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Enhanced Biodegradation of Hexachlorocyclohexane in Soil by Application of Exogenous Amendments

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Abstract In this study, bioaugmentation and biostimulation were used to explore the efficiency of bioremediation of hexachlorocyclohexane (HCH)-contaminated soil in the laboratory. A complex microbial community (germ A, abbreviated as A) was used for bioaugmentation. The KNO_3 and K_2HPO_4 (NP), wheat straw (JG), and glucose (PU) were used as exogenous amendments for biostimulation. Five treatments were set up: the control (CK), NP, A + NP, A + NP + JG, and A + NP + PU. After 28 days of remediation, the removal rate of total HCH (HCHs) in NP, A + NP, A + NP + JG, and A + NP + PU was 27.0%, 32.2%, 43.4%, and 45.3%, respectively. The results indicated that the removal of HCHs in A + NP, A + NP + JG, and A + NP + PU was higher than NP, indicating that bioaugmentation combined with biostimulation was more effective than biostimulation. Furthermore, compared with A + NP, A + NP + JG and A + NP + PU were more conducive to the degradation of HCHs. Because applying inorganic and organic nutrient substances simultaneously can effectively improve the living environment of microorganisms and increase microbial quantities in HCH-contaminated soil, therefore, the enzymatic reaction of microorganisms is accelerated, resulting in the efficiency of HCHs degradation improvement. The results indicated that bioaugmentation with germ A combine with biostimulation by organic and inorganic nutrients simultaneously constitutes a promising method for restoring soils contaminated with HCHs. This study would provide the basis for improving the bioremediation efficiency of HCH-contaminated soil.

Keywords Hexachlorocyclohexane · Bioaugmentation · Biostimulation · Exogenous amendments

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1 Introduction

Hexachlorocyclohexane (HCH) is an organochlorine pesticide that has been extensively used to minimize economic losses caused by pests and diseases. It was used worldwide from the 1950s to the 1980s. During this period, large amounts of wasted and discarded HCHs entered the environment. HCHs are hard to dissipate in soil naturally because of its recalcitrance, hydrophobicity, and ecotoxicity, which caused long persistence in soil. HCHs can enter the human body by the food chain, accumulating in the human body and impairing human's health. HCHs have been listed as one of the persistent organic pollutants in

many countries due to its properties with relatively high toxicity, long persistence, and bioaccumulation (Sun et al., 2015; Vijgen et al., 2011). Industrial-grade HCHs are a mixture of α, β, γ, and δ-HCH (Vijgen et al., 2011; Willett et al., 1998). Among the four isomers of HCH, the longest persistence in soil environment was observed in β-HCH, followed by δ-HCH, γ-HCH, and α-HCH, respectively (Deo et al., 1994; Qiao et al., 2019). The persistence and toxicity of HCH isomers are mainly determined by the spatial orientation of chlorine atoms on the cyclohexane ring (Beurskens et al., 1991). The structures of HCH isomers were shown in Fig. 1 (Deo et al., 1994). The more chlorine atoms in the ring structure plane, the more stable the isomer is (Beurskens et al., 1991; Vijgen et al., 2019). The chlorine atoms of β-HCH lie in the ring plane, which makes β-HCH more stable and persistent. The previous report proved that because δ-HCH has more chlorine atoms in the ring planet, it is more stable and persistent than α-HCH and γ-HCH (Beurskens et al., 1991). Although HCHs have been banned for decades, HCHs still have been detected on many sites. Bhattacharya et al. (2003) reported that the highest levels of HCHs in the sediments were detected in the estuaries of tropical mangrove forests in India. Gao et al. (2006) found that the average HCHs residue in agricultural soil was 627 ng/g in Guangzhou, China. Long-term HCHs pollution causes severe carcinogenic, teratogenic, and mutagenic risks to soil organisms and humans, thus greatly restricting soil utilization worldwide (Zungu et al., 2020). Therefore, the remediation of HCH-contaminated soils is urgent.

The remediation technologies of HCH-contaminated soil mainly include physical, chemical, and bioremediation (Bhatt et al., 2009; Cruz-Gonzalez et al., 2018; Dominguez et al., 2018). Both physical and chemical methods have the shortcomings of the adverse effect of soil properties and high cost. In the past decades, bioremediation has emerged in organic pollution remediation for its advantages of being cost-effective and environment-friendly (Bhatt et al., 2020; Zhao et al., 2020). Bioremediation is mainly decomposing organic pollutants in soil by microbial metabolism. Microorganisms are the most active part of soil organisms because they are widely distributed, abundant, and diverse in the whole soil ecosystem. Once pollutants are into the soil, they will cause influences on soil microorganisms inevitably. Some soil microorganisms can use organic pollutants as energy substances and then effectively decompose organic pollutants (Sun et al., 2015).

Biodegradation was recognized as the main mechanism during the HCHs removal (Chishti et al., 2013). The degradation of HCHs by microorganisms is actually a series of enzymatic reactions (Gaur

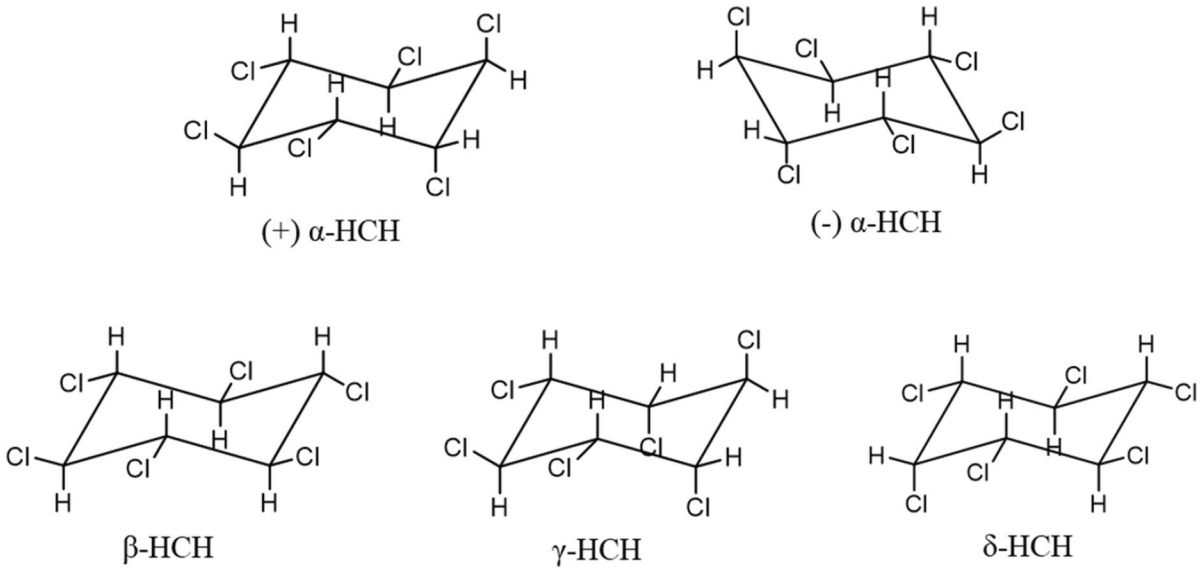

Fig. 1 Structures for HCH isomers

et al., 2018; Rao et al., 2014; Zhou et al., 2020). Hence, soil microbial activity and enzyme activity are closely related to the bioremediation process (Li et al., 2019). Enzymes produced by soil microorganisms are the main source of soil enzymes. Among the soil enzymes, catalase and dehydrogenase have received the most attention. The catalase can promote the decomposition of hydrogen peroxide, which effectively prevents soil and microbial from being poisoned by hydrogen peroxide produced during metabolism. And dehydrogenase reflects microbial activity and directly indicates the degradation ability of microbial to degrade matrix (Gao et al., 2020; Liu et al., 2020).

However, HCH is a synthetic compound, which poses a challenge to bioremediation because it is difficult to be used by microorganisms in a short time due to its complex structure and strange biology. In order to improve the bioremediation efficiency of HCH in soil, some methods have been developed, such as bioremediation by biostimulation with organic or inorganic amendments (i.e., animal manures, straw, glucose, compost, nutrients) and bioremediation by bioaugmentation (Alessandrello et al., 2017). It is essential to understand the primary mechanism involved in the microbial degraded pesticide by using exogenous amendments. Currently, bioremediation studies by simultaneous application of bioaugmentation with high efficiency degrading microbial and biostimulation with exogenous amendments to remediation of HCHs in the soil contaminated by the chemical industry are scarce.

The aims of this study were (1) to determine the conditions of maximum HCHs removal in soil by simultaneous application of bioaugmentation with efficiency degrading microbial and biostimulation with exogenous amendments. The KNO_3 and K_2HPO_4 (NP), wheat straw (JG), and glucose (PU) were selected as exogenous amendments to explore the biodegradation of HCHs in contaminated soil. (2) to compare the possible mechanism of the amendments that promote HCHs degradation from the perspective of the quantity of microbial, dehydrogenase and catalase activity, it would provide a theoretical basis for improving the bioremediation of HCHs contamination in chemical contaminated sites.

2 Materials and Methods

2.1 Microorganisms and Culture Media

Luria–Bertani medium (LB), used to culture the microbial, made the microbial can be multiplied to meet the requirements. The composition of LB is established as follows: casein tryptone, 10.0 g/L; yeast extract, 5.0 g/L; and NaCl, 10.0 g/L.

Plate count agar (PCA, Britania) has been employed to enumerate total heterotrophic microorganisms; the composition of PCA is established as follows: yeast extract, 2.5 g/L; tryptone, 5.0 g/L; glucose, 1.0 g/L; and agar, 15.0 g/L.

All medium were adjusted to pH 7.0 ± 0.2 before autoclaving at 121 °C for 20 min.

Complex microbial community (germ A) was purchased from Atel Biochemical Co. LTD (Japan). The complex microbial community was solid grey powder, and its living bacterial count was over 10^9 cfu/g. The optimum growth temperature of the microbial inoculant ranges from 0 to 50 ℃, and the optimum growth pH ranges from 5.0 to 10.0. These microbes in germ A were *Corynebacterium* and *Pseudomonadaceae*, and most of them are aerobe/facultative aerobe. The SEM image and Gram's stained image

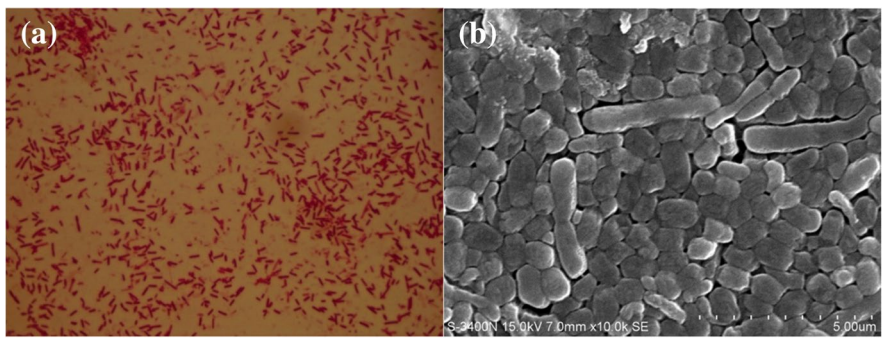

Fig. 2 Gram staining and electron microscopic photographs of germ A (**a**) Gram staining of germ A and (**b**) electron microscopic photographs of germ A

of germ A incubated in LB medium can be found in Fig. 2. It could be observed from the microscope (Fig. 2a) that the microbe in germ A was Gram-negative. And the SEM image indicated that germ A mainly consisted of *Bacillus* and cocci (Fig. 2b).

2.2 Soil and Wheat Straw Samples Preparation

HCH-contaminated soil samples (topsoil 0–20 cm) were collected from an abandoned chemical plant site in Nanning, Guangxi Zhang autonomous region, China. After all the clutters were removed, the soil was put into a plastic bag and transferred to laboratory for further treatments. The soil sample was air-dried naturally and passed through a 2-mm sieve in the laboratory and divided into two parts. One part was for later determinations of physical and chemical properties of soil. The other part was stored in refrigerator at 4 ℃ for the bioremediation experiment.

The soil pH was measured using a pH meter (soil: water = 1:2.5, w/v). And potassium dichromate method was employed to determine the organic matter (Berehe et al., 2013). Kjeldahl method was applied for determining the total nitrogen (Huang et al., 2020). Total phosphate was evaluated by $HClO_4$-H_2SO_4 digesting molybdenum-antimony colorimetric method (Gao et al., 2021). The soil properties are shown in Table 1.

The wheat straw was obtained from Dezhou, Shandong province, China, and ground and passed through a 60-mesh sieve for later experiments. The organic carbon content was 46.50%, the total nitrogen content was 0.48%, and the total phosphate content was 0.11%. Before addition, the straw powder was sterilized by steam at 120 ℃ for 30 min.

The glucose and other chemical reagents used in the experiment were all analytical grade.

2.3 Bioremediation Experiments

Bioremediation experiments were performed in glass bottles containing 50.00 g of HCH-contaminated soil. Five treatments are set up, which are presented in Table 2. The sterilized distilled water was added to the soils to maintain a maximum soil water-holding capacity of 30%. Then, the treated soil was put into a biochemical incubator for dark incubation at 30 ℃. During the incubation, soil moisture capacity was maintained by adding sterilized distilled water using the weighing method every 2 days. Sampling was conducted on the

Table 1 Physico-chemical characteristics of soil

Parameters	pH	Organic carbon (g/kg)	Total nitrogen (g/kg)	Total phosphorus (g/kg)	HCHs (mg/kg)	α-HCH (mg/kg)	β-HCH (mg/kg)	γ-HCH (mg/kg)	δ-HCH (mg/kg)
Soil	7.80	5.48	0.22	0.26	80.56	48.14	31.22	0.68	0.52

Table 2 Experimental design scheme for bioremediation experiments

NO	Abbreviation	Treatments					
		Soil (g)	Nitrogen source (g)	Phosphorus source (g)	Germ A (g)	Wheat straw (g)	Glucose (g)
1	CK	50.00	-	-	-	-	-
2	NP	50.00	0.25	0.025	-	-	-
3	A + NP	50.00	0.25	0.025	0.15	-	-
4	A + NP + JG	50.00	0.25	0.025	0.15	0.75	-
5	A + NP + PU	50.00	0.25	0.025	0.15	-	0.83

The addition of glucose in treatment A + NP + PU is based on the carbon content of A + NP + JG, making the carbon content in these two treatments at the same level. The nitrogen source was added in the form of KNO_3; the phosphorus source was added in the form of K_2HPO_4

day of 7, 14, and 28, respectively. The soil sample was divided into two parts: one was freeze dried for analyzing the concentration of residual HCH in the soil, and the least was stored in refrigerator at 4 ℃ until the related microbial indicator determination. All treatments were conducted in triplicates.

2.4 Analytical Determinations

2.4.1 The Extract and Analysis of HCH in the Soil

The bioremediation experiment was lasting for 28 days, and soil samples were collected at the 7th day, the 14th day, and the 28th day. Residual HCHs in the soil were extracted through the ultrasonic extraction method. A 2-g aliquot of soil sample (oven dry weight equivalent) was added to 30 mL acetone/petroleum ether mixture (v: v = 1:1). The mixture was homogenized and placed under ultrasonic agitation for 30 min at 25 °C and then centrifuged at 5000 rpm for 10 min. The supernatant was collected, and the solid residue was extracted with fresh acetone/petroleum ether mixture twice. The acetone/petroleum extract was combined and extracted with 10 mL acetone three times. Collect all the acetone extract in a 250-mL round-bottomed flask and evaporated to dryness under vacuum on a rotavapor after dehydration by anhydrous sodium sulfate. The residue was recovered in 3 mL petroleum ether/ethyl acetate (v/v = 95:5), repeating three times. The solution above was collected, and after filtering the above solution with a Florisil purification column, the solution was re-evaporated to dryness by rotavapor. Finally, using n-hexane to rinse the round bottom flask, collect the eluent, and dilute to 10 mL with n-hexane. And then, passed the diluted solution through a 0.45-μm Teflon syringe filter before HCHs and HCH isomers were analyzed on gas chromatograph (GC-2010PLUS).

Gas chromatography analysis was conducted by the injector and was operated at 290 ℃, and the extracts (1 mL) were injected in a 5:1 shunt splitless mode. Chromatographic separations of the four isomers were performed with a fused silica capillary column HP-5MS (30 m 0.25 mm × 0.25 mm) under the following temperature program: 150 ℃ (held for 1 min), then an increase to 240 ℃ (at 8 ℃/min and held for 1 min), and finally an increase to 290 ℃ (at 25 ℃/min and held for 2 min). The column carrier gas was N_2. The ECD detector was operated at 300 ℃.

The HCH isomers were distinguished according to the time when their characteristic peaks appeared in gas chromatography (α-HCH, 6.835 min; β-HCH, 7.354 min; γ-HCH, 7.513 min; δ-HCH, 7.988 min).

2.4.2 Determination of Microbial Indicators

The microbial quantities in the soil were determined by the plate count method (Fan et al., 2021). The activity of catalase was determined by the titration method (Raimondo et al. 2021). The colorimetric method was employed to determine the activity of dehydrogenase (Campos et al., 2019).

2.4.3 Statistical Analysis

Determinations were carried out in triplicate, and the results were expressed as mean values. One-way ANOVA (Dunnett's test, $p < 0.05$) was employed

to compare among treatments, considering HCHs removal, HCH isomers removal, microbial quantities, and soil enzymatic activities. The correlation analysis was conducted in SPSS 22.0 using the Spearman method.

3 Results

3.1 Degradation of HCHs in Soil

The variation of HCHs content in each treatment is shown in Fig. 3a. The concentration of HCHs in CK treatment has a slight decrease, after 28-day incubation. At the end of incubation, the removal rate of HCHs in NP, A + NP, A + NP + JG, and A + NP + PU was 27.0%, 32.1%, 43.4%, and 45.3%, respectively. The removal rate of HCHs in NP was 21.4% higher than CK. It proved that the lack of nutrients was an important factor limiting the degradation of HCHs by native microorganisms. Compared with NP, the removal rate of HCHs in A + NP was increased by 5.6%, which indicated that bioaugmentation with germ A could improve the degradation of HCHs. The removal of HCHs in A + NP + JG and A + NP + PU was 11.3% and 13.2% higher than that of A + NP, indicating that HCHs degradation affected by adding inorganic and exogenous organic amendments was better than that by adding inorganic amendments alone. The A + NP + JG and A + NP + PU treatment can efficiently remove HCHs in soil compared with CK treatment, while there was no significant difference between the application of glucose and wheat straw ($p > 0.05$).

3.2 Degradation of α-HCH in Soil

α-HCH is an isomer of HCH, accounting for 59.8% of the HCHs content. The content of α-HCH in each treatment is shown in Fig. 3b. The removal rate of α-HCH in NP, A + NP, A + NP + JG, and A + NP + PU was 26.3%, 36.7%, 46.0%, and 55.7%, respectively,

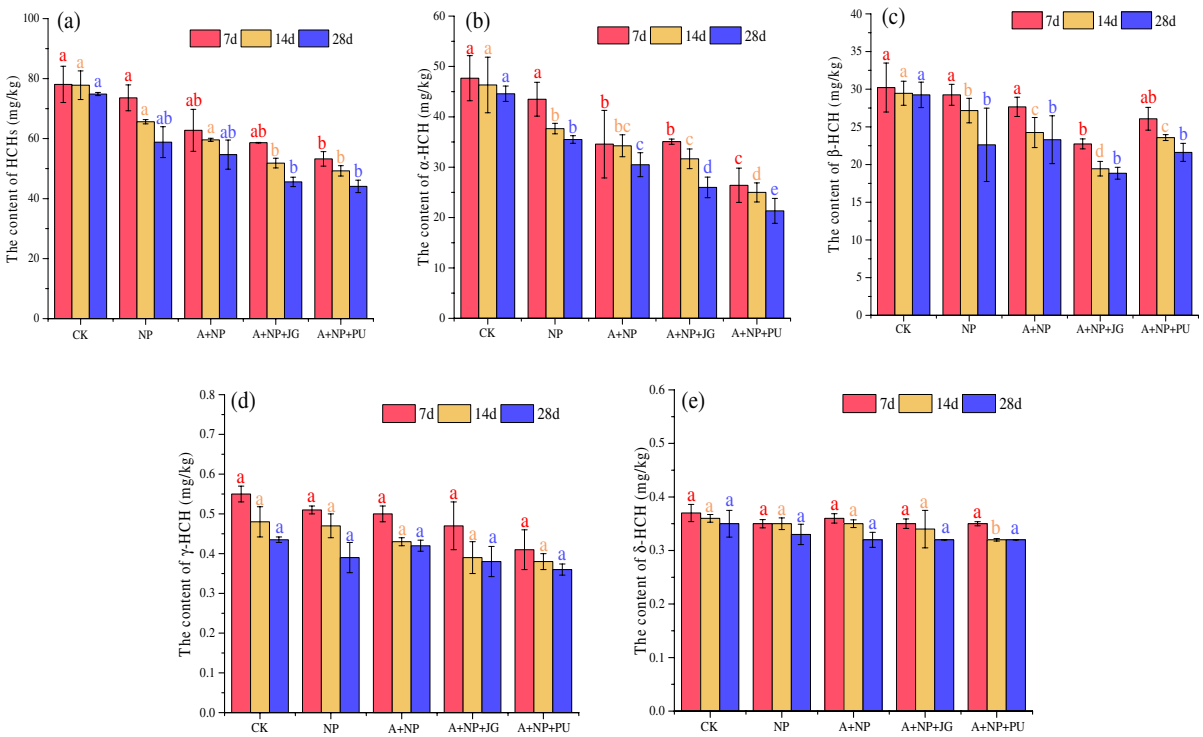

Fig. 3 The content of HCHs and its isomer in soil under different treatments. (a) The content of HCHs. (b) The content of α-HCH. (c) The content of β-HCH. (d) The content of γ-HCH. (e) The content of δ-HCH. Results were represented as mean ± SD (n = 3). Different letters above columns with the same color indicate that the treatments are significant according to Dunnett's test at $p < 0.05$ level

on the 28th day. Compared with NP, the content of α-HCH in A + NP was significantly ($p < 0.05$) reduced. It was indicated that germ A could significantly ($p < 0.05$) increase the removal rate of α-HCH. The removal rates of A + NP + JG and A + NP + PU within 28 days increased 9.3% and 19%, respectively, compared with the treatment of A + NP. It demonstrated that adding exogenous organic amendments significantly ($p < 0.05$) enhanced the degradation of α-HCH by microorganisms. And compared with wheat straw, adding glucose as exogenous organic amendment was more conducive to the degradation of α-HCH.

3.3 Degradation of β-HCH in Soil

The variation of β-HCH content in each treatment is shown in Fig. 3c. At the end of the incubation, the removal of β-HCH in NP, A + NP, A + NP + JG, and A + NP + PU was 27.5%, 25.4, 39.6%, and 30.7%, respectively. Compared with CK, the content of β-HCH in these four treatments decreased significantly ($p < 0.05$), especially A + NP + JG and A + NP + PU. It was indicated that both bioaugmentation and biostimulation significantly promoted the degradation of β-HCH. Compared with A + NP, the removal rate of β-HCH in A + NP + JG and A + NP + PU increased by 14.2% and 5.3%, respectively, on the 28th day. It was shown that exogenous organic amendments could promote the degradation of β-HCH. Compared with glucose, straw is more conducive to promote the degradation of β-HCH in soil.

3.4 Degradation of γ-HCH in Soil

After 28 days of incubation, the content of γ-HCH has decreased in all treatments (Fig. 3d), but no significant difference ($p > 0.05$) was observed among different treatments. At the end of the incubation, the removal of β-HCH in NP, A + NP, A + NP + JG, and A + NP + PU was 42.6%, 38.2%, 44.1%, and 47.1%, respectively. Compared with A + NP, the removal rate of γ-HCH in A + NP + JG and A + NP + PU decreased by 5.9% and 8.8%, respectively.

3.5 Degradation of δ-HCH in Soil

δ-HCH was more resistant to degradation and slightly was degraded in the NP, A + NP, A + NP + JG, and A + NP + PU at the end of the incubation process. As shown in Fig. 3e, at the end of incubation, the treatment of A + NP + JG and A + NP + PU enhances the degradation of δ-HCH with the removal rates of 26.9% and 30.8%.

3.6 Microbial Quantities in Soil

The variation of microbial quantities is shown in Fig. 4a. The quantity of microbial in CK and NP decreased continuously within 28 days, and there were no significant differences between these two treatments ($p > 0.05$). The quantity of microbial in A + NP decreased at 7–14 days and then increased at 14–28 days. On the contrary, the microbial quantities in the treatments of A + NP + JG and A + NP + PU increased in 7–14 days but decreased in 14–28 days,

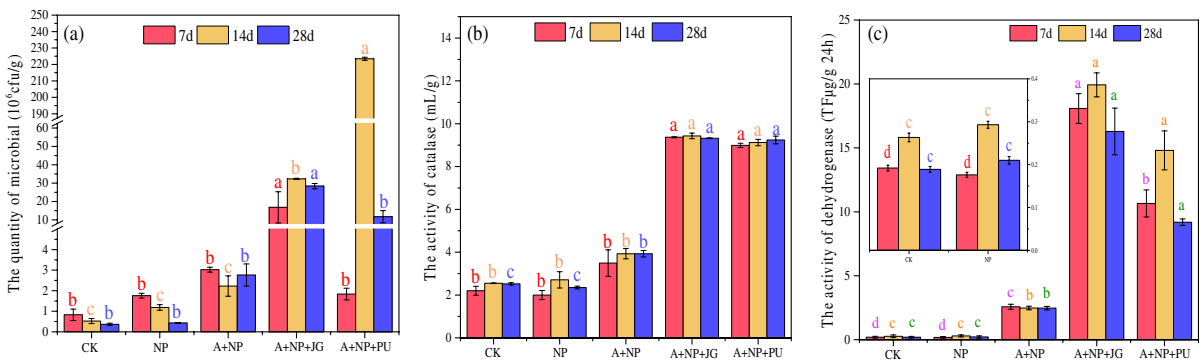

Fig. 4 The microbiological indexes in soil under different treatments. (a) The variation of bacteria number. (b) The activity of catalase. (c) The activity of dehydrogenase. Results were represented as mean ± SD (n = 3). Different letters above columns with the same color indicate significance between the treatments according to Dunnett's test at $p < 0.05$ level

and the maximum can be observed on the 14th day. On the 14th day, compared with CK, the microbial quantities in NP, A + NP, A + NP + JG, and A + NP + PU were increased, corresponding to increased 1.27-fold, 3.26-fold, 60.8-fold, and 427-fold, respectively. At the end of incubation, the microbial quantities in A + NP + JG and A + NP + PU decreased by 12.3% and 94.8%, respectively, compared with the 14th day. Even though the microbial quantities in A + NP + JG and A + NP + PU decreased at 14–28 days, the microbial quantities in A + NP + JG and A + NP + PU remained significantly ($p < 0.05$) higher than other treatments. It was indicated that both glucose and straw could significantly increase ($p < 0.05$) the microbial quantities compared with A + NP, but the effect of straw is more permanent and stable. Consequently, adding straw is more beneficial to the growth of microorganisms than adding glucose.

3.7 Soil catalase Activity

The changes in catalase activity are shown in Fig. 4b. There were no significant ($p > 0.05$) differences between CK and NP, indicating that only adding inorganic amendments to HCH-contaminated soil did not improve the catalase activity. Microbial bioaugmentation assisted by biostimulation with exogenous amendments can promote the catalase activity in the soil, especially in A + NP + JG and A + NP + PU, which significantly increased catalase activity ($p < 0.05$) compared with CK. Relative to that of CK, wheat straw and glucose led to a 3.70-fold and 3.67-fold increase in the catalase activity in soils, respectively. Compared with A + NP, the catalase activity of A + NP + JG and A + NP + PU has been a 2.70-fold increase and 2.67-fold increase, respectively. Adding wheat straw and glucose significantly ($p < 0.05$) promoted the activity of catalase in soil.

3.8 Soil dehydrogenase Activity

The activity of dehydrogenase in soil could reflect the degradation of HCH ((Liu et al., 2020). The result of the activity of dehydrogenase is shown in Fig. 4c. High dehydrogenase activity could be observed in the treatments with adding organic amendments. However, in CK and NP, dehydrogenase activity was extremely low, and there was no significant difference ($p > 0.05$) between them. Therefore, it was indicated that only adding nitrogen and phosphorus could not improve soil dehydrogenase activity in HCH-contaminated soil. Compared with CK, dehydrogenase activity in A + NP, A + NP + JG, and A + NP + PU treatments increased significantly ($p < 0.05$). Among them, dehydrogenase activity in A + NP + JG and A + NP + PU increased in 7–14 days and then decreased in 14–28 days. On the 14th day, dehydrogenase activity reached the peak, whose increment was 76-fold and 56-fold, respectively, compared with CK.

4 Discussion

According to the changes of HCHs and its isomers contents, exogenous amendments promoted the degradation of HCHs and its isomers by native microorganisms. Microorganisms are the most sensitive fraction of the soil. When contaminants enter the soil, microbes can sense and respond to it. Some specific microorganisms will produce the enzyme that can decompose HCHs under the stress of HCHs and then degrade and transform it (Bose et al., 2021). Similarly, Sun et al. (2015) found that indigenous microorganisms could metabolize HCHs after the soil was contaminated for the long term. Shen et al. (2005) found that fresh PAH entering long-term PAH-contaminated soil could be used as a carbon source by native soil microorganisms and could positively stimulate microbial activity. Nitrogen and phosphorus are the essential nutrients for microorganism growth (Banet et al., 2021). The lack of nitrogen and phosphorus in the contaminated soil inhibited the degradation of organic pollutants by indigenous microorganisms (Hubalek et al., 2007; Qian et al., 2019). Adding inorganic nutrients (nitrogen and phosphorus) improved the living environment of indigenous microorganisms and facilitated their population, thus promoting the degradation of pollutants by indigenous microorganisms. This result was following previous studies that biostimulation with exogenous amendments can effectively improve the native microorganisms to repair organic-contaminated soil (Dadhwal et al., 2009; Garg et al., 2016; Qian et al., 2019). This study found that although native microorganisms could degrade HCH, they did so inefficiently.

Bioaugmentation is practiced when native microorganisms cannot break down the contaminant or do

so inefficiently (Zawierucha et al. 2011). The removal rate of HCHs in contaminated soil was improved in A + NP compared with NP, indicating that germ A could promote the degradation of HCHs in soil. It has already been reported that applying bioaugmentation with efficiency degrading microbial can elevate the removal of contaminants. For instance, Raimondo et al. (2020) found that bioaugmented with actinobacteria consortium improves organic contaminant degradation. Qian et al. (2019) also reported that adding degradation bacteria to HCH-contaminated soil increased the removal of HCHs. In general, bioaugmentation by bacterial consortia was found to be more efficient than by mono-cultures.

Applying bioaugmentation or biostimulation alone usually fails to remove persistent organic pollutants in the soil effectively. Generally speaking, bioaugmentation and biostimulation are used simultaneously in realistic bioremediation (Chang et al., 2021; Patowary et al., 2016). In bioremediation, there must be enough nutrients such as nitrogen and phosphorus and a suitable growth substrate as a carbon source (Banet et al., 2021). The exogenous organic amendments can be used as the cooperative carbon source for microorganisms, which can improve the activity of microbial enzymes and then enhance the degradation efficiency of microorganisms (Bastida et al., 2016; Margesin et al., 2000; Raimondo et al., 2020). It was shown in this work that the addition of straw or glucose as co-metabolites could effectively promote the degradation of HCHs by microorganisms. And there was no significant ($p > 0.05$) difference in the effect between the two exogenous organic amendments on the degradation of HCHs. Glucose is the simplest monosaccharide carbon source in nature. It can provide energy for the biochemical reaction of microorganisms directly (Nguyen & Guckert, 2001). Hence, when glucose was added to the soil, the quantity of microbial in A + NP + PU reached the maximum at the initial stage (7–14 days), but the soil microbial population decreased rapidly with the depletion of glucose at the later stage (14–28 days). Wheat straw is a common agricultural waste, which contains a lot of carbon and phosphorus and nitrogen (Siedt et al., 2021). These nutrients are necessary for the synthesis of proteins and nucleic acids, so the straw is able to promote microbial activity and growth. Adding straw enhanced the degradation of HCHs, since straw could be decomposed under the action of microorganisms and converted into small molecules of fatty acids and alcohol. These small molecule substances could be used as electron donors for HCHs reduction dechlorination (Han & He, 2010; Stemmer et al., 1999).

The effects of various organic amendments on the isomer's degradation were different. The degradation of α-HCH and β-HCH was significantly ($p < 0.05$) improved by adding glucose and straw. However, no significant ($p > 0.05$) differences were found between γ-HCH and δ-HCH. In this study, it was found that adding glucose was more conducive to the degradation of α-HCH. Because glucose added to the soil improve the content of the labile organic matter in the soil, which rapidly increases the quantity of microorganisms in a short time (Ren et al., 2018). Furthermore, α-HCH is most easily degraded by microorganisms among the isomers of HCH, so that microorganisms will prioritize the degradation of α-HCH. Additionally, the increase of the content of labile organic matter further improves the contaminants' water solubility, thus increasing the bioavailability of α-HCH and making it easier to be degraded by microorganisms (Bhatt et al., 2009). However, the degradation of β-HCH by the treatment of adding straw was better than that of glucose. It has been demonstrated that β-HCH is most resistant to degradation than other isomers, and it takes longer for microorganisms to degrade (Phillips et al., 2005; Qian et al., 2019). Therefore, it is crucial to maintain a continuous supply of nutrients and provide a long-term stable living environment for microorganisms in microbial degradation of β-HCH. On the one hand, as a carbon source, the decomposition process of straw is slow and lasting, which can provide stable and sustainable energy for microbial degradation of β-HCH. On the other hand, during bioremediation, both microbial growth and nutrient diffusion through the cell wall require water (Schimel et al., 2007). The low water content will limit oxygen migration, while high water content will saturate the pores between soil particles and reduce oxygen migration (Haghollahi et al., 2016; Niti et al., 2013). Straw possesses loose and porous structure and good water-holding capacity. It not only improves soil structure and porosity, contributing to the diffusion of oxygen (Alessandrello et al., 2017), but also enhances microbial colonization. It provides a long-term stable environment for microorganisms,

which increases microbial contact with pollutants and promoting microbial remediation activity (Siedt et al., 2021).

The quantity of microbial in the soil cannot directly reflect the metabolic status of microorganisms (Humberto et al., 2021; Zungu et al., 2020). The degradation of organic pollutants by microorganisms is mainly through a series of enzymatic reactions (Fan et al., 2021; Kong et al., 2014). Therefore, the activity of metabolic microorganisms can be evaluated by the enzyme activity in the soil. Both soil dehydrogenase and catalase are typical soil enzymes (Achuba & Peretiemo-Clarke, 2008). Dehydrogenase is a representative of microbial intracellular enzymes, and catalase is a typical extracellular enzyme (Campos et al., 2019; Gao et al., 2020; Sun et al., 2021). Previous studies were demonstrated that the activity of dehydrogenase and catalase was closely related to the quantity and activity of microorganisms in the soil (Achuba & Peretiemo-Clarke, 2008; Lee et al., 2008; Teng et al., 2010). Soil enzyme activity is closely related to the degradation of pollutants in soil (Jastrzebska, 2011; Tejada et al., 2011). The contents of HCHs and its isomers were negatively correlated with the quantity of microorganisms and the activities of catalase and dehydrogenase in soil (Table 3), indicating that microorganisms and soil enzyme activities played an important role in the degradation of HCHs (Bhatt et al., 2009). In soils that have been contaminated for a long time and lack of nutrients materials, the reproduction of microorganisms is inhibited, and the degradation rate of pollutants is meager. In the early stage, after adding nutrients (7–14 days), there were abundant nutrients in the soil for microorganisms to use. At this time, the growth and metabolism of microorganisms were most vigorous, and the quantity of microorganisms also begins to increase. On the 14th day, the quantity of microorganisms reached the highest, and soil catalase and dehydrogenase activities were the largest. It was consistent with the results of the correlation analysis, further indicating that microorganisms were the main contributors to soil enzymes (Zhang et al., 2020). Therefore, the growth of microorganism's population will inevitably lead to the increase of soil enzyme activity. As catalysts for various biochemical reactions in soil, soil enzymes participate in many important metabolic processes, and their activity can directly reflect the degradation intensity of pollutants (Liu et al., 2020; Xu et al., 2021). The increase in soil enzyme activity promoted the enzyme reaction, thereby promoting the degradation of pollutants (Banet et al., 2021). In 14–28 days, due to the continuous consumption of nutrients, the energy materials available for microbial utilization gradually decreased. As a result, the microbial population declined and the enzyme activity also decreased.

5 Conclusions

This study demonstrated that the optimal condition to bioremediate the HCH-contaminated soil was bioaugmentation with germ A combined with biostimulation by organic and inorganic nutrients simultaneously. Because applying inorganic and organic nutrient substance simultaneously can effectively improve the living environment of microorganisms and increase microbial quantities in HCH-contaminated soil, therefore, the enzymatic reaction of microorganisms is

Table 3 The content of HCH and its isomers is correlated with the number of bacteria, catalase activity, and dehydrogenase activity

	Quantity of bacteria	Dehydrogenase activity	Catalase activity
The content of α-HCH	−0.973**	−0.878**	−0.774**
The content of β-HCH	−0.587**	−0.555**	−0.696**
The content of γ-HCH	−0.271	−0.419*	−0.490**
The content of δ-HCH	−0.248	−0.244	−0.301*
The content of HCH	−0.706**	−0.778**	−0.776**

*Significant at $p < 0.05$ level, **significant at $p < 0.01$ level

accelerated, resulting in the efficiency of HCHs degradation improvement.

Wheat straw is a non-expensive alternative for the biostimulation of soils contaminated with HCH by significantly improving the pesticide removal in the bioaugmented microcosms. Therefore, microbial bioaugmentation assisted by biostimulation with exogenous amendments, especially organic, just applied in the previously optimized conditions, represents a promising biotechnological strategy for the restoration of hexachlorocyclohexane-contaminated soils.

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Data Availability All the data used in the paper are presented in the form of table and/or figure.

Declarations

Conflict of Interest The authors declare no competing interests.

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