suitable external environmental condition, it will make up for this deficiency.
The binding energy of the ligand molecule bound to the receptor enzyme represents the strength of the interaction between the ligand molecule and the enzyme [42]. The present study revealed that compared to the calculation performed without setting the external environmental stimulation conditions, the calculation performed under suitable external environmental conditions enhanced the binding effect of the seven PCBs as a whole for binding to plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85) by 25.60%, 7.32%, and 10.71%, respectively. This indicated that the most significant improvement was obtained in the binding effect of the seven PCBs as a whole docked with the plant absorption enzyme (6QIM), followed by the improvement in the case of microbial mineralization enzyme (1B85) and plant degradation enzyme (3GZX), respectively (Table 7). This is because the effect of the plant remediation of the organically contaminated soil directly depends on its absorption efficiency for organic pollutants. Therefore, the increased strength of the binding action of the PCBs on the plant absorption enzyme (6QIM) provided the assist for the increased in vivo degradation of PCBs in plants and promoted the efficiency of plants to remediate the PCB-contaminated soil.

In summary, although the strength of the binding effect of PCB pollutants bound to the three enzymes varied, the effect of improving the external environmental conditions for the plant-microbe combined remediation of PCB-contaminated soil was not negligible.

3.3.2 Plant-microbe combined remediation of PCB-contaminated soil under external environmental conditions

In the last part, we studied the effect of plant degradation of PCBs under different external conditions and the effect of microbe degradation of PCBs under different external conditions. In this part, the degradation of PCBs by plant-microbe remediation system under different external conditions was studied. Firstly, the plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme were docked to generate the 6QIM-3GZX-1B85 enzyme receptor containing the three enzymes. Secondly, the seven PCBs as a whole were docked to the 6QIM-3GZX-1B85 enzyme receptor to calculate the binding energy under 32 different sets of external environmental factors (Table 8).

Analysis of the suitable external environmental conditions for the binding effect of the seven PCBs as a whole docked with the 6QIM-3GZX-1B85 enzyme receptor. As presented in Table 9,
the significance ranking of the external environmental conditions promoting the binding effect of PCBs docked with the 6QIM-3GZX-1B85 enzyme receptor, and their optimum levels were as follows: pH (9), catalyst concentration (200 mg/L), oxygen concentration (150 mg/L), temperature (15 °C), and the N:P ratio (10:1). The binding energy of the seven PCBs and the 6QIM-3GZX-1B85 enzyme receptor without suitable external environment conditions was 302.290 kJ/mol. In contrast to this, compared to which the binding energy (709.235 kJ/mol) of the seven PCBs as a whole docked with the 6QIM-3GZX-1B85 enzyme receptor under the suitable external environmental conditions exhibited an increase by 2.35 times. This indicated that the suitable external environmental factors contributed significantly to the binding effect of the seven PCBs as a whole docked with the 6QIM-3GZX-1B85 enzyme receptor. However, it was only 0.89 times the binding energy (796.195 kJ/mol) of the seven PCBs docked to the plant degradation enzyme (3GZX) under the suitable external environmental conditions.

In summary, it was further demonstrated that the interspecies variability of the three enzymes rendered it impossible to achieve the ideal improvement in degrading the PCBs under consistent external environmental conditions.

### 3.3.3 Analysis of the binding energy of PCBs as a whole docked with the multifunctional plant degradation enzymes under suitable external environmental conditions

We carried out the degradation of PCBs by multifunctional enzymes with three function roles under the same suitable external conditions. Molecular dynamics calculations performed for the binding effect of the six PCBs as a whole docked with the six multifunctional plant degradation enzymes under suitable external environmental conditions for the degradation of PCBs by the plant degradation enzyme (3GZX) revealed that the binding energies of the seven PCB molecules as a whole docked with the six multifunctional plant degradation enzymes (3GZX-1, 3GZX-2, 3GZX-3, 3GZX-4, 3GZX-5, and 3GZX-6) under suitable external environmental conditions were 812.184 kJ/mol, 726.134 kJ/mol, 763.428 kJ/mol, 710.642 kJ/mol, 684.633 kJ/mol, and 709.468 kJ/mol, respectively. The binding effect of the seven PCB molecules as a whole docked with the multifunctional plant degradation enzyme 3GZX-1 was the most significant (812.184 kJ/mol), and its degree of improvement was 2.01% of the binding energy of the seven PCB molecules as a whole docked to the plant degradation enzyme (3GZX) under suitable external environmental conditions (796.195 kJ/mol), 14.52% of the binding energy (709.235 kJ/mol) of the seven PCB
molecules as a whole docked to the 6QIM-3GZX-1B85 enzyme under suitable external environmental conditions, 12.87% of the binding energy (719.592 kJ/mol) of the seven PCB molecules as a whole docked to the multifunctional plant degradation enzyme (3GZX-1) under no external environmental conditions, and 168.68% of the binding energy (302.290 kJ/mol) of the seven PCB molecules as a whole docked to the 6QIM-3GZX-1B85 enzyme under no external environmental conditions (Table 10).

In summary, the binding effect of the seven PCB molecules and the six multifunctional plant degradation enzymes under the same external environmental conditions exhibited significant improvement. It indicated that the multifunctional plant degradation enzymes designed in the present study, possessing a combination of the three functional roles, could be used for the plant-microbe combined remediation of PCB-contaminated soils instead of other enzymes.

3.4 Selective analysis of the degradation of PCBs by the multifunctional plant degradation enzymes

In order to study the preference of different PCBs degraded by multifunctional enzymes, four types of dioxin PCBs (Tetrachlorobiphenyl: PCB-77, Pentachlorobiphenyl: PCB-126, Hexachlorobiphenyl: PCB-168, and Heptachlorobiphenyl: PCB-189) were selected as ligands, and the three representative multifunctional plant degradation enzymes (one kind of the three mutations was VAL residues: 3GZX-2; two kinds of the three mutations were VAL residues: 3GZX-1; all three mutations were VAL residues: 3GZX-4) were used as receptors for the selective analysis of the degradation of the PCBs by the multifunctional plant degradation enzymes under the complex action of PCBs. First, the L8 Taguchi experimental design table with four factors (PCB-77, PCB-126, PCB-168, and PCB-189) and two levels [{0, 1}, where zero represented no molecular action of the PCBs and one represented that the molecular action of PCBs exists] was generated using the Taguchi experimental design method. Next, the binding energy of the dioxin-like PCBs with the multifunctional plant degradation enzymes under different compound actions was calculated in combination with the molecular dynamics’ methods. Finally, the Taguchi experimental design method was used for generating the signal-to-noise ratio (SNR) parameters that could represent the intensity of the interaction between the different PCBs and the multifunctional plant degradation enzymes, and to conduct a preferential analysis of the degradation of the PCBs by these multifunctional plant degradation enzymes. The L8 Taguchi
design table and the binding energy of the four kinds of dioxin-like PCBs docked with the three kinds of multifunctional plant degradation enzymes are listed in Table 11. An analysis of the effect diagram of the signal-to-noise ratio parameters of the actions of the four kinds of dioxin-like PCBs with the three kinds of multifunctional plant degradation enzymes (Figure 4) revealed that the four dioxin-like PCBs acted with 3GZX-1 in the following order of priority: Heptachlorobiphenyl: PCB-189, Pentachlorobiphenyl: PCB-126, Tetrachlorobiphenyl: PCB-77, Hexachlorobiphenyl: PCB-168; and, all the four dioxin-like PCBs interacted with 3GZX-2 and 3GZX-4 in the following order of priority: Pentachlorobiphenyl: PCB-126, Tetrachlorobiphenyl: PCB-77, Heptachlorobiphenyl: PCB-189, Hexachlorobiphenyl: PCB-168, indicating that the multifunctional plant degradation enzymes had certain differences in their degradation preferences for the different PCBs. However, the range of variation in the SNR parameters for the action of the four dioxin-like PCBs with 3GZX-1, 3GZX-2, and 3GZX-4 (1.66%–5.91%, 4.60%–8.60%, 5.37%–9.50%, respectively) was not significant. In summary, it indicated that there was no significant difference in the degradation selectivity for the other PCBs.

3.5 Applicability analysis of the multifunctional plant degradation enzymes in degrading PCBs

To study the applicability of multifunctional enzyme degradation of different PCBs, using the multifunctional plant degradation enzyme receptor 3GZX-1 as an example, eleven PCBs (four dioxin PCBs and seven typical PCBs) were selected as ligands, and the binding energies of their interactions with the 6QIM-3GZX-1B85 enzyme receptor and the 3GZX-1 enzyme were calculated. The binding energies of the four dioxin-like PCBs docked with the 6QIM-3GZX-1B85 enzyme receptor and the 3GZX-1 enzyme, and the binding energies of the seven PCBs docked with 3GZX-1 were calculated and are listed in Table 12. According to Table 12, there was no significant difference between the binding energy of the four kinds of dioxin-like PCBs alone docked with 3GZX-1 and the binding energy of the 7 kinds of PCBs alone docked with 3GZX-1, indicating that the designed multifunctional plant degradation enzyme 3GZX-1 had the same ability to degrade PCBs. In addition, the binding energy of the four dioxin-like PCBs, alone and as a whole, dock with the 6QIM-3GZX-1B85 enzyme receptor and the 3GZX-1 enzyme demonstrated antagonistic and synergistic effects, respectively. In comparison to the 6QIM-3GZX-1B85 enzyme receptor, the multifunctional plant degradation enzyme (3GZX-1)
exhibited a 12% increase in its degradation capacity against the four dioxin PCBs as a whole, which was consistent with the degree of synergistic effect.

In summary, the binding energy of the seven PCBs as a whole docked with the enzyme 3GZX-1 was significantly greater than that of the seven PCBs as a whole docked with the 6QIM-3GZX-1B85 enzyme receptor (138%), indicating that the designed multifunctional degradation enzyme had a significantly enhanced ability to degrade the seven PCBs as a whole compared to the 6QIM-3GZX-1B85 enzyme receptor, although the designed multifunctional degradation enzyme did not exhibit significantly enhanced degradation of the four dioxin PCBs as a whole, the reasons for which have to be further analyzed.
4 Conclusion

The present study combined molecular docking and molecular dynamics methods to explore the remediation capacity of the plant absorption enzyme, plant degradation enzyme, and microbial mineralization enzyme for PCB-contaminated soil. The results revealed that the binding energy of the combined action of the seven PCBs as a whole on the plant degradation enzyme (3GZX) was the highest, which indicated that the plant degradation enzyme (3GZX) is more suitable for application in the remediation of PCB-contaminated soils. Therefore, the plant degradation enzyme was selected as the template for enzyme modification using amino acid recombination and site-directed mutagenesis. We have achieved the design of six multifunctional plant degradation enzymes which can degrade the PCBs significantly effective. Molecular dynamics assisted by the Taguchi experimental design method was used for determining the suitable external environmental conditions (such as pH, temperature, organic matter content (nitrogen and phosphorus dosing ratio), oxygen promoter concentration, and catalyst concentration) that would promote the plant-microbe combined remediation of the PCB-contaminated soil. The multifunctional plant degradation enzymes constructed in the present study and the suitable external environmental conditions determined for the different remediation methods can meet the design requirements for enhanced efficiency of the enzymes in degrading PCBs.

In this paper, we studied a method to strengthen the repair ability of enzymes, and the multifunctional plant degradation enzyme has a combination of the three functions roles of absorption, degradation, and mineralization. It has not been studied before to solve the three functions with one enzyme. The presented method is design concept of transgenic plants and microbial improvement. The application of this method has practical significance for developing an improved plant-microbe remediation technique in the soil environment.

Conflicts of 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.

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Intramolecular Hydrogen Bond and Some Thermodynamic Properties of Polyhydroxylated Anthraquinones. Journal of Chemical and Engineering Data, 2012, 57: 2442-2455.
Table 1 Taguchi experimental design table covering 32 groups under different external environment conditions

| NO. | Temperature (℃) | pH | N:P  | Oxygen promoter (mg/L) | Catalyst (mg/L) |
|-----|-----------------|----|------|------------------------|-----------------|
| 1   | 15              | 5  | 1:1  | 50                     | 150             |
| 2   | 15              | 5  | 1:1  | 50                     | 200             |
| 3   | 15              | 5  | 1:1  | 350                    | 150             |
| 4   | 15              | 5  | 1:1  | 350                    | 200             |
| 5   | 15              | 5  | 10:1 | 50                     | 150             |
| 6   | 15              | 5  | 10:1 | 50                     | 200             |
| 7   | 15              | 5  | 10:1 | 350                    | 150             |
| 8   | 15              | 5  | 10:1 | 350                    | 200             |
| 9   | 15              | 9  | 1:1  | 50                     | 150             |
| 10  | 15              | 9  | 1:1  | 50                     | 200             |
| 11  | 15              | 9  | 1:1  | 350                    | 150             |
| 12  | 15              | 9  | 1:1  | 350                    | 200             |
| 13  | 15              | 9  | 10:1 | 50                     | 150             |
| 14  | 15              | 9  | 10:1 | 50                     | 200             |
| 15  | 15              | 9  | 10:1 | 350                    | 150             |
| 16  | 15              | 9  | 10:1 | 350                    | 200             |
| 17  | 35              | 5  | 1:1  | 50                     | 150             |
| 18  | 35              | 5  | 1:1  | 50                     | 200             |
| 19  | 35              | 5  | 1:1  | 350                    | 150             |
| 20  | 35              | 5  | 1:1  | 350                    | 200             |
| 21  | 35              | 5  | 10:1 | 50                     | 150             |
| 22  | 35              | 5  | 10:1 | 50                     | 200             |
| 23  | 35              | 5  | 10:1 | 350                    | 150             |
| 24  | 35              | 5  | 10:1 | 350                    | 200             |
| 25  | 35              | 9  | 1:1  | 50                     | 150             |
| 26  | 35              | 9  | 1:1  | 50                     | 200             |
| 27  | 35              | 9  | 1:1  | 350                    | 150             |
| 28  | 35              | 9  | 1:1  | 350                    | 200             |
| 29  | 35              | 9  | 10:1 | 50                     | 150             |
| 30  | 35              | 9  | 10:1 | 50                     | 200             |
| 31  | 35              | 9  | 10:1 | 350                    | 150             |
| 32  | 35              | 9  | 10:1 | 350                    | 200             |
Table 2 The binding energies of the seven PCB molecules as a whole to the plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and microbial mineralization enzyme (1B85).

|                      | 7 PCBs molecules as a whole to plant absorption enzyme (6QIM) | 7 PCBs molecules as a whole to plant absorption enzyme (3GZX) | 7 PCBs molecules as a whole to plant absorption enzyme (1B85) |
|----------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Binding energy (kJ/mol) | 289.018                                                      | 741.904                                                      | 611.667                                                      |
Table 3 Multifunctional plant degradation enzyme modification schemes and the overall binding capacity of the seven PCBs in interaction with these enzymes

| No. | Multifunctional plant degradation enzyme | Site-directed mutagenesis | Binding energy (kJ/mol) |
|-----|------------------------------------------|--------------------------|------------------------|
| 1   | 3GZX-1                                   | ASP230>VAL, ALA234>HIS, GLY319>VAL | 719.592                |
| 2   | 3GZX-2                                   | ASP230>HIS, ALA234>LEU, GLY319>VAL | 716.383                |
| 3   | 3GZX-3                                   | ASP230>VAL, ALA234>LEU, GLY319>VAL | 698.524                |
| 4   | 3GZX-4                                   | ASP230>VAL, ALA234>VAL, GLY319>VAL | 692.853                |
| 5   | 3GZX-5                                   | ASP230>LEU, ALA234>VAL, GLY319>VAL | 687.546                |
| 6   | 3GZX-6                                   | ASP230>ILE, ALA234>VAL, GLY319>VAL | 669.489                |
**Table 4** Evaluation of the affinity between the six multifunctional plant degradation enzymes and mutant amino acids and their thermal stability.

|                | Type (kcal/mol) | 3GZX-1 | 3GZX-2 | 3GZX-3 | 3GZX-4 | 3GZX-5 | 3GZX-6 |
|----------------|----------------|--------|--------|--------|--------|--------|--------|
| Mutation energy of affinity | -1.06         | -1.00  | -0.90  | -0.87  | -0.78  | -0.73  |
| Mutation energy of thermal stability | -1.90         | -4.10  | -4.19  | -2.73  | -3.36  | -1.70  |
### Table 5
Calculation of the binding energy and the signal-to-noise ratio of the seven PCBs as a whole docked with 6QIM, 3GZX, and 1B85 under different external environmental conditions

| No. | Binding energy of plant absorption enzyme (6QIM) (kJ/mol) | SNR | Binding energy of plant degradation enzyme (3GZX) (kJ/mol) | SNR | Binding energy of microbial mineralization enzyme (1B85) (kJ/mol) | SNR |
|-----|---------------------------------------------------------|-----|----------------------------------------------------------|-----|-----------------------------------------------------------------|-----|
| 1   | 625.350                                                 | 55.92 | 743.835                                                  | 57.43 | 295.499                                                         | 49.41 |
| 2   | 622.546                                                 | 55.88 | 749.715                                                  | 57.50 | 246.719                                                         | 47.84 |
| 3   | 590.936                                                 | 55.43 | 777.544                                                  | 57.81 | 226.996                                                         | 47.12 |
| 4   | 638.607                                                 | 56.10 | 731.505                                                  | 57.28 | 266.275                                                         | 48.51 |
| 5   | 555.335                                                 | 54.89 | 712.176                                                  | 57.05 | 237.134                                                         | 47.50 |
| 6   | 623.663                                                 | 55.90 | 766.976                                                  | 57.70 | 192.570                                                         | 45.69 |
| 7   | 655.967                                                 | 56.34 | 796.037                                                  | 58.02 | 311.193                                                         | 49.86 |
| 8   | 656.930                                                 | 56.35 | 760.688                                                  | 57.62 | 276.254                                                         | 48.83 |
| 9   | 636.611                                                 | 56.08 | 729.560                                                  | 57.26 | 290.614                                                         | 49.27 |
| 10  | 566.367                                                 | 55.06 | 755.958                                                  | 57.57 | 243.647                                                         | 47.74 |
| 11  | 649.125                                                 | 56.25 | 761.545                                                  | 57.63 | 295.539                                                         | 49.41 |
| 12  | 614.123                                                 | 55.77 | 796.195                                                  | 58.02 | 266.730                                                         | 48.52 |
| 13  | 603.428                                                 | 55.61 | 766.824                                                  | 57.69 | 264.182                                                         | 48.44 |
| 14  | 611.955                                                 | 55.73 | 747.563                                                  | 57.47 | 237.812                                                         | 47.52 |
| 15  | 604.998                                                 | 55.64 | 741.921                                                  | 57.41 | 221.756                                                         | 46.92 |
| 16  | 644.980                                                 | 56.19 | 720.779                                                  | 57.16 | 235.527                                                         | 47.44 |
| 17  | 637.442                                                 | 56.09 | 666.925                                                  | 56.48 | 218.026                                                         | 46.77 |
| 18  | 619.528                                                 | 55.84 | 745.985                                                  | 57.45 | 229.575                                                         | 47.22 |
| 19  | 677.174                                                 | 56.61 | 697.410                                                  | 56.87 | 189.269                                                         | 45.54 |
| 20  | 677.059                                                 | 56.61 | 746.299                                                  | 57.46 | 328.696                                                         | 50.34 |
| 21  | 597.988                                                 | 55.53 | 703.717                                                  | 56.95 | 279.959                                                         | 48.94 |
| 22  | 625.784                                                 | 55.93 | 685.926                                                  | 56.73 | 363.019                                                         | 51.20 |
| 23  | 612.194                                                 | 55.74 | 698.597                                                  | 56.88 | 304.206                                                         | 49.66 |
| 24  | 552.240                                                 | 54.84 | 705.736                                                  | 56.97 | 289.304                                                         | 49.23 |
| 25  | 609.323                                                 | 55.70 | 701.543                                                  | 56.92 | 198.126                                                         | 45.94 |
| 26  | 611.777                                                 | 55.73 | 778.388                                                  | 57.82 | 260.638                                                         | 48.32 |
| 27  | 646.348                                                 | 56.21 | 761.141                                                  | 57.63 | 286.584                                                         | 49.15 |
| 28  | 616.131                                                 | 55.79 | 753.604                                                  | 57.54 | 249.055                                                         | 47.93 |
| 29  | 605.392                                                 | 55.64 | 737.427                                                  | 57.35 | 227.525                                                         | 47.14 |
| 30  | 617.103                                                 | 55.81 | 771.160                                                  | 57.74 | 216.614                                                         | 46.71 |
| 31  | 630.064                                                 | 55.99 | 729.713                                                  | 57.26 | 320.407                                                         | 50.11 |
| 32  | 620.615                                                 | 55.86 | 771.563                                                  | 57.75 | 284.691                                                         | 49.09 |
Table 6 Significance ranking of the external environmental conditions promoting the interaction of PCBs docked with 6QIM, 3GZX, and 1B85.

| Level | Temperature | pH   | N:P | Oxygen concentration | Catalyst concentration |
|-------|-------------|------|-----|----------------------|------------------------|
| 1     | 55.82       | 55.88| 55.94| 55.71                | 55.85                  |
| 2     | 55.87       | 55.82| 55.75| 55.98                | 55.84                  |
|       |             |      |     |                      |                        |
| Order | 4           | 3    | 2   | 1                    | 5                      |

| Level | Temperature | pH   | N:P | Oxygen concentration | Catalyst concentration |
|-------|-------------|------|-----|----------------------|------------------------|
|       | 57.54       | 57.26| 57.42| 57.32                | 57.29                  |
|       | 57.24       | 57.52| 57.36| 57.46                | 57.49                  |
|       |             |      |     |                      |                        |
| Order | 4           | 3    | 2   | 1                    | 5                      |

| Level | Temperature | pH   | N:P | Oxygen concentration | Catalyst concentration |
|-------|-------------|------|-----|----------------------|------------------------|
|       | 48.13       | 48.35| 48.06| 47.85                | 48.20                  |
|       | 48.33       | 48.10| 48.39| 48.60                | 48.26                  |
|       |             |      |     |                      |                        |
| Order | 4           | 3    | 2   | 1                    | 5                      |
| External environmental conditions | Binding energy of PCBs and 6QIM (kJ/mol) | Change rate (%) | Binding energy of PCBs and 3GZX (kJ/mol) | Change rate (%) | Binding energy of PCBs and 1B85 (kJ/mol) | Change rate (%) |
|-----------------------------------|------------------------------------------|-----------------|------------------------------------------|-----------------|------------------------------------------|-----------------|
| Non                              | 289.018                                  | -               | 741.904                                  | -               | 611.667                                  | -               |
| Suitable                         | 363.019                                  | 25.60           | 796.195                                  | 7.32            | 677.174                                  | 10.71           |
Table 8 Calculations of the binding energy of the seven PCBs as a whole docked with the 6QIM-3GZX-1B85 enzyme receptor under different external environmental factors.

| No. | Binding energy of seven PCBs and 6QIM-3GZX-1B85 enzyme receptor (kJ/mol) | SNR  |
|-----|------------------------------------------------------------------------|------|
| 1   | 703.034                                                                | 56.94|
| 2   | 646.508                                                                | 56.21|
| 3   | 625.203                                                                | 55.92|
| 4   | 638.518                                                                | 56.10|
| 5   | 619.512                                                                | 55.84|
| 6   | 633.331                                                                | 56.03|
| 7   | 655.492                                                                | 56.33|
| 8   | 662.995                                                                | 56.43|
| 9   | 706.350                                                                | 56.98|
| 10  | 661.386                                                                | 56.41|
| 11  | 660.901                                                                | 56.40|
| 12  | 631.976                                                                | 56.01|
| 13  | 704.026                                                                | 56.95|
| 14  | 640.422                                                                | 56.13|
| 15  | 650.864                                                                | 56.27|
| 16  | 665.263                                                                | 56.46|
| 17  | 610.218                                                                | 55.71|
| 18  | 644.960                                                                | 56.19|
| 19  | 661.792                                                                | 56.41|
| 20  | 631.542                                                                | 56.01|
| 21  | 653.142                                                                | 56.30|
| 22  | 638.717                                                                | 56.11|
| 23  | 665.149                                                                | 56.46|
| 24  | 671.408                                                                | 56.54|
|    |       |       |
|----|-------|-------|
| 25 | 664.369 | 56.45 |
| 26 | 709.235 | 57.02 |
| 27 | 646.758 | 56.21 |
| 28 | 709.014 | 57.01 |
| 29 | 622.689 | 55.89 |
| 30 | 700.319 | 56.91 |
| 31 | 622.781 | 55.89 |
| 32 | 702.994 | 56.94 |
Table 9 Significance ranking of the external environmental conditions for the effect of the seven PCBs as a whole dock with the 6QIM-3GZX-1B85 enzyme receptor.

| Level | Temperature | pH   | N:P  | Oxygen concentration | Catalyst concentration |
|-------|-------------|------|------|----------------------|------------------------|
| 1     | 56.34       | 56.22| 56.37| 56.38                | 56.31                  |
| 2     | 56.38       | 56.50| 56.34| 56.34                | 56.41                  |
| Sort  | 4           | 1    | 5    | 3                    | 2                      |
Table 10 The improvement degree of the binding energy of seven PCBs as a whole docked to the multifunctional plant degradation enzymes under the suitable external environmental conditions.

| Type                                                                 | Binding energy (kJ/mol) | Degree of improvement (%) |
|----------------------------------------------------------------------|-------------------------|---------------------------|
| The binding effect of seven PCBs as a whole dock with 3GZX-1 under the suitable external environmental conditions | 812.184                 | -                         |
| The binding effect of seven PCBs as a whole dock with 3GZX under the suitable external environmental conditions | 796.195                 | 2.01                      |
| The binding effect of seven PCBs as a whole dock with the 6QIM-3GZX-1B85 enzyme under the suitable external environmental conditions | 709.235                 | 14.52                     |
| The binding effect of seven PCBs as a whole dock with 3GZX-1 under no external environmental conditions | 719.592                 | 12.87                     |
| The binding effect of seven PCBs as a whole dock with the 6QIM-3GZX-1B85 enzyme under no external environmental conditions | 302.290                 | 168.68                    |
**Table 11** Taguchi design table and the binding energy of the four kinds of dioxin-like PCBs and the multifunctional plant degradation enzymes.

| No. | PCB-77 | PCB-126 | PCB-168 | PCB-189 | Binding energy of dioxin-like PCBs and 3GZX-1 | Binding energy of dioxin-like PCBs and 3GZX-2 | Binding energy of dioxin-like PCBs and 3GZX-4 |
|-----|--------|---------|---------|---------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| 1   | 0      | 0       | 0       | 0       | 0.000                                       | 0.000                                       | 0.000                                       |
| 2   | 0      | 0       | 1       | 1       | 264.371                                     | 175.860                                     | 167.913                                     |
| 3   | 0      | 1       | 0       | 1       | 228.830                                     | 234.555                                     | 241.202                                     |
| 4   | 0      | 1       | 1       | 0       | 186.974                                     | 212.910                                     | 200.786                                     |
| 5   | 1      | 0       | 0       | 1       | 232.469                                     | 241.509                                     | 222.508                                     |
| 6   | 1      | 0       | 1       | 0       | 182.771                                     | 190.304                                     | 181.207                                     |
| 7   | 1      | 1       | 0       | 0       | 241.211                                     | 266.755                                     | 250.917                                     |
| 8   | 1      | 1       | 1       | 1       | 448.567                                     | 426.349                                     | 430.761                                     |
Table 12 Binding energy (kJ/mol) of the PCBs docked with the 6QIM-3GZX-1B85 enzyme and the 3GZX-1 enzyme.

| Type                  | Dioxin-like PC77 docked with 6QIM-3GZX-1B85 | Dioxin-like PC126 docked with 6QIM-3GZX-1B85 | Dioxin-like PC168 docked with 6QIM-3GZX-1B85 | Dioxin-like PC189 docked with 6QIM-3GZX-1B85 | 4 dioxin-like PCBs as a whole docked with 6QIM-3GZX-1B85 |
|-----------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|--------------------------------------------------|
| Binding energy        | 105.342                                    | 114.165                                    | 84.637                                      | 109.595                                     | 401.105                                          |
| Degree of compound action | Antagonistic                                | Antagonistic                                | Antagonistic                                | Antagonistic                                | Antagonistic                                     |

| Type                  | PCB-77 docked with 3GZX-1                  | PCB-126 docked with 3GZX-1                 | PCB-168 docked with 3GZX-1                 | Dioxin-like PC189 docked with 3GZX-1        | 4 dioxin-like PCBs as a whole docked with 3GZX-1 |
|-----------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|--------------------------------------------------|
| Binding energy        | 112.826                                    | 106.042                                    | 70.763                                      | 104.307                                     | 448.567                                          |
| Degree of compound action | Synergistic                                 | Synergistic                                 | Synergistic                                 | Synergistic                                 | Synergistic                                     |

| Type                  | PCB-28 docked with 3GZX-1                  | PCB-52 docked with 3GZX-1                 | PCB-101 docked with 3GZX-1                 | PCB-118 docked with 3GZX-1                 | PCB-138 docked with 3GZX-1                      | PCB-153 docked with 3GZX-1                      | PCB-180 docked with 3GZX-1                      | 7 PCBs as a whole docked with 3GZX-1                     |
|-----------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|--------------------------------------------------|
| Binding energy        | 125.101                                    | 83.186                                     | 119.748                                     | 100.866                                     | 99.135                                      | 98.635                                      | 121.449                                     | 719.592                                          |
| Degree of compound action | Antagonistic                                 | Antagonistic                                 | Antagonistic                                 | Antagonistic                                 | Antagonistic                                 | Antagonistic                                 | Antagonistic                                 | Antagonistic                                     |
**Figure 1** The binding energies of the seven PCBs to plant absorption enzyme (6QIM), plant degradation enzymes (3GZX), and microbial mineralization enzyme (1B85).
Figure 2 The recombination of the key amino acid residues within the active regions of the plant absorption enzyme (6QIM), plant degradation enzyme (3GZX), and mineralization enzyme (1B85).
Figure 3 Ramachandran conformation maps for the six multifunctional plant degradation enzymes.
Figure 4 Effect diagram of the change in the signal-to-noise ratio parameters of the dioxin-like PCBs docked with the multifunctional plant degradation enzymes.