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CORRESPONDENCE

Current insights into environmental acetochlor toxicity and remediation strategies

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Abstract Acetochlor is a selective pre-emergent herbicide that is widely used to control annual grass and broadleaf weeds. However, due to its stable chemical structure, only a small portion of acetochlor exerts herbicidal activity in agricultural applications, while most of the excess remains on the surfaces of plants or enters ecosystems, such as soil and water bodies, causing harm to the environment and human health. In recent years, researchers have become increasingly focused on the repair of acetochlor residues. Compared with traditional physical and chemical remediation methods, microorganisms are the most effective way to remediate chemical pesticide pollution, such as acetochlor, because of their rich species, wide distribution, and diverse metabolic pathways. To date, researchers have isolated and identified many high-efficiency acetochlor-degrading strains, such as *Pseudomonas oleovorans*, *Klebsiella variicola*, *Bacillus subtilus*, *Rhodococcus*, and *Methylobacillus*, among others. The microbial degradation pathways of acetochlor include dechlorination, hydroxylation, *N*-dealkylation, C-dealkylation, and dehydrogenation. In addition, the microbial enzymes, including hydrolase (ChlH), debutoxylase (Dbo), and monooxygenase (MeaXY), responsible for acetochlor biodegradation are also being investigated. In this paper, we review the migration law of acetochlor in the environment, its toxicity to nontarget organisms, and the main metabolic methods. Moreover, we summarize the latest progress in the research on the microbial catabolism of acetochlor, including the efficient degradation of microbial resources, biodegradation metabolic pathways, and key enzymes for acetochlor degradation. At the end of the article, we highlight the existing problems in the current research on acetochlor biodegradation, provide new ideas for the remediation of acetochlor pollution in the environment, and propose future research directions.

Keywords Biodegradation · Metabolic pathways · Key enzymes · Toxicity · Remediation

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Introduction

Acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl) acetamide) (molecular formula: $C_{14}H_{2}OClNO_{2}$; relative molecular mass: 269.8) is a chloroacetanilide herbicide that Monsanto developed in 1971. Acetochlor is a long-chain fatty acid inhibitor that inhibits the growth of weed seedlings by inhibiting the pyrophosphorylase activity, and it can be used for the pre-emergence control of weeds, such as annual grass and broadleaf weeds (Huang et al., 2020; Couto Petro et al., 2020). Acetochlor, with a stable structure and a half-life of 40–70 days, is primarily used for weed control in soybean, corn, peanut, and other crop fields (Yi-Zhu et al., 2016; Bedmar et al., 2017). Acetochlor has become one of the most commonly used herbicides in China (Li et al., 2016).

Due to the stable chemical structure of acetochlor, only a small portion of it exerts herbicidal activity in agricultural applications, with most of the excess remaining on the plant surface or in the soil (Li et al., 2016; Liu et al., 2024; Chandrasekaran & Paramasivan, 2024). Acetochlor migrates to groundwater and rivers with rainwater, which increases its pollution in the environment (Tatarková et al., 2014). Moreover, its major metabolite, quinonimine, has carcinogenic effects, and the US Environmental Protection Agency has identified it as a Group 2B carcinogen (Li et al., 2013). The European Commission has also decided not to register acetochlor. However, in China, acetochlor is still used as the main herbicide, and the country has not issued any corresponding laws or regulations for its supervision or control. As a result, China faces a particularly challenging situation regarding the environmental residue issue of acetochlor. Wang et al. (2019) found that acetochlor may cause brain abnormalities in zebrafish embryos, affect the motor behavior of zebrafish larvae, and induce neurotoxicity in the early developmental stages of zebrafish. In addition, acetochlor damages the cardiovascular, immune, and endocrine systems of mammals, and it can induce oxidative stress behavior and apoptosis (Jiang et al., 2015; Liu et al., 2017; Chatterjee & Roy, 2022; Valencia-Quintana et al., 2022).

Researchers have frequently detected acetochlor degradation products and residues in groundwater, and often at concentrations that exceed the European Union drinking water limit of 0.1 μg/L (Malaguerra et al., 2012; Shishaye et al., 2021). The dissipation and degradation time of acetochlor in the environment often varies under different conditions, but it typically has a long residence time and undergoes slow degradation in the environment, which makes it highly pollutive (Kucharski et al., 2014). Due to the direct or indirect consumption of water and food contaminated with acetochlor, the health of nontarget organisms, and even human beings, is seriously threatened. Therefore, understanding the degradation behavior of acetochlor to improve its degradation efficiency is of critical importance for improving the environmental and human health problems that are associated with it.

Researchers have confirmed that we can effectively remove or degrade acetochlor using physical and chemical methods, such as radiation induction, activated carbon, and photochemistry (Chenyi et al., 2018; Wang et al., 2021a, 2021b; García-Delgado et al., 2020; Sim et al., 2022). However, the use of bioremediation to remove chemical pesticide residues is gradually becoming the most promising restoration method for environmental pollutants due to its high efficiency, low cost, and high ecological benefits (Bhatt et al., 2023a; Huang et al., 2023; Mishra et al., 2021a; Zhang et al., 2023). Furthermore, bioremediation has also become a research hotspot in the field of environmental science. Bioremediation includes phytoremediation and microbial remediation (Bhatt et al., 2023b; Huang et al., 2017; Lü et al., 2024; Mulla et al., 2018; Zhong et al., 2023). However, there have been limited reports on the application of phytoremediation for the treatment of acetochlor contamination. Chu et al. introduced the gene (*cndA*) that encodes the oxygenase component of the acetochlor dealkylase system, CndABC, into *Arabidopsis*. According to the results, the chloroplast transformants could effectively degrade the acetochlor residues in soil and water (Chu et al., 2020). Microbial remediation is currently the most important method for acetochlor remediation (Chen et al., 2023a; Lin et al., 2021). The microbial degradation of acetochlor primarily occurs through a series of enzymatic reactions that primarily include hydrolysis, oxidation, and deoxygenation. In addition, we can also remove acetochlor from the environment through the cometabolism, bioconcentration, and mineralization of microorganisms (Chen et al., 2023a; Lei et al., 2023). Researchers have isolated and screened many kinds of acetochlor-degrading bacteria, such as *Cupidesulfovibrio*, *Sphingomonas*, *Pseudomonas*, and phosphate-solubilizing *Bacillus* (Li et al., 2020; Liu et al., 2022a, 2022b; Luo et al., 2015; Wang et al., 2018; Xu et al., 2013).

To date, there has been a lot of research on the microbial degradation of acetochlor and its degradation enzymes (Chu et al., 2020; Li et al., 2020; Liu et al., 2022a, 2022b; Luo et al., 2015; Wang et al., 2018). However, there is currently no general overview of the microbial degradation and degradation mechanisms of acetochlor. Here, in this review we report on the acetochlor residues in the natural environment, along with its toxic effects on nontarget organisms. We review the degradation mechanisms of high-efficiency acetochlor-degrading bacteria, and we also summarize the key enzymes that are involved in microbial degradation to confirm their roles. This work provides a rich dataset for a better understanding of the microbial degradation of acetochlor herbicide.

Environmental residues and toxicity

Environmental residues of acetochlor

The herbicide acetochlor is widely used throughout the world. Once in the environment, the herbicide follows many different pathways (Fig. 1), including transformation degradation, adsorption–desorption, volatilization, plant uptake, runoff into surface water, and transport to groundwater (Chu et al., 2020; Gao et al., 2021; Lyu et al., 2024; Tan et al., 2024).

In Guangxi, China, researchers detected acetochlor in 33.3% of the water samples from sugarcane growing areas, with the highest detection level reaching 0.311 mg/L (Li et al., 2018a, 2018b). Moreover, researchers also detected acetochlor residues in Northeast China (Fu et al., 2018). Sun et al. studied the occurrence and distribution of acetochlor in sediments and riparian soils during and before the rainy season in the Songhua River Basin (Sun et al., 2011). According to the results, the acetochlor concentration in the sediment was 0.47–11.76 μg/kg, and the concentration in the riparian soil was 0.03–709.37 μg/kg. In surface sediments, there is a substantial correlation between the acetochlor concentration and total organic carbon. Yu et al. investigated the acetochlor residual levels in 145 water sources and 209 factory waters in 36 key cities in China from 2009 to 2015. According to the results, the detection rate of acetochlor in the water sources was 66.9%, and the average concentration was 33.9% (Yu et al., 2014).

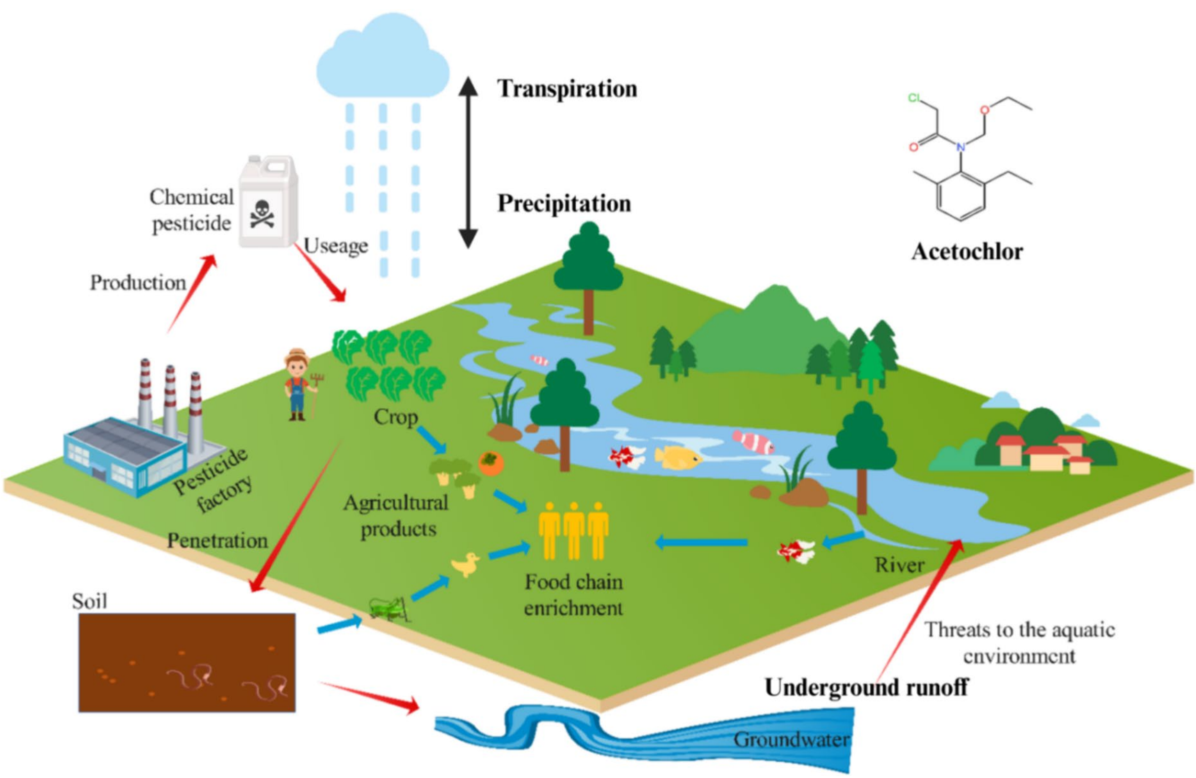

Fig. 1 Fate and occurrence of acetochlor in environment

In the United States, acetochlor is a major source of potential herbicide contamination in the rivers of the Midwest, and the detection range of acetochlor in the surface water is from 0.02 to 2.5 mg/L (Nowell et al., 2018; Carroll et al., 2024; Skalaban et al., 2024). The US Geological Survey collected and analyzed the acetochlor distribution in the hydrological system in 1994. According to the results, acetochlor was present in 29% of the rain samples from 4 locations in Iowa, and in 17% of the stream samples from 51 locations in 9 states. The acetochlor concentrations increased in the rainwater and streams following its application to corn in the United States Midwest, with acetochlor present in 75% of the rainwater samples and 35% of the stream samples during this period.

In addition, researchers have detected acetochlor in the soils of the agricultural region of Belgrade, Serbia, and the Vilaine Bay region (the Atlantic coast, south Brittany, and France), in the surface waters of the maize-producing areas of the western part of South Africa, as well as in the coastal waters of the Liaodong Peninsula of China (Caquet et al., 2013; Huaijun, 2019; Marković et al., 2010; Kurt-Karakus et al., 2023; Ren et al., 2024).

Acetochlor toxicity to non-target organisms

Transformation and degradation are two of the key processes that control the environmental fate and transport of pesticides, including abiotic degradation (e.g., oxidation, hydrolysis, and photolysis) and biodegradation (Chen et al., 2023b; Fan et al., 2023; Pang et al., 2023). In these processes, pesticide residues that are not fully mineralized can pose risks to the entire ecosystem (Bilal et al., 2021; Birolli et al., 2022; Cycoń et al., 2017).

Acetochlor itself has a variety of toxic effects, including on the reproductive, endocrine, and cardiovascular systems, as well as immunotoxicity effects on cells and various model organisms (Wang et al., 2024; Wang et al., 2023; Chang et al., 2020; Lu et al., 2023a, 2023b). Acetochlor disrupted the expressions of the nervous system genes and apoptosis-related genes in zebrafish embryos, ultimately leading to apoptosis and morphological deformities (Wang et al., 2019), and it altered the endocrine concentrations by altering the expressions of the key genes in the endocrine systems of zebrafish (Jiang et al., 2015; Sun & Li, 2019; Von Hellfeld et al., 2020). Acetochlor is not only harmful to aquatic organisms, but it also has toxic effects on terrestrial animals. In rats, the long-term exposure to acetochlor caused liver and kidney damage, pantothenic acid synthesis, fatty acid biosynthesis, and antioxidant system dysfunction, and in mouse liver, acute and subacute exposure to acetochlor directly inhibited the fatty acid oxidation (Counihan et al., 2017; Li et al., 2016; Cao et al., 2022; Song et al., 2019).

The acetochlor toxicity to animals makes us aware of its harm to humans and reminds us that we need to be more careful when it comes to pesticide residues (Bhatt et al., 2020, 2021a). Humans are particularly vulnerable to pesticide residues during the early stages of development, representing a critical period for exposure management and risk assessment. Pesticide exposure can adversely affect the nervous, reproductive, endocrine, and immune systems of humans (de Gavelle et al., 2016). For instance, Huang et al. found that acetochlor was cytotoxic to human liver carcinoma cells (HepG2) (Huang et al., 2020; Wang et al., 2021b). When exposed to acetochlor, the intracellular production of reactive oxygen species (ROS), mitochondrial dysfunction, cell cycle arrest, etc., eventually lead to HepG2 apoptosis. We present the main toxic effects of acetochlor on nontarget organisms in Table 1.

In addition, in some cases, the degradation products of the pesticide may be more toxic than the parent compound (Chen et al., 2014a, 2014b, 2015, 2023a, 3b; Ji et al., 2020; Mahler et al., 2021; Dong, 2024). Therefore, considering the risk to animals and humans, we cannot ignore the potential harm caused by residual acetochlor, nor the importance of removing it from the environment.

Acetochlor photodegradation and chemical oxidation

Photodegradation is an important means through which organic pesticides, such as acetochlor, degrade in the environment (Li et al., 2021b; Mishra et al., 2021b; Wu et al., 2023a; Zhan et al., 2018). Photodegradation is a process in which high-molecular-weight organic substances are gradually oxidized into low-molecular-weight substances under the irradiation of a light source, which finally results in the formation of CO_2 and H_2O. Acetochlor is a chloroacetanilide

Table 1 Toxicity of acetochlor on non-target organisms

No	Target	Results	References
1	Zebrafish larvae	The target organ of acetochlor's toxicity to zebrafish is the cardiovascular system, and the main phenotypes include bradycardia, pericardial edema, circulatory disturbance, and thrombosis	Liu et al., (2017)
2	Earthworms (*Eisenia fetida*)	Hydroxyl radical (•OH) content, superoxide dismutase (SOD), and antioxidant enzyme catalase (CAT) activities, as well as cytochrome P450 content, were significantly increased. Acetylcholinesterase (AchE) activity was significantly inhibited after exposure to both Acetochlor enantiomers Acetochlor enantiomer induced lipid peroxidation and DNA damage	Liu et al., (2021)
3	Embryonic zebrafish	Acetochlor has the potential to induce acute toxicity, result in developmental abnormalities, and impact the expression of proteins and critical genes associated with the innate immune system in zebrafish embryos	Xu et al., (2016)
4	Red Swamp Crayfish (*Procambarus clarkii*)	Crayfish that were subjected to acetochlor displayed symptoms such as body twitching, abdominal arching, loss of balance, body and appendage rocking, and lethargy. Notably, exposure to a concentration of 72.62 mg/L resulted in significant histopathological alterations	Yu et al., (2017)
5	Goldfish (*Carassius auratus*) larvae	The concurrent exposure to acetochlor and copper resulted in heightened toxicity towards goldfish larvae compared to individual exposure, leading to various adverse effects such as growth inhibition, tissue damage, oxidative stress, and suppression of antioxidant-related gene expression	Xue et al., (2021)
6	Bighead Carp (*Aristichthys nobilis*)	Acetochlor causes oxidative stress in bighead carp. Bighead carp treated with acetochlor showed significant DNA damage, higher levels of oxidative biomarkers, and significantly lower cellular protein concentrations their gills, liver, brain, and kidneys	Mahmood et al., (2022)
7	Male mouse	Acetochlor exposure reduced spermatogonia viability, altered oxidative stress levels and increased cell apoptosis in male mice	Jiang et al., (2020)

herbicide. Yasmine et al. proposed a dechlorination process for the photodegradation of chloroacetanilide ions (Yasmine et al., 2013), as shown in Fig. 2.

Acetochlor herbicides are relatively stable in the environment, and they have a slow photolysis rate. However, researchers have still performed the

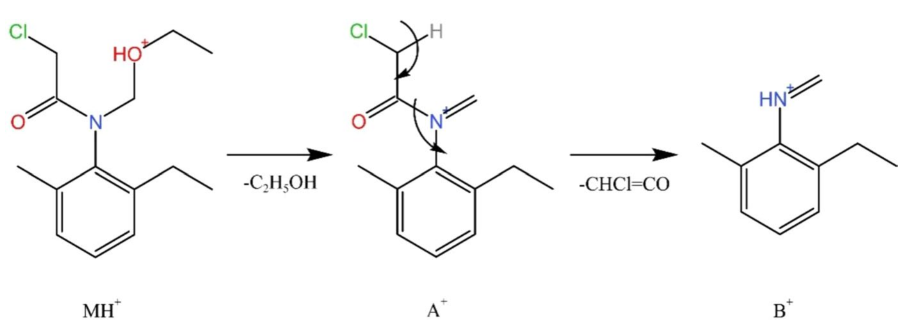

Fig. 2 Dechlorination process of chloroacetanilide ion in photodegradation

corresponding research. The main photolysis product of acetochlor is hydroxylated acetochlor, and its rate-limiting step is dehalogenation. Together, dehalogenation, hydroxylation, and cyclization constitute the main processes of acetochlor photolysis (Yasmine et al., 2013). Jablonski found at least five photodegradation products of acetochlor: *N*-(2-ethyl-6-methylphenyl)fomamide; 2-chloro-*N*-(2-ethyl-6-methylphenyl)acetamide; *N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl) acetamide; 2-hydroxy-N-(2-ethyl-6-methylphenyl) acetamide; 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(propyloxymethyl)acetamide. Kiss et al. found that the main acetochlor photodegradation steps in water are the cleavage of the ester-bond of the *N*-ethoxy-methyl group and the breaking off of the chloro and hydroxyl groups, which result in 2-ethyl-6-methyl-*N*-methyl-aniline. There are several major photodegradation products, such as 2-chloro-*N*-hydroximethyl-*N*-(2-ethyl-6-methylphenyl)acetamide, *N*-hydroxi-methyl-*N*-(2-ethyl-6-methylphenyl) acetamide, and *N*-methyl-*N*-(2-ethyl-6-methylphenyl) acetamide (Kiss & Virag, 2009). We present the degradation pathways in Fig. 3.

At present, the research on the photodegradation method for the removal of acetochlor in the environment is not mature enough, and we still require more experimental research (Yasmine et al., 2013; Yuan et al., 2018). In addition to photodegradation, acetochlor can also be degraded via chemical oxidation. The chemical oxidation method refers to the addition of chemical oxidants to the polluted environment (Mishra et al., 2020, 2022; Chen et al., 2022). Researchers have employed the strong oxidizing properties of the oxidants to degrade the pollutants and convert acetochlor into substances with low toxicities and mobilities (Baiqing et al., 2021; Fu et al., 2019; Souissi et al., 2013; Yuan et al., 2018). Zhang et al. studied the degradation reaction of acetochlor in water using single oxidation devices (hydrogen peroxide, potassium peroxymonosulfonate, and Fenton reagent), and then they combined these single

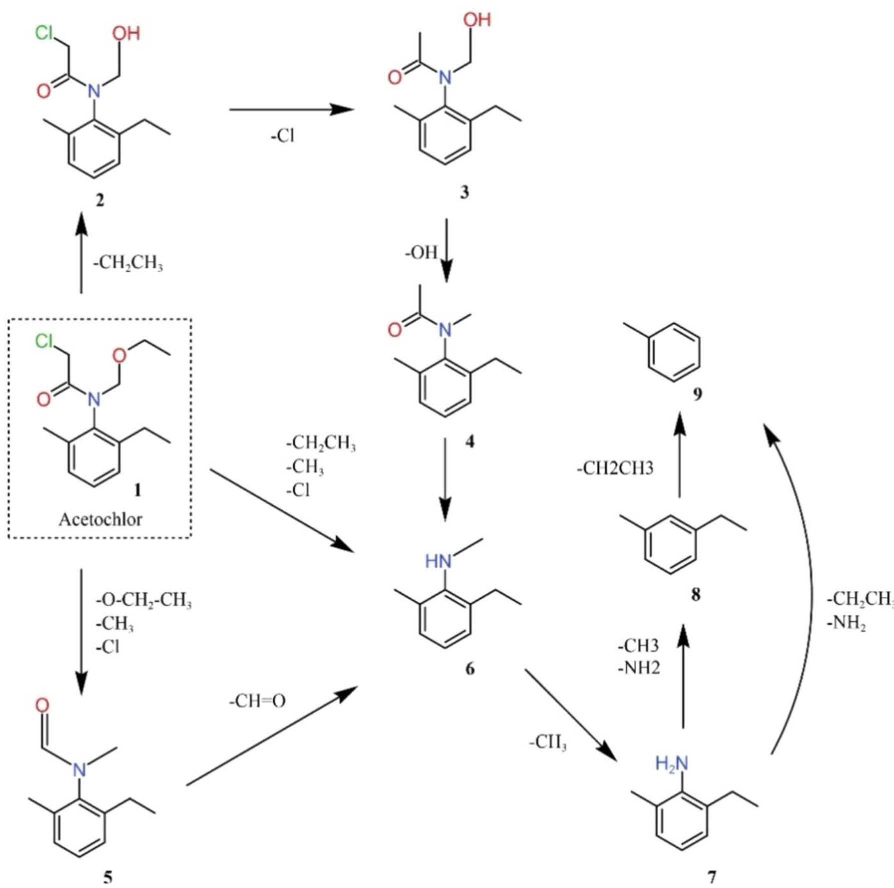

Fig. 3 Proposed acetochlor photodegradation pathways

oxidation devices. Through potassium peroxymonosulfonate and Fenton with the combined action of reagents, researchers increased the degradation effect of acetochlor to 90% in 90 s (Yuehua et al., 2012). Friedman et al. used anodic electro-Fenton technology to treat acetochlor-contaminated water (Friedman et al., 2006; Barzoki et al., 2023; Pacheco-Álvarez et al., 2023). After the electrochemical oxidation, the acetochlor completed the dechlorination, and the biodegradability of the polluted water was considerably enhanced.

The anodic Fenton treatment (AFT) is an electrochemical treatment method in which the Fenton reaction is utilized to generate hydroxyl radicals, which are powerful oxidants that are capable of degrading organic compounds by hydrogen abstraction (Friedman et al., 2006; Lu et al., 2023b; Olvera-Vargas et al., 2019). We present the general acetochlor pathway under the action of strong oxidants in Fig. 4. The acetochlor undergoes hydroxylation at all available phenyl sites via hydroxyl radical addition to form free radicals, which are then oxidized by Fe^{3+} to form phenolic compounds. However, this method has a large investment and is not suitable for the remediation of large-scale acetochlor-contaminated soil. In contrast, the cost-effectiveness and environmental friendliness of microbial remediation technologies promise extensive utility and significant potential for widespread application.

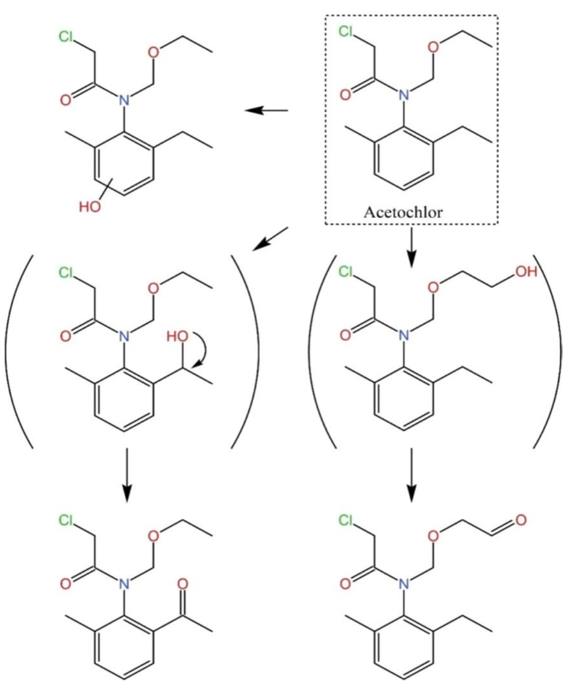

Fig. 4 Acetochlor degradation by anodic Fenton treatment

Microbial acetochlor degradation and key enzymes

In view of the serious environmental toxicity and ecological threat of acetochlor, the development of reasonable and efficient acetochlor pollution environmental remediation technology is urgent. In recent years, due to their high efficiency, low cost, and high ecological benefits, the use of microorganisms to remove chemical pesticide residues has gradually become the most promising restoration method for environmental pollution control, and it is also a research hotspot in the field of environmental science (Bhatt et al., 2021b; Ruan et al., 2024; Wu et al., 2023b; Zhang et al., 2022; Zhao et al., 2022a, 2022b).

High-efficiency microbial resources for acetochlor biodegradation

Many researchers have isolated pure cultures with acetochlor-degrading abilities from acetochlor-polluted soil, and they have further isolated and identified high-efficiency acetochlor-degrading strains, such as *Rhodococcus*, *Klebsiella variicola*, *Bacillus subtilus*, and *Methylobacillus* (Huang et al., 2022; Li et al., 2013, 2022; Yingying et al., 2011) (Table 2).

Ni et al. isolated chlorinated amide herbicide-degrading bacteria that belong to the *Paracoccus* genus from the activated sludge produced by the biological wastewater treatment tank of an acetochlor pesticide plant, and they named it Y3B-1 (Yingying et al., 2011; Feng et al., 2023; Liu et al., 2023; Yang et al., 2022). The inoculation amount of the strain is proportional to the acetochlor degradation rate, which can reach 86.7%. Luo et al. isolated and identified the bacterium JD115, which belongs to the genus *Pseudomonas aeruginosa* (Han et al., 2021; Liu et al., 2022a; Luo et al., 2015). The bacterium can rapidly degrade acetochlor under the optimal growth conditions of a temperature of 37 °C and a pH of 7. With the addition of nutrients, the degradation rate can reach 95.4%.

Table 2 Microbial resources for biodegradation of acetochlor

No	Strains	Degradation conditions	Degradation effect	References
1	*Ensifer adhaerens* A-3	Inorganic salt with 10 mg/L acetochlor as sole nitrogen source	The degradation rate of acetochlor can reach 35% within 10 days	Bin and Feng (2011)
2	*Stenotrophomonas* sp. M-3	Acetochlor as the sole carbon source	The degradation rate of acetochlor at a concentration of 50 mg/L within 5 days can reach 76.6%	Lei et al., (2013)
3	*Sphingomonas* sp. DC-6	Acetochlor 100 mg/L liquid basal salt medium	The degradation rate of acetochlor in 48 h was 93.6%	Qing et al., (2013)
4	*Bacillus* sp. ACD-9	Optimal degradation of acetochlor in solution at pH 6.0 and 42 °C	The degradation rate of 30 mg/L acetochlor in 2 days can reach 56.78%	Li et al., (2020)
5	*Shinella* sp. Y-4	With acetochlor as the sole carbon source and energy, the optimum pH value is 8.0, and the optimum temperature is 30 °C	The degradation rate of 50 mg/L acetochlor can reach 83.3% within 48 h	Jun et al., (2011)
6	*Burkholderia* sp. WN-3	Optimal degradation of acetochlor in solution at pH 6.0 and 35 °C	The degradation rate of 50 mg/L acetochlor in 7 days can reach 38.3%	Shuang et al., (2015)
7	*Rhodococcus* sp. MZ-3	Utilize acetochlor as the exclusive carbon, nitrogen, and energy source to support growth	Completely degrades 200 mg/L of acetochlor in 12 h	Zhang et al., (2016)
8	*Rhodococcus* sp.AC-1	The ideal temperature is 30 °C, while the optimal pH level is 7.5	Completely degrades 0.2 mM acetochlor within 48 h, but fails to mineralize acetochlor	Zhou et al., (2016)
9	*Klebsiella* sp. B-2	Inorganic salt medium with acetochlor as the sole carbon source	The degradation rate of acetochlor can reach 90.31% at 30 °C for 5 days	Deping et al., (2016)
10	The bacterial consortium T3 consists of *Rhodococcus* sp. T3-1, *Delftia* sp. T3-6, and *Sphingobium* sp. MEA3-1	No data	Completely degrade 100 mg/L acetochlor within 6 days	Hou et al., (2014)
11	*Pseudomonas* sp. A-1	The addition of carbon and nitrogen sources can improve the degradation rate of acetochlor	The degradation rate of acetochlor (5 ~ 10 mg/L) can reach 72% ~ 80%	Wei et al., (2016)
12	L201-4	No data	The degradation rate of 50 mg/L acetochlor in 7 days was 58.62%	Hu et al., (2015)
13	*Achromobacter* sp. D-12	Optimal degradation of acetochlor in inorganic salt media (MSM) at pH 7.0 and 30 °C	The degradation rate of 10 mg/L acetochlor in 5 days was 95%	Xu et al., (2013)
14	*Sphingobium quisquiliarum* DC-2, *Sphingobium baderi* DE-13	No data	No data	Li et al., (2013)
15	Cyanobacteria	No data	The degradation rate in water is much higher than that in soil	El-Nahhal et al., (2013)
16	*Pseudomonas oleovorans* LCa2	The optimal growth temperature and pH are 35 °C and 8.0, respectively	The strain can degrade 98.03% of acetochlor at a concentration of 7.6 mg/L within 7 days	Xu et al., (2006)

Table 2 (continued)

No	Strains	Degradation conditions	Degradation effect	References
17	*Pseudomonas aeruginosa* JD115	Acetochlor was best degraded at a pH of 7.0 and a temperature of 37 °C	Degradation of 95.4% acetochlor with a concentration of 50 mg/L	Luo et al., (2015)
18	*Bacillus subtilis* L3	The bacterial solution with a final concentration of 5×10^8 CFU/g soil had the highest degradation rate of acetochlor	The degradation rate of acetochlor after 50 days was 92.65%	Zhi et al., (2016)
19	*Pseudomonas fluorescens* KT3	No data	Degradation of 100% acetochlor with a concentration of 100 mg/L	Duc and Oanh (2019)
20	*Serratia* sp. QSxin4	The bacterium *Serratia* sp. QSxin4 has the capability to utilize acetochlor as its exclusive carbon source, exhibiting robust growth in a medium containing acetochlor at a concentration of 500 mg/L and lead at 200 mg/L	*Serratia* sp. QSxin4 can degrade acetochlor from 500 to about 4.5 mg/L in 48 h with a maximum degradation rate of 12 ± 0.1 mg/mL/h	Yufeng et al., (2021)

However, the biodegradation of acetochlor in real polluted environments occurs under more complex and harsher degradation conditions. Because the degradation of microorganisms is affected by various factors, such as the temperature, pH, and soil oxygen content, the addition of these microorganisms to the soil alone cannot effectively decompose acetochlor (Bin and Feng, 2011; Chen et al., 2013; Duc and Oanh, 2019; He et al., 2015; Huang et al., 2022; Li et al., 2022), which means higher requirements for the environmental tolerance of the degrading strains. Taking the *Rhodococcus* sp. T3-1 as an example, the optimum temperature for the degradation of acetochlor by the strain was 37 °C, and the optimum pH range was 6–10. Ba^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, and Cu^{2+} have strong inhibitory effects on acetochlor degradation, while Ca^{2+}, Li^{+}, Mg^{2+}, and Ni^{2+} can accelerate the acetochlor degradation efficiency (Ying et al., 2013). Therefore, Liu et al. proposed a new degradation strategy to enhance the acetochlor degradation efficiency using inactive composites or immobilized active materials (Liu et al., 2022a, 2022b). Using this method, the maximum acetochlor degradation rate can reach about 98%, and the immobilized synthetic microbial consortium (SMC) system exhibits remarkable environmental robustness and reusability (Kang et al., 2022; Li et al., 2021a).

Hence, the creation of a suitable living environment for microorganisms, or enhancing their tolerance, has become the main research direction for the bioremediation of organic pollutants, including acetochlor.

Metabolic biodegradation pathways of acetochlor

The acetochlor degradation process via soil microorganisms is a complex biochemical process, and its complete metabolic pathway is still unclear. Therefore, we require further studies on the complete acetochlor degradation pathway in the environment and its degradation products.

Xu et al. isolated a microorganism that is capable of degrading acetochlor from acetochlor-contaminated soil (LCa2), and they identified the acetochlor biodegradation products using GC–MS. The main possible degradation pathways involve dechlorination, hydroxylation, *N*-dealkylation, C-dealkylation, and dehydrogenation (Xu et al., 2006). Luo et al. also identified acetochlor-degradation products using GC–MS, and they speculated that the intermediate metabolite of acetochlor was catechol, which was finally degraded after 5 days (Luo et al., 2015). Hou et al. studied a bacterial population that was composed of *Rhodococcus* sp. T3-1, *Delftia* sp. T3-6, and *Sphingobium* sp. MEA3-1 that could fully mineralize acetochlor via biochemical synergy. T3-1 converts acetochlor to 2-chloro-*N*-(2-ethyl-6-methyl benzene) acetamide (CMEPA) via deethoxymethylation, and *Sphingobium* sp. MEA3-1 fully mineralizes the metabolite 2-methyl-6-ethylaniline (MEA) to CO_2 and H_2O (Hou et al., 2014). The combined degradation ability of these three strains is much higher than that of a single pure culture, and it can completely degrade 100 mg/L of acetochlor within 6 days. We present the specific degradation mechanism in Fig. 5. Based on the original upstream metabolic pathway, Cheng et al. found that the downstream 2-methyl-6-ethylaniline (MEA) pathway was initiated by the hydroxylation of the aromatic rings. The MEA finally achieved complete acetochlor mineralization under the action of *Sphingobium baderi* DE-13 (Cheng et al., 2017).

In addition to the aerobic dealkylation pathway, acetochlor can also pass through the anaerobic dechlorination pathway. Firstly, acetochlor forms 2-ethyl-6-methyl-*N*-(ethoxymethyl)-acetanilide (EMEMA) by removing the chlorine atom in the chloroacetyl group, and further by the ethoxymethyl removal of the radical to form *N*-(2-methyl-6-ethylphenyl)acetamide (MEPA). The obtained intermediate product (MEPA) can be converted into *N*-2-ethylphenylformamide (EPF) through the removal of the aromatic ring methyl group and the methylation of the acetyl group, and further through the hydroxylation of the formyl group to generate 2-ethyl-*N*-carboxyaniline (ECA) (Fig. 5). However, studies on the anaerobic acetochlor dechlorination pathway are still lacking, and we have yet to identify the key target genes and corresponding enzymes.

Key enzymes in acetochlor biodegradation pathway

To deal with the risk of acetochlor in the external environment, nontarget organisms may have a mechanism that biotransforms acetochlor. The enzyme system that plays an important role in the detoxification

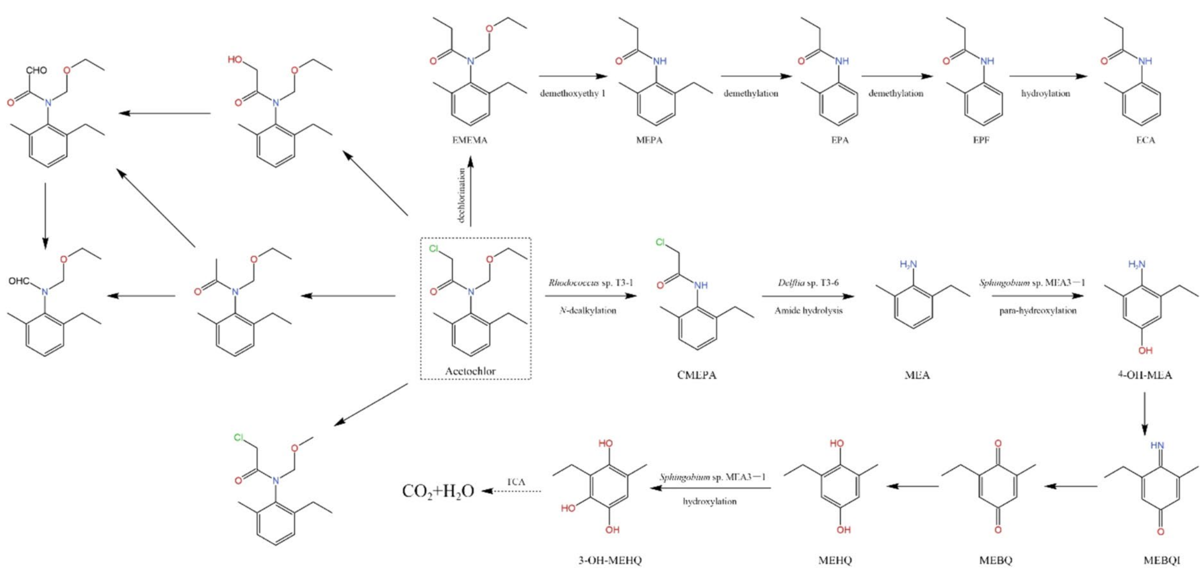

Fig. 5 Proposed acetochlor metabolic degradation pathways. Acetochlor was converted into 2-chloro-*N*-(2-ethyl-6-methyl benzene) acetamide (CMEPA) via the *N*-dealkylation reaction, and CMEPA was further converted into 2-methyl-6-ethylaniline (MEA) via the amide hydrolysis reaction. Subsequently, MEA was able to form 2-methyl-3-hydroxy-6-ethylhydroquinone (3-OH-MEHQ) through spontaneous hydrolysis and hydroxylation, was then transformed into the tricarboxylic acid cycle (TCA) with the ring-opening reaction of the benzene ring, and was eventually degraded into CO_2 and H_2O (Cheng et al., 2017; Hou et al., 2014)

and metabolism of herbicides in animals and plants is cytochrome P450 monooxygenase (Liu et al., 2021; Su et al., 2023; Wang et al., 2015). Cytochrome P450, a heme iron-sulfur protein found in various organisms, can bind with carbon monoxide in its reduced form, with an absorption peak at 450 nm. This enzyme system is crucial for metabolizing and detoxifying many herbicides (Christ et al., 2019; Hansen et al., 2021; Gergel et al., 2023). The most common P450 catalytic reactions are aromatic ring hydroxylation, alkyl hydroxylation, *N*-dealkylation, O-dealkylation, and epoxidation (Dutour et al., 2018; Pathak et al., 2024; Tsutsumi et al., 2018).

The plants that are transgenic for mammalian cytochrome P450 obtained by genetic engineering technology have excellent herbicide tolerances, among which CYP2B6 is the more prominent. Hirose et al. transferred the human CYP2B6 gene into rice, and the transgenic rice that expressed the human CYP2B6 gene had a strong tolerance to acetochlor (Hirose et al., 2005). Coleman et al. report that acetochlor forms 2-chloro-*N*-(2-methyl-6-ethylphenyl) acetamide (CMEPA) under the action of mouse P450 enzymes (most likely CYP2B6 and CYP3B4), and then 2-methyl-6-ethylaniline (MEA) is formed under the action of arylamidase (Dutour et al., 2018; Pathak et al., 2024; Tsutsumi et al., 2018). We present the inferred metabolic pathway of acetochlor in Fig. 6.

In addition, in *Rhodococcus*, cytochrome P450 was also involved in acetochlor biodegradation. Wang et al. purified a three-component enzyme (the cytochrome P450 system) from *Rhodococcus*. According to the phylogenetic tree of the proteins, the closest related proteins were ferredoxin reductase EthA (99% identity), cytochrome P-450 EthB (98% identity), and ferredoxin EthD (96% identity) (Fig. 7) (Wang et al., 2015). The enzyme has the activity of acetochlor *N*-deethoxymethylase and can convert acetochlor to CMEPA.

Dealkylation is the initial reaction of aerobic acetochlor microbial degradation, and *N*-dealkylase and C-dealkylase can catalyze it. Chen et al. report a three-component Rieske nonheme iron oxygenase (RHO) system that consists of homo-oligomer oxygenase, [2Fe-2S]ferredoxin, and GR-type reductase, which catalyzes the *N*-dealkylation of acetochlor and its conversion to CMEPA and ethoxymethanol (Chen et al., 2014a, 2014b). Gao et al. isolated the *Bacillus* sp. strain hys-1 from activated sludge, and they cloned a *Debutoxylase* (Dbo) gene that encodes a decarboxylase from the strain that can catalyze acetochlor *C*-dealkylation (Gao et al., 2015).

Amide hydrolysis can hydrolyze CMEPA to form MEA. Li and Wang obtained the amidohydrolases DamH and CmeH from the *Sphingobium quisquiliarum* DC-2 and *Delftia* sp. T3-6 strains, respectively. These two enzymes can efficiently hydrolyze the substrate CMEPA to MEA, and they are the key enzymes in microbial acetochlor degradation (Li et al., 2013; Wang et al., 2014). Subsequently, MEA is converted into 3-OH-MEAQ through a series of benzene ring hydroxylation processes, and it is finally fully mineralized through the catechol degradation pathway. Dong et al. identified the key genes involved in the degradation process of MEA (MeaA and MeaB), and the corresponding encoded oxygenase MeaA and reductase MeaB constitute a novel flavin monooxygenase system that catalyzes MEHQ hydroxylation and converts it to 3-OH-MEHQ (Dong et al., 2015). Table 3 provides a summarization of the key enzymes responsible for acetochlor biodegradation pathways.

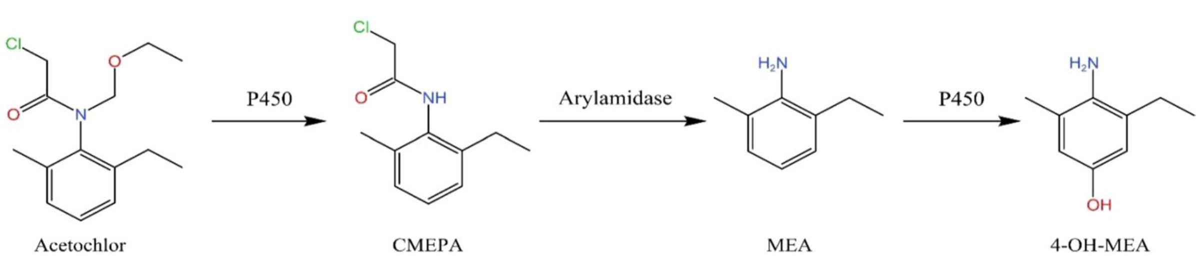

Fig. 6 Speculation on acetochlor metabolic pathway under action of mouse P450 enzymes

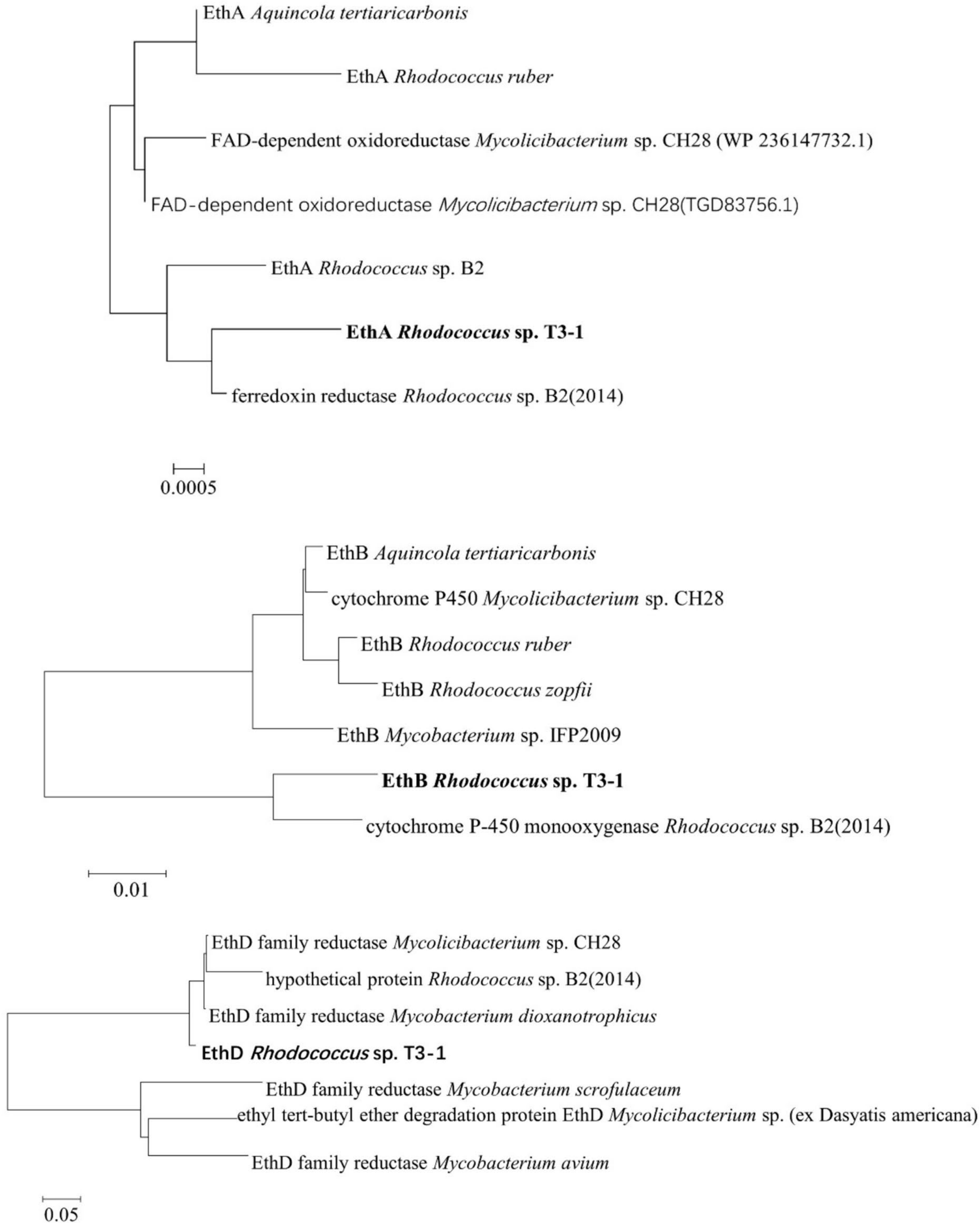

Fig. 7 The phylogenetic tree of EthABD and related proteins constructed using neighbor-joining method. We collapsed branches corresponding to partitions reproduced in less than 50% of bootstrap replicates. We eliminated all positions with less than 60% site coverage

Table 3 Key enzymes involved in biodegradation process of acetochlor

Enzymes	Source	Classification	Catalytic reaction	References
Rieske non-heme iron oxygenase	*Sphingomonads* DC-6, DC-2	Rieske non-heme iron oxygenase type *Iva*	*N*-dealkylation	Chen et al., (2014a, 2014b)
Hydrolase (ChlH)	*Rhodococcus* sp. B1	No data	*N*-dealkylation	Liu et al., (2012)
Debutoxylase (Dbo)	*Bacillus* sp. Hys-1	No data	C-dealkylation	Gao et al., (2015)
N-ethoxymethylase	*Rhodococcus* sp. T3-1	Carboxylesterase	*N*-deethoxymethylation	Wang et al., (2015)
Amide hydrolase (DamH)	*Delftia* sp. T3-6	Amidase	Amide hydrolysis reaction	Wang et al., (2014)
Amidohydrolase (CmeH)	*Sphingobium quisquiliarum* DC-2	Amidase	Amide hydrolysis reaction	Li et al., (2013)
Flavin-dependent monooxygenase (MeaBA)	*Sphingobium* sp. MEA3-1	Flavin monooxygenase	Hydroxylation of 2-methyl-6-ethylaniline (MEA)	Dong et al., (2015)
Two-component monooxygenase (MeaXY)	*Sphingobium baderi* DE-13	Riboflavin monooxygenase	Hydroxylation of MEA	Cheng et al., (2017)

Acetochlor phytoremediation

Phytoremediation is also a promising approach to the reduction or elimination of heavy metal residues and organic pollutants (Bhatt et al., 2022; Lin et al., 2022; Sarwar et al., 2017). However, the plant degradation of toxic compounds, such as acetochlor, is difficult due to the absence of various metabolic genes and enzymes (Li et al., 2018a, 2018b). Transgenic engineering offers the opportunity to improve the abilities of plants to use exogenous genes to remove pollutants, which presents new possibilities for phytoremediation research (Rai et al., 2020). In the past two decades, researchers have isolated many microorganisms that are capable of degrading acetochlor from the environment. These related metabolic enzyme systems provide valuable resources for the development of bioremediation engineering plants.

Chu et al. introduced the *Sphingomonas wittichii* DC-6 *cndA* genes that encode the acetochlor *N*-dealkylase system into *Arabidopsis thaliana* to obtain cytoplasmic transformants and chloroplast transformants, as shown in Fig. 8. According to the results, the chloroplast transformants exhibited high acetochlor degradation rates and strong tolerances. They could transform 94.3% of 20 μmol/L of acetochlor in water within 48 h, and they could remove 80.2% of 5 mg/kg of acetochlor in soil within 30 days (Chu et al., 2020). The results of this study have an important reference value for the phytoremediation of acetochlor residues in real polluted environments. Su et al. also studied a genetically engineered plant that removed and degraded acetochlor in a growth medium (Su et al., 2019). The researchers identified the acetochlor removal from transgenic rice plants that overexpressed an unidentified glycosyltransferase (IRGT1). According to the results, IRGT1 conferred acetochlor resistance on the plants, and the IRGT1-gene-transformed lines removed 39.8–53.5% of the acetochlor from the growth medium.

The essence of acetochlor phytoremediation is the introduction of the key genes to realize its catalytic degradation in plants, as well as the construction of transgenic plants with acetochlor-degradation abilities.

Summary and outlook

In agriculture, acetochlor is widely used for the pre-emergence control of weeds. However, the extensive use of this herbicide has resulted in a series of environmental contamination problems. Therefore, the need for effective strategies to remove acetochlor herbicides from the environment is extremely urgent. Traditional physical repair is simple and fast; however, the removal rate is low, which often results in residues. The chemical remediation technology is complex, the investment is large, and it often leads to secondary pollution. Recently, microbial remediation is gaining heightened interest as an effective and eco-friendly technique.

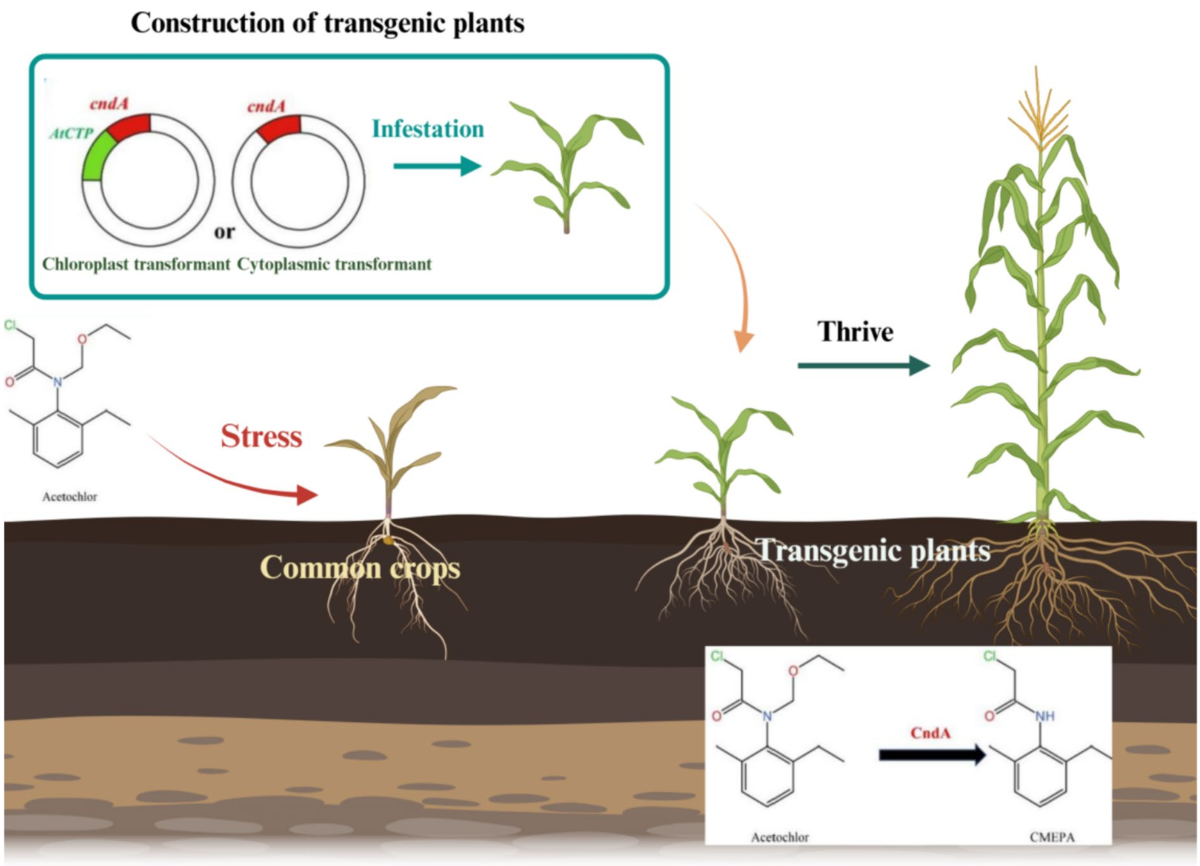

Fig. 8 Remediation of acetochlor-contaminated soil by transgenic plants

Bioremediation includes phytoremediation and microbial remediation. Microorganisms can convert acetochlor into low-toxic or nontoxic compounds via a series of enzymatic reactions. However, due to the lack of genes and enzymes available for pesticide metabolism, the plant degradation of toxic substances, such as acetochlor, is difficult. We can remove the pesticide residues in environmental soil by introducing the acetochlor-degradation genes of microorganisms to construct transgenic plants, and at the same time, we can reduce the adverse effects of acetochlor on crops.

At present, many types of microorganisms degrade acetochlor, and researchers have studied the relevant degradation paths and key enzymes. However, most of the degradation experiments remain in the laboratory simulation stage due to the real environmental physical and chemical factors (pH, moisture, oxygen, indigenous microorganisms, etc.), which have an important influence on the microbial enzyme activity. Therefore, improving the degradation and mineralization efficiencies of acetochlor in real polluted environments is the key to the application of microbial remediation technology. We can use the method to isolate the degrading microorganisms from complex external environments by providing them with a mild microenvironment via microbial immobilization. In addition to microbial immobilization, enzyme immobilization has also been applied to bioremediation. Immobilized enzymes offer advantages such as high stability, easy recovery, reusability, and low cost as compared to free enzymes. The use of enzyme modification technology (sequential error-prone PCR, combinatorial active-site saturation test, terative saturation mutagenesis, etc.) can substantially enhance the catalytic efficiencies and stabilities of enzymes, which provides more options for the practical application of acetochlor and other pollutants for microbial remediation in real environments.

Complete acetochlor degradation and mineralization are often achieved through the synergy of multiple microorganisms, such as complementary metabolic pathways, production of growth-promoting signaling substances, or enzyme systems. However, building a mixed bacterial system by simply mixing microbial strains cannot maximize the efficiency of the labor division and cooperation between strains. Therefore, in the future, researchers should focus on building efficient and controllable bacterial community systems to realize the labor division and cooperation between microorganisms. Mixed bacterial system can improve resistance to the external environment and broaden the spectrum of degradation substrates for different environmental pollutants.

Author contribution Wen-Juan Chen, Shao-Fang Chen, and Xidong Zhang wrote the manuscript. Shao-Fang Chen, Haoran Song, Zeren Li, Xiaofang He, and Xiaofan Zhou facilitated discussions and revised the manuscript. All authors approved the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declaration

Competing interest The authors declare no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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