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ORIGINAL PAPER

Dual-stage fenton–anaerobic treatment achieving complete elimination of diazinon and its toxic intermediates

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Abstract

A sequential Fenton-anaerobic biodegradation process was developed for efficient removal of the organophosphorus pesticide diazinon from aqueous solution. In the first stage, Fenton oxidation achieved 48.9% removal of diazinon within 20 min at optimized reagent dosages, generating toxic intermediates such as diazoxon and sulfotep. The pretreated effluent was subsequently treated by anaerobic biodegradation using diazinon-adapted sludge, resulting in complete elimination of these intermediates and an overall removal efficiency of 94.4% for an initial 22.4 mg L^{-1} diazinon solution over 96.3 h. GC–MS confirmed the transformation of hazardous by-products into less harmful hydrocarbons, including 1-tetradecene, 1-nonadecene, and 1-octadecene. Microbial analysis showed a shift from Gram-negative to Gram-positive bacteria after exposure, reflecting selective survival under pesticide stress. These results demonstrate that coupling Fenton pretreatment with anaerobic biodegradation provides a cost-effective strategy for complete detoxification of diazinon-contaminated water.

Keywords Organophosphorus pesticide · Anaerobic biodegradation · GC–MS analysis · Wastewater treatment

Introduction

Water pollutant is due to illegal and incomplete treatment has been caused by many environmental concerns (Chen and Dai 2024; Diab et al. 2025; Li et al. 2025). The use of organophosphorus pesticides in modern agriculture has expanded to produce high-quality products, and as a result, water pollution by pesticides is an integral part of agricultural activities (Mirsoleimani-azizi et al. 2018; Qi et al. 2018). Therefore, the development of water and wastewater treatment technologies is an essential element for eliminating these pollutants from the environment (Huang et al.

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2017; Fan et al. 2025). Studies have shown similar concerns with other pollutants, such as the migration of heavy metals in soils (Bai et al. 2020) and the use of industrial by-products for water remediation (Bai et al. 2022), highlighting the broad need for innovative strategies.

Diazinon (O,O-diethyl O-[6-methyl-2-(1-methylethyl)−4-pyrimidinyl] phosphorothioate) is one of the most famous and most used organophosphorus pesticides that are relatively soluble in water (40 mg/l at 25 °C). This pesticide was introduced in 1952 and classified by the World Health Organization (WHO) in a "moderately hazardous" category (Shemer and Linden 2006; Zhang et al. 2010). Researches have been showed these kinds of pesticides cause chronic deleterious effects and developmental disruption (Poirier et al. 2017). Therefore, the treatment of such wastewaters is necessary (Barzoki et al. 2023; Azizi et al. 2021; Pourali et al. 2023; Samarghandi et al. 2024). Several studies have explored different pollutants' removal using adsorption and advanced oxidation methods (Dargahi et al. 2019, 2021a; Latif et al. 2025; Nazir et al. 2026; Samarghandi et al. 2019). For example, Pirsaheb et al. (Pirsaheb et al. 2014) reported that granular activated carbon achieved 88% diazinon and 90% 2,4-D removal within 50 min at pH 6, indicating the potential of adsorption for pesticide treatment.

Advanced oxidation processes (AOPs) are increasingly applied as powerful tools to degrade a wide range of toxic organic pollutants in water. Because hydroxyl radicals have very high oxidizing power, they cause complete destruction of most pollutants (Hodges et al. 2018). More broadly, cooperative activation strategies are being developed across chemical sciences to enable efficient and sustainable transformations, such as photoactivation-assisted arylgermylation of alkenes (Cao et al. 2024). However, challenges have arisen in the face of limitations such as iron sludge production, acidic pH for the process, high peroxide consumption and intermediate production. Similar stability concerns have been reported in remediation materials exposed to corrosive conditions (Bai et al. 2024), emphasizing the importance of addressing secondary pollution risks.

In relation to the treatment of toxic wastewater, AOPs are a better option and efficient to clean up toxic and nonbiodegradable substances (Olalla and José 2007). But complete mineralization by advanced oxidation processes is often costly and, in some cases, leads to the production of toxic intermediates (Ballesteros Martín et al. 2009). Thus, the combination of both AOPs and biological oxidation is an economically and environmentally friendly method for destroying the target contaminant, since the cost of biological treatment is usually less than AOPs (Olalla and José 2007; Sonwani et al. 2018).

The Fenton process is more attractive than other advanced oxidation methods due to its low cost (Gomez-Herrero et al. 2019). Recent studies have shown that the process of Fenton in acidic pH is an effective process for the removal of organic compounds. In this process, hydrogen peroxide is used as the oxidizing agent and the ferrous ions are used as a promoter. The decomposition of hydrogen peroxide in the presence of Fe(II) involves a series of chain reactions, with Eq. 1 being the most important. In this context, Fe(II) functions as a promoter, since it is consumed and transformed into Fe(III) (Wang et al. 2016).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH \quad (1)$$

Biological processes are the preferred option for wastewater treatment owing to unrequiring chemicals. But in the face of toxic and nonbiodegradable compounds, they lose their effectiveness. biodegradability can be obtained through the use of advanced oxidation processes. For example, in research conducted by Zhang et al. (Zhang et al. 2019) the ratio of BOD_5/COD to the effluent after the Fenton process increased from 0.078 to 0.463. Thus, the combination of two methods of advanced oxidation and biocidal oxidation is an economical and suitable method for destroying the target pollutant.

Several studies have demonstrated that coupling these processes enhances overall degradation efficiency, reduces costs, and improves mineralization of hazardous compounds (Li et al. 2026; Qin et al. 2023; Chen et al. 2024). In this study, the by-products derived from the Fenton process and their removal in the anaerobic biodegradation process stage were identified and a possible mechanism for the destruction of diazinon and intermediate formation was suggested. Also, the effect of different concentrations and Fenton process time was investigated in the combined method. The potential for removal of diazinon was evaluated by each of the combined treatment steps. Finally, the gram stain test was carried out for the sludge in the anaerobic biodegradation process before and after contact with diazinon.

Experimental

Chemicals

Diazinon (98%), hydrogen peroxide (30%) and methanol (99%) were purchased from Sigma-Aldrich. Chloroform (99%) and hydrochloric acid were provided by Merck and Ferric sulfate were from AppliChem.

Fenton process

A 500 ml Erlenmeyer Flask was used for the Fenton process. Different concentrations of Diazinon were subjected to the Fenton process. 0.0016 g of weighted iron sulfate and 4.9 µl of hydrogen peroxide were added to different Diazinon solution. Sulfuric acid was applied to adjust pH (about 2.8–3), which is introduced in the resources as an optimum value for Fenton oxidation (Gomez-Herrero et al. 2019). The process was run inside the incubator at 30 °C with 200 rpm stirring. This process was carried out in a dark place.

All experiments were carried out in triplicate to ensure reproducibility. For each operating condition, three independent samples were prepared and analyzed. Reported results represent the mean of these replicates. This approach was adopted to reduce experimental variability and provide statistically reliable data.

Biological oxidation

The activated sludge was prepared from local dairy Industries company and was adapted to the diazinon pesticide. In order to create an oxygen-free environment, in addition to passing nitrogen gas through sludge solution, the biological reactor was placed in a dark environment. Because the light causes photosynthesis process, which it changes the anaerobic condition to aerobic (Nguyen et al. 2019; Barclay and Crawford 1982). The pH of the pre-treated effluent was adjusted using sodium bicarbonate at a range of 7–8. The volume of mixed liquor and the processing time for each experiment were 220 mL and 96 h, respectively.

The overall procedure of combined diazinon treatment was illustrated in Fig. 1.

Gram stain test procedure

The Gram stain test was performed according to the sources (Moyes et al. 2009; Zhang et al. 2025).

Adaptation procedure

At first, the concentration of 1 ppm of diazinon in a volume of 150 ml of sludge (50 ml of sludge + 100 ml of a solution including diazinon, urea, potassium dihydrogen phosphate

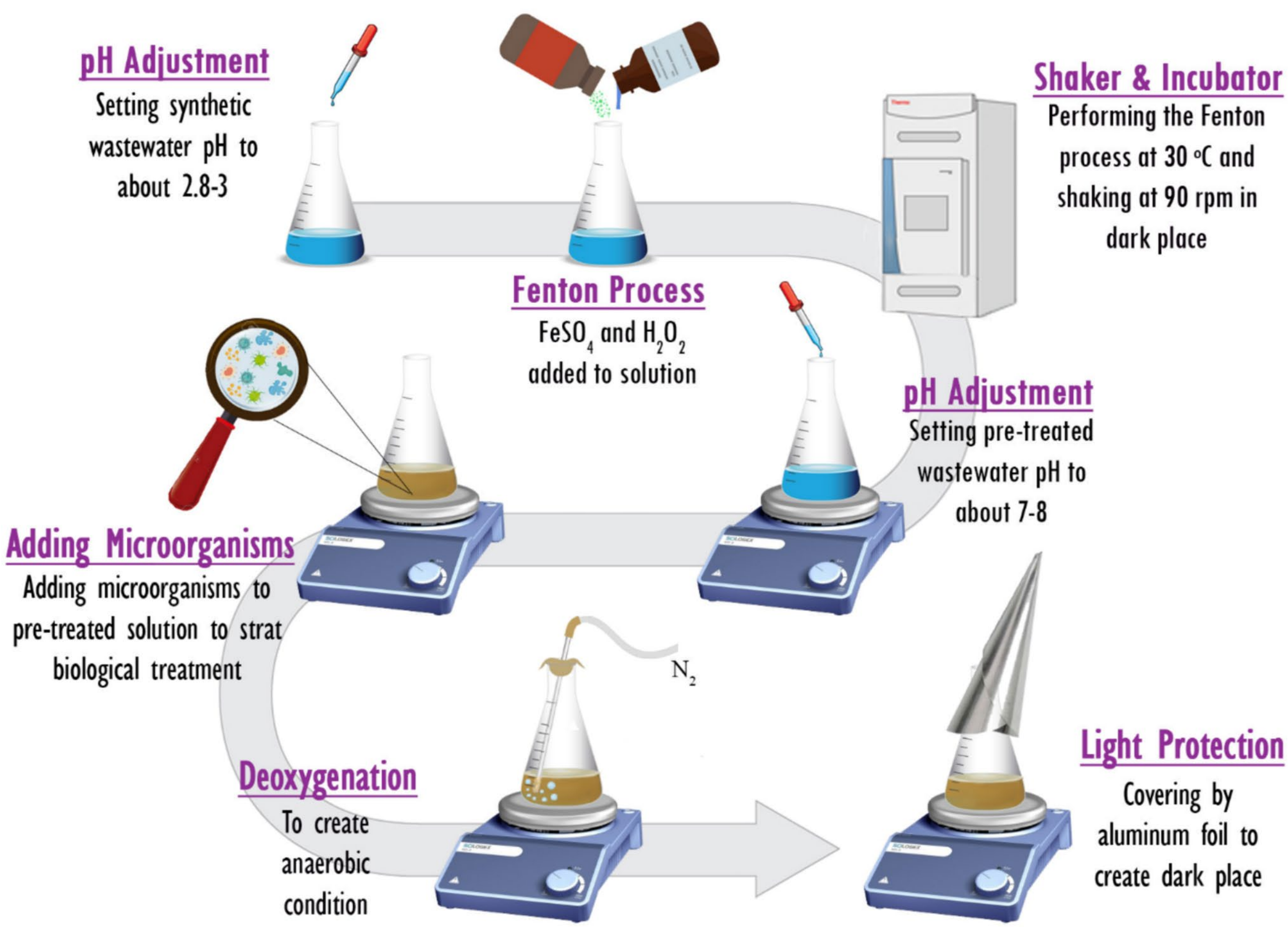


Fig. 1 The overall procedure of combined diazinon treatment

and glucose) was in contact with each other for 72 h. This process continued to a concentration of 7 ppm. The details of the steps are in Table 1.

DLLME procedure

The samples in the aqueous phase are not suitable for injection and will cause the GC detector to shut down. Thus, samples were condensed in an organic phase by using dispersive liquid–liquid microextraction (DLLME). In this way, a mixture of 1 ml methanol and 60µl chloroform are injected rapidly to 5 ml of an aqueous solution containing the sample by using 5 ml Hamilton syringe. diazinon in an aqueous sample was extracted into the fine droplets of chloroform. After the formation of a cloudy solution, the mixture was centrifuged at 5000 rpm for 5 min. The fine droplets were sedimented in the bottom of the conical test tube. 1µl of sedimented phase was injected into GC-FID by using microsyringe (zero dead volume, Hamilton).

GC analysis

A gas chromatograph with a split/split less injector system, and a flame ionization detector and GC–MS (Agilent 6890 N GC and Agilent 5973 mass detector, USA) were used for separation and determination of Diazinon. Ultrapure helium (99.9999%, Air Products, UK) passed through a molecular sieve trap and oxygen trap (Chromatography Research Supplies, USA) was used as the carrier gas at a constant linear velocity of 30 cm/s. The injection port was held at 100 °C and used in the splitless/split mode with a purge time 1 min. Separation was carried out on a CBP5 (25 m × 0.22 mm id) capillary column with a 0.25 µm stationary film thickness (Shimadzu, Japan) and an HP5MS (5% phenyl methyl siloxane, 30 m × 0.25 mm id, 0.25 µm film thickness) capillary column (Hewlett-Packard, USA) in GC-FID and GC–MS, respectively. The column oven temperature was initially held at 50 °C for 5 min. then raised to 300 °C at a rate of 10 °C min^1. The FID temperature was maintained at 300 °C. Hydrogen gas was generated by a hydrogen generator (OPGU-2200s, Shimadzu, Japan) for FID at a flow rate of 30 mL/min. The flow rate of zero air (99.999%, Air Products, UK) for FID was 300 mL/min.

Table 1 Adaptation conditions for microorganisms

	Step 1	Step 2	Step 3	Step 4
Diazinon (ppm)	1	3	7	10
Glucose (g)	0.15	0.12	0.075	0.06
Urea (g)	0.0074	0.006	0.0037	0.003
Potassium dihydrogen phosphate (g)	0.0068	0.0054	0.0033	0.0027

Results and discussion

Adaptation process

The adaptation of microorganisms increases the performance of the biological treatment process. Microorganisms adaptation to Diazinon has already been approved in studies (Felsot xxxx). But the duration of the adaptation in the resources varied for several hours to several days. For this purpose, various concentrations of Diazinon were exposed to microorganisms over several days. Considering that the adaptation time is 12 days. therefore, at the last stage of adaptation procedure, variation in concentration of Diazinon were investigated to ensure microorganisms were active. By comparing Fig. 2A and Fig. 2B it is found that the concentration of Diazinon after 72 h has been significantly reduced by microorganisms. The results for investigation of the chromatograms confirmed the removal of 97 percent of Diazinon by microorganisms. The decrease in the concentration of diazinon by microorganisms indicates the activity of microorganisms in the presence of the Diazinon. Due to the fact that during the adaptation stages of microorganisms, their nutritional changes were associated with a decrease in glucose and increased Diazinon, thus, at the last stage of the adaptation process, microorganisms mainly used diazinon as a carbon source to their metabolism. In a study conducted by Ochoa-Herrera et al. (Ochoa-Herrera et al. 2011) the gradual reduction of copper concentration in the culture medium was identified as one of the reasons for microbial adaptation Table 2.

Intermediates

Intermediates generation in the fenton process

In order to investigate the produced intermediates, a 20 ppm Diazinon solution was placed under the Fenton process. The Fenton process was performed for 30 min inside the incubator at 30 °C. Table 3 shows the results of the GC–MS analysis. The compounds of Sulfotep and Diazoxon were identified at retention times of 16.25 and 17.64, respectively.

According to sources, the results show that most of the intermediates are poisonous, which Diazoxon toxicity 10 times higher than that of Diazinon and one of the most well-known intermediates of Diazinon (Wang and Shih 2016).

Destruction of intermediates in anaerobic biodegradation

At this stage, the pre-treated solution (the effluent resulted from Fenton process) was placed under anaerobic biological treatment. At the end of the biological process, there was no significant reduction in sludge volume. As it can be seen

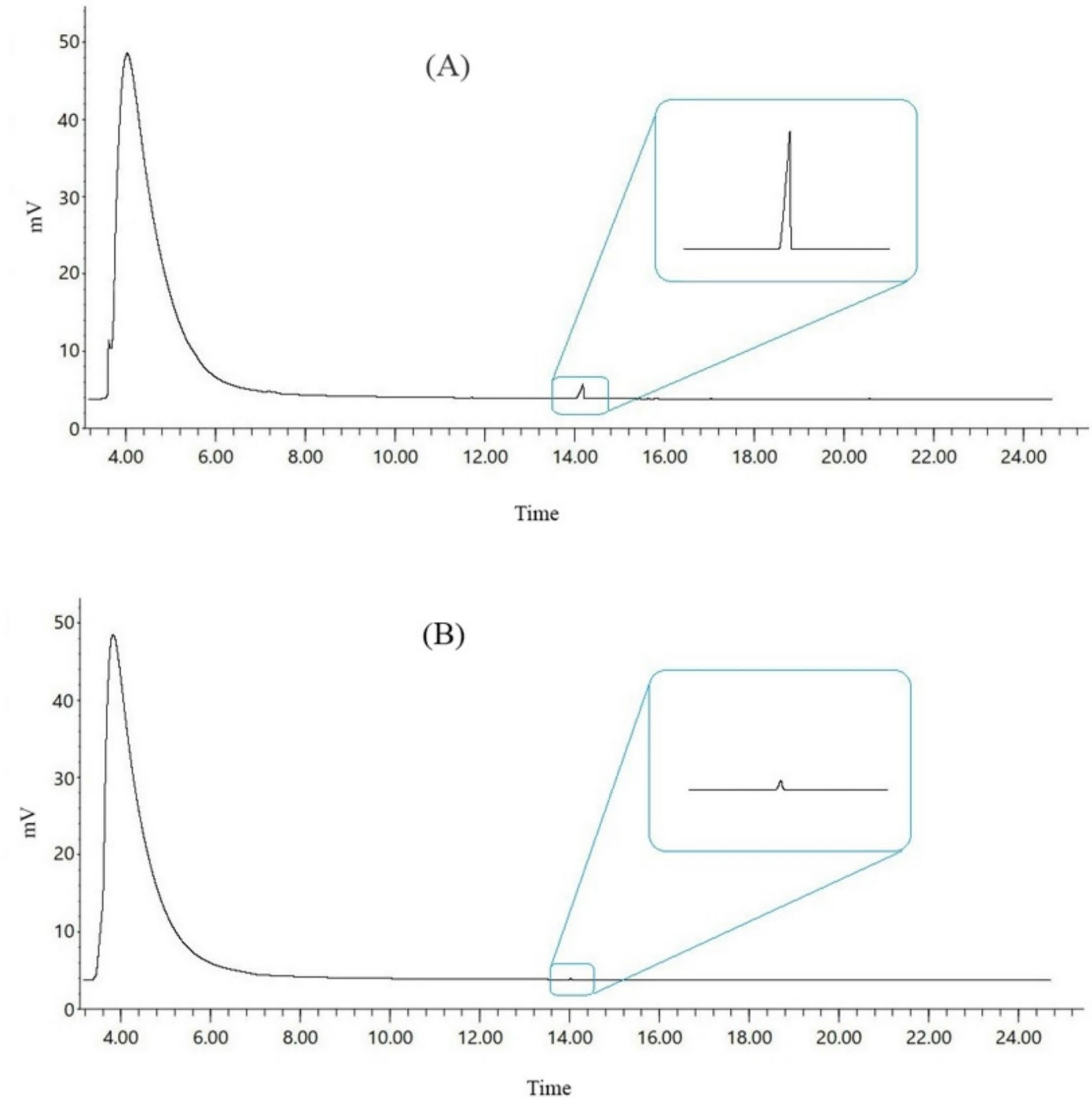


Fig. 2 Chromatogram of Diazinon at the beginning (**A**) and (**B**) end of the adaptation stage

Table 2 Diazinon's peak information

	R_t (min)	Area	Height	Area to height ratio
A	14.183	5208/72	1367.77	3.8082
B	14.183	148.193	63.82	2.3

in Table 4 the intermediates 1-Tetradecene, 1-Nonadecene, and 1-Octadecene have been obtained after completion of the anaerobic biodegradation. retention times of 13.15, 15.68 and 17.96, respectively.

As it is seen, the by-products from the advanced oxidation stage, degraded in combined with the biological process, and what is observed after the anaerobic biodegradation, are 1-Tetradecene, 1-Nonadecene, and the like.

Many studies have been performed on the removal of Diazinon from wastewater, which is illustrated below. As noted earlier, the toxicity of Diazoxon was greater than that of Diazinon, and in all cases listed in Table 5, presence of Diazoxon as the primary intermediate at the end of the processes has been inevitable. In the present project,

Table 3 Identified intermediates by GC–MS analysis in the Fenton process

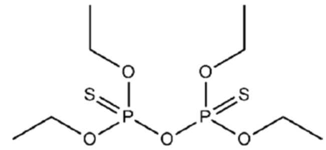
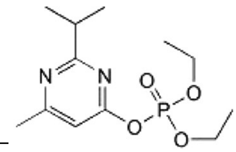
No	R_t (min)	Name	Structure
1	16.25	Sulfotep	
2	17.64	Diazoxon	

Table 4 Identified intermediates by GC–MS analysis in the anaerobic biodegradation

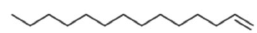
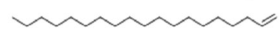
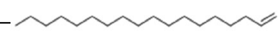
No	R_t (min)	Name	Structure
1	13.15	1-Tetradecene	
2	15.68	1-Nonadecene	
3	17.96	1-Octadecene	

Table 5 Comparison of diazinon degradation metabolism using combined chemical and chemical-biological methods

Treatment process	Method of identification	By-products of diazinon	Reference
Sono-Fenton processes	GC–MS	Hydroxydiazinon Diethyl phosphonate 2-Isopropyl-6-methyl-4-pyrimidinol Diazoxon	Wang and Shih 2015)
UV and H_2O_2 treatment	UPLC-ESI–MS/MS	Diethyl thiophosphate Diethyl phosphate Diazoxon	Li et al. 2015)
Ultrasonic irradiation	GC–MS	Hydroxydiazinon Diazinon methyl ketone 2-Hydroxydiazinon 2-Isopropyl-6-methyl-4-pyrimidinol Diazoxon	Zhang et al. 2011)
TiO_2 induced photocatalysis	GC–MS/MS and LC–MS	Diazinon aldehyde Hydroxydiazoxon Hydroxydiazinon 2-Isopropyl-6-methyl-4-pyrimidinol Diazinon methyl ketone 2-Hydroxydiazinon 2-Hydroxydiazoxon Diazoxon	Kouloumbos et al. 2003)
Fenton-anaerobic biodegradation	GC–MS	1-Tetradecene 1-Nonadecene 1-Octadecene	This study

the elimination of the Diazoxon compound was achieved by combining the two chemical and biological methods as a desirable treatment method. This work demonstrates the efficacy of biological treatment as post-treatment in a combined method.

After the Fenton process, the pH of the effluent was adjusted to neutral (7–8) before entering the anaerobic stage. Under these conditions, most of the soluble Fe(III) generated during the oxidation step precipitates as ferric hydroxide. The resulting suspension was not subjected to any further iron removal, and the precipitate remained in the mixed liquor during biological treatment. Previous studies have reported that such levels of residual iron generally do not inhibit anaerobic microbial activity and may even provide micronutrients that support microbial metabolism (He et al. 2021; Wang et al. 2024; Sun et al. 2022). In agreement with these reports, no adverse effects were observed in our experiments, as indicated by the successful microbial adaptation and high diazinon removal efficiency obtained in the combined process.

The possible adsorption and precipitation of Fe species after the Fenton stage can be partly explained by surface chemistry principles, as demonstrated in studies where hydrated Al^{3+} and Lu^{3+} ions showed preferential inner-layer adsorption on kaolinite, governed by ion size and hydration energy (Yan et al. 2022). This suggests that residual Fe(III) could similarly interact with mineral or sludge surfaces during the neutralization step.

Table 6 Diazinon removal results in each step of the combined method. Fenton process time = 30 min, anaerobic biodegradation process time = 96 h

Test	Diazinon mass removed in Fenton process (mg)	Diazinon mass removed in anaerobic biodegradation process (mg)	Final Residual Diazinon mass (mg)	Removal percentage in combination method
F-1	1.908	0.208	0.17	93.39
F-2	2.315	0.341	0.61	83.86
F-3	2.627	0.615	1.17	77.95

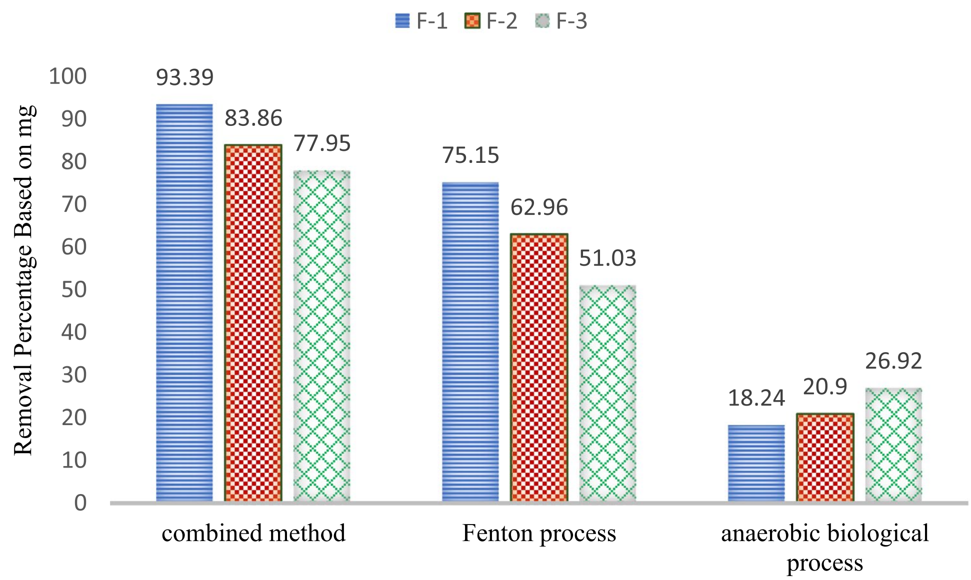


Fig. 3 Diazinon removal in each step of combined treatment, Fenton process time: 30 min, anaerobic biodegradation process time: 96 h

Investigation of different parameters on diazinon removal by the combined method

Effect of diazinon concentration in the combined method

In many reported combined processes, the advanced oxidation stage is applied after biological treatment to remove residual recalcitrant compounds (Dargahi et al. 2021b). In contrast, the present study employed the Fenton process as a pretreatment step before the anaerobic stage. This strategy was adopted because diazinon and its immediate intermediates, such as diazoxon and sulfotep, are highly toxic and strongly inhibit microbial activity, thereby reducing the efficiency of direct biological degradation. By applying the Fenton process first, these compounds were rapidly decomposed into smaller and more biodegradable intermediates, which facilitated microbial adaptation and ensured the complete elimination of hazardous by-products in the subsequent anaerobic process. This sequencing not only enhanced the removal efficiency but also minimized the risk of biological inhibition, supporting the rationale for selecting the Fenton–anaerobic pathway in this work.

At this stage, the removal of various concentrations of diazinon was investigated by a Fenton-anaerobic biodegradation method. To do this, various experiments were carried out with 11.54 ppm (F-1), 16.71 (F-2), and 23.4 ppm (F-3) concentrations. The operational conditions of Fenton and the anaerobic biodegradation were considered constant at all of these experiments. Conditions and test results are briefly summarized in Table 6 .

In all of these experiments, adapted anaerobic sludge has been used. Figure 3 shows the removal percentage of Diazinon in each of the combined treatment steps compared to the untreated sample in F-1, F-2, and F-3 experiments.

At Fenton process stage, choosing the proper concentration of reagents plays a key role. To oxidize resistant compounds, more amount of hydrogen peroxide is needed. But, on the one hand, hydrogen peroxide reduces the efficiency of the bio-activity and, on the other hand, the best efficiency for the Fenton process is when the amount of hydrogen peroxide is higher than iron (II). It is worth noting that, with increasing the iron (II) concentration, the radical hydroxyl production decreases. Similarly, the high concentration of hydrogen peroxide will also spontaneously deactivate hydroxyl radicals (Olalla and José 2007).

As the results show, the maximum removal of diazinon in the Fenton process is related to the F-1 test. Under reported circumstances in the F-1 test, 75.15% of diazinon removal was obtained during the Fenton process. In this experiment, the elimination of Diazinon by combined treatment was more than 93%. As can be seen, the contribution of the Fenton process to the removal of diazinon is high. In the F-2 and F-3 tests, 83.86% 77.95% of the diazinon was eliminated by the combined method, respectively. which decreased

Table 7 Residual Diazinon in each treatment step

Test	Fenton process time (min)	Diazinon mass removed in Fenton process (mg)	Diazinon mass removed in anaerobic biodegradation process (mg)	Residual Diazinon mass (mg)	Removal percentage in combination method
F-4	10	1.897	0.705	1.48	71.29
F-5	20	2.413	1.007	0.30	94.39
F-6	60	2.861	0.820	0.27	95.10

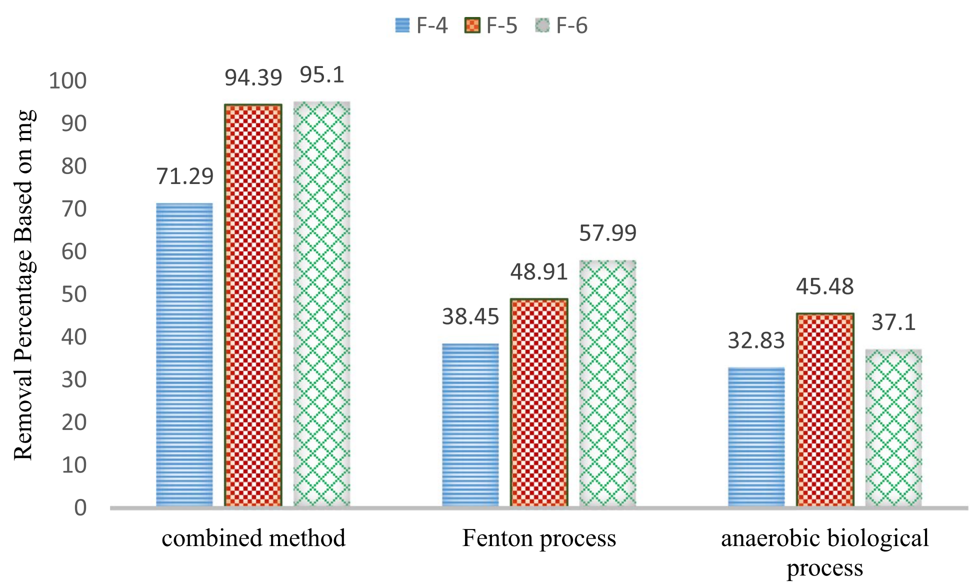


Fig. 4 Diazinon removal in each step of combined treatment, anaerobic biodegradation process time: 96 h

compared to the F-1 test. Reducing the efficiency of the Fenton process in the amount of promoter and hydrogen peroxide is probably due to the increase in the concentration of Diazinon since the production of hydroxyl radicals is constant in all three experiments, but the concentration of Diazinon increases, and the removal percentage results from the Fenton process. Degradation of Diazinon in the F-1 test is 18.24%, and the reason for the low bio-activity is probably due to its high pre-oxidation. As noted in Sect. "Intermediates generation in the Fenton process", the intermediates produced by the Fenton process are poisonous, which also affects the efficiency of the biodegradation.

Bio-degradation was highest in the F-3 test, so more experiments were conducted to investigate the effect of Fenton process time on the elimination of Diazinon on F-3 sample.

Effect of fenton process time in the combined method

F-4, F-5 and F-6 tests were designed to investigate the effect of Fenton process time on Diazinon degradation. In these experiments, the concentration of Diazinon and Fenton reagents was constant at 20 ppm and only the Fenton process time was considered as a variable parameter. an anaerobic biodegradation process was carried out at 96 h, neutral pH and under a temperature of 30 °C. Table 7 illustrates the results of these tests.

In all of these experiments, adapted anaerobic sludge has been used. Figure 4 shows the removal percentage of Diazinon in each step of the combined method compared to the untreated sample in the F-4, F-5, and F-6 experiments.

It is observed that in the F-4 and F-5 tests, the removal in the anaerobic biodegradation process is somewhat close to the Fenton process. Results of the F-4, F-5, and F-6 experiments show that the highest bio-degradation is related to the F-5 test. In this study, the reaction time of 20 min for the Fenton process has been concluded as the optimal prediction. In the F-6 test, despite the greater Fenton time, less biodegradation than the F-5 test has been achieved, which can involve several factors. More pre-oxidation is likely to result in higher intermediate production, and, as noted in Sect. "Intermediates generation in the Fenton process", Diazinon's intermediates more toxic than the diazinon itself. Therefore, the high concentrations of these substances will disrupt bio-activity. Table 8 was prepared to evaluate the combined Fenton and anaerobic biodegradation process in the removal of Diazinon by other combined methods. As can be seen by the integration of chemical methods with each other, even at low concentrations of diazinon, a good result has not been obtained. However, the method introduced in the present project yields the highest removal rate for diazinon.

It is recognized that the operation of the Fenton process under acidic pH (~2.8–3.0) may increase the cost of large-scale wastewater treatment due to the need for pH

Table 8 Comparison of diazinon removal in different processes

Process	Diazinon initial concentration (mg/L)	Diazinon removal (%)	Ref
Photocatalysis processes	11	40.9	Gar Alalm et al. 2015)
(UV/TiO_2) Photo-Fenton	11	50.9	
Ultrasonic irradiation	2.4	Less than 50	Zhang et al. 2011)
Ozonation	10	64	Ayoubi-Feiz et al. 2019)
Photo-electro catalysis	10	76–81	
Photo–Fenton–like process ($UV/H_2O_2/Fe^0$)	10	83	Kazemizad et al. 2016)
Electrospun nanofibrous membrane	10	83.7	Pordel et al. 2019)
Fenton-anaerobic biodegradation	22.42	95.1	This study

Table 9 Comparison of dia and estimated treatment cost by different processes

Process	Removal efficiency (%)	Estimated cost (USD/m^3)	Reference
Photocatalysis (UV/TiO_2)	40–50	1.2–1.5	Gar Alalm et al. 2015)
Sono-Fenton process	60–70	1.0–1.3	Wang and Shih 2015)
UV/H_2O_2 treatment	70–80	1.5–2.0	Li et al. 2015)
Ozonation	~64	1.3–1.6	Ayoubi-Feiz et al. 2019)
Electrospun nanofibrous membrane	~83.7	~1.0	Pordel et al. 2019)
Fenton-anaerobic biodegradation	94–95	0.5–0.8	This study

adjustment and control. Nevertheless, integration with biological treatment significantly reduces reagent consumption and treatment time, thereby lowering overall operational costs. Previous studies have also demonstrated that Fenton and Fenton-like systems are feasible at pilot- and full-scale when coupled with economical neutralization and sludge management strategies (Ballesteros Martín et al. 2009; Gomez-Herrero et al. 2019). This evidence highlights the practical potential of the combined process for real wastewater treatment involving large volumes.

Cost study

To further evaluate the technological potential of the proposed approach, a comparative assessment of diazinon removal efficiency and approximate treatment costs with other reported methods is presented in Table 9. The costs are based on reagent consumption reported in the literature, which typically represent the dominant operating expenses in advanced oxidation processes. While several AOPs achieve moderate to high removal efficiencies, their costs often range from 1–2 USD/m^3 due to continuous consumption of oxidants and energy inputs (Mousset et al. 2021; Martínez et al. 2018; Pera-Titus and V. García-Molina, M.A. Baños, J. Giménez, S. Esplugas 2004). In contrast, the present Fenton-anaerobic process achieves over 94% removal efficiency with lower reagent requirements, since the biological stage ensures the degradation of toxic intermediates. This integration reduces reliance on oxidants and neutralization reagents, improving overall economic feasibility, consistent with broader trends in replacing conventional reagents with greener and more stable alternatives, such as sodium thiocyanate in mineral flotation (Zhou et al. 2024). Similar to advances in other engineering domains where numerical models guide process optimization (Ni et al. 2025), such integrative approaches highlight the role of mechanistic insights in achieving sustainable solutions. Comparable advances are reported in resource utilization, such as the development of red mud-fly ash geopolymers under optimized curing conditions (Bai et al. 2023), reinforcing the potential of sustainable material-based solutions.

The present study demonstrates several strengths compared to similar works. First, the sequential Fenton-anaerobic process achieved a higher overall diazinon removal efficiency (94.4%) than adsorption (Pirsaheb et al. 2014) or single AOPs such as UV/H_2O_2 and ozonation, which typically report 60–80% removal (Li et al. 2015; Ayoubi-Feiz et al. 2019). Second, the combined system reduced reagent consumption by transferring part of the degradation burden to the biological stage, making the process more cost-effective. Third, GC–MS analysis confirmed that toxic intermediates were fully degraded into less harmful compounds, ensuring detoxification rather than mere transfer of pollutants.

Despite these advantages, some limitations remain. The requirement for acidic pH in the Fenton stage increases operational costs due to the need for neutralization before the biological step. In addition, iron sludge precipitation is inevitable and requires proper management for large-scale application. Addressing these challenges through process optimization and integration with cost-effective neutralization strategies will be essential for scaling up the technology.

Adopting microenvironment or confinement engineering (as reviewed by Fang et al. (Fang et al. 2025)) could potentially lead to improved control over Fenton reagent utilization, reduced Fe sludge deactivation, and enhanced selectivity toward less harmful intermediates in combined oxidation–biodegradation systems.

Gram stain test results

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan, and as a result are stained purple by

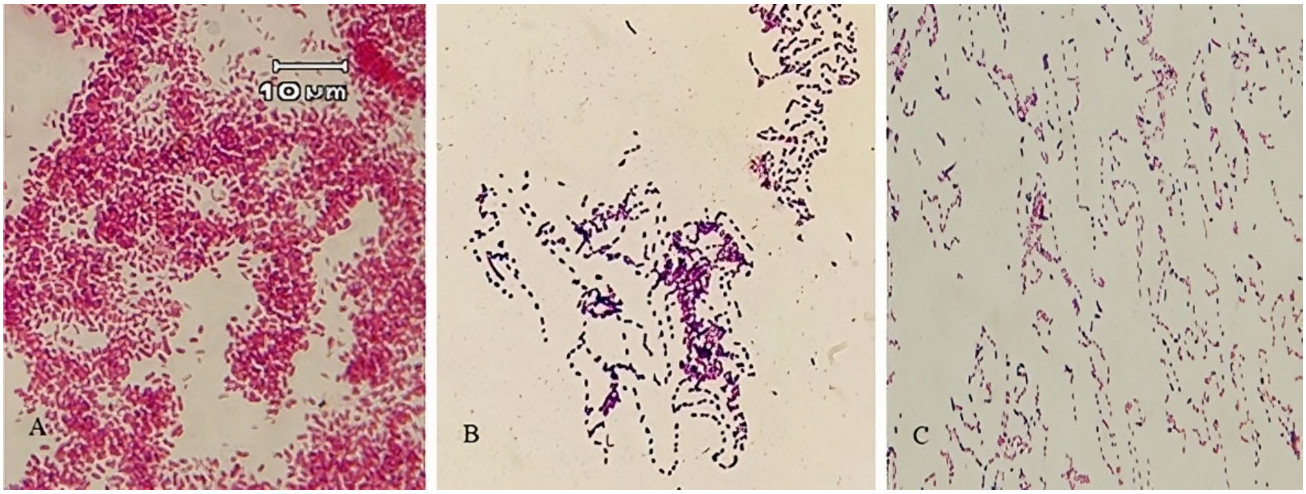


Fig. 5 Gram stain test on bacteria before the adaptation process (**A**), after the adaptation process (**B**) and after the combined process (**C**)

crystal violet, whereas Gram-negative bacteria have a thinner layer, so do not retain the purple stain and are colored as pink by safranin (Peck and Badrick 2017; E. Team 2019). Figure 5 shows the results of Gram stain test. It was found that bacteria before contact with Diazinon were mostly in the form of Gram-negative, but after contact with Diazinon, Germ-positive species appeared. As expected, the frequency of Gram-negative bacteria decreased after exposure to Diazinon due to their poor resistance. Gram-negative bacteria also have a slower growth due to more nutritional needs and limit their growth (Zhang et al. 2019). After the end of the combined process due to the exposure of microorganisms to toxins such as Diazinon and Diazoxon, the frequency of gram-negative bacteria has decreased and as the results show, the only dominant species is Gram-positive bacteria.

Conclusion

This study demonstrates that coupling Fenton oxidation with anaerobic biodegradation offers a powerful and sustainable route for the removal of diazinon from aqueous systems. The Fenton process provided rapid pre-oxidation, breaking down diazinon into more biodegradable intermediates, while the subsequent anaerobic stage ensured complete elimination of toxic by-products. Under optimized conditions, the integrated method achieved over 94% overall removal efficiency, with GC–MS confirming the disappearance of hazardous intermediates such as diazoxon. The microbial analysis revealed a selective shift toward Gram-positive species, highlighting microbial adaptation and resilience under pesticide stress. Compared with conventional single-step treatments, this dual approach not only enhanced removal efficiency but also minimized secondary pollution risks. The findings underscore the potential of combining advanced oxidation with biological treatment as a practical, cost-effective, and environmentally sound strategy for managing pesticide-contaminated wastewater. These results align with a wider scientific trend toward exploring both engineered and natural compounds as sustainable resources for addressing human and environmental challenges (Wei et al. 2024). Future studies may extend this approach to pilot-scale systems and explore its applicability to other persistent agrochemicals in real wastewater matrices.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper.

Declarations

Conflict 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.

References

Ayoubi-Feiz B, Mashhadizadeh MH, Sheydaei M (2019) Degradation of diazinon by new hybrid nanocomposites N-TiO2/graphene/Au and N-TiO2/graphene/Ag using visible light photo-electro catalysis and photo-electro catalytic ozonation: optimization and comparative study by Taguchi method. Sep Purif Technol 211:704–714. https://doi.org/10.1016/j.seppur.2018.10.032

Azizi A, Dargahi A, Almasi A (2021) Biological removal of diazinon in a moving bed biofilm reactor – process optimization with central composite design. Toxin Rev 40:1242–1252. https://doi.org/10.1080/15569543.2019.1675708

Bai B, Xu T, Nie Q, Li P (2020) Temperature-driven migration of heavy metal Pb^{2+} along with moisture movement in unsaturated soils. Int J Heat Mass Transf 153:119573. https://doi.org/10.1016/j.ijheatmasstransfer.2020.119573

Bai B, Bai F, Li X, Nie Q, Jia X, Wu H (2022) The remediation efficiency of heavy metal pollutants in water by industrial red mud particle waste. Environ Technol Innov 28:102944. https://doi.org/10.1016/j.eti.2022.102944

Bai B, Bai F, Nie Q, Jia X (2023) A high-strength red mud–fly ash geopolymer and the implications of curing temperature. Powder Technol 416:118242. https://doi.org/10.1016/j.powtec.2023.118242

Bai B, Chen J, Bai F, Nie Q, Jia X (2024) Corrosion effect of acid/alkali on cementitious red mud-fly ash materials containing heavy metal residues. Environ Technol Innov 33:103485. https://doi.org/10.1016/j.eti.2023.103485

Ballesteros Martín MM, Sánchez Pérez JA, García Sánchez JL, Casas López JL, Malato Rodríguez S (2009) Effect of pesticide concentration on the degradation process by combined solar photo-Fenton and biological treatment. Water Res. 43:3838–3848. https://doi.org/10.1016/j.watres.2009.05.021

Barclay AM, Crawford RMM (1982) Plant growth and survival under strict anaerobiosis. J Exp Bot 33:541–549. https://doi.org/10.1093/jxb/33.3.541

Barzoki HR, Dargahi A, Shabanloo A, Ansari A, Bairami S (2023) Electrochemical advanced oxidation of 2,4-D herbicide and real pesticide wastewater with an integrated anodic oxidation/heterogeneous electro-Fenton process. J Water Process Eng 56:104429. https://doi.org/10.1016/j.jwpe.2023.104429

Cao J, Liu Y, Wang Z, Liu L (2024) Arylgermylation of alkenes by a cooperative photoactivation and hydrogen atom transfer strategy. Org Chem Front 11:7098–7106. https://doi.org/10.1039/D4QO01497A

Chen X, Duan F, Yu X, Xie Y, Wang Z, El-Baz A, Ni B-J, Ni S-Q (2024) One-stage anammox and thiocyanate-driven autotrophic denitrification for simultaneous removal of thiocyanate and nitrogen: pathway and mechanism. Water Res 265:122268. https://doi.org/10.1016/j.watres.2024.122268

Chen X, Dai A (2024) Quantifying contributions of external forcing and internal variability to Arctic warming during 1900–2021. Earths Future. https://doi.org/10.1029/2023EF003734

Dargahi A, Hasani K, Mokhtari SA, Vosoughi M, Moradi M, Vaziri Y (2021a) Highly effective degradation of 2,4-dichlorophenoxyacetic acid herbicide in a three-dimensional sono-electro-Fenton (3D/SEF) system using powder activated carbon (PAC)/Fe_3O_4 as magnetic particle electrode. J Environ Chem Eng 9:105889. https://doi.org/10.1016/j.jece.2021.105889

Dargahi A, Ansari A, Nematollahi D, Asgari G, Shokoohi R, Reza Samarghandi M (2019) Parameter optimization and degradation mechanism for electrocatalytic degradation of 2,4-diclorophenoxyacetic acid (2,4-D) herbicide by lead dioxide electrodes. RSC Adv 9:5064–5075. https://doi.org/10.1039/C8RA10105A

Dargahi A, Shokoohi R, Asgari G, Ansari A, Nematollahi D, Reza Samarghandi M (2021) Moving-bed biofilm reactor combined with three-dimensional electrochemical pretreatment (MBBR–3DE) for 2,4-D herbicide treatment: application for real wastewater, improvement of biodegradability. RSC Adv 11:9608–9620. https://doi.org/10.1039/D0RA10821A

Diab MA, Ali ABM, Abdulrahman A, BaQais A, Khan MI, Abduvokhidov A, Karimov M, Mukhitdinov O, Mahariq I (2025) Design and synthesis of a novel Fe_3O_4/ZIF-67/$CuCo_2S_4$ composite for efficient ciprofloxacin and Ni^{2+} removal from wastewater: Characterization and mechanistic insights. Surf Inter 73:107517. https://doi.org/10.1016/j.surfin.2025.107517

Fan J, Zhang X, He N, Song F, Wang X (2025) Investigation on novel deep eutectic solvents with high carbon dioxide adsorption performance. J Environ Chem Eng 13:117870. https://doi.org/10.1016/j.jece.2025.117870

Fang Q, Sun Q, Ge J, Wang H, Qi J (2025) Multidimensional engineering of nanoconfined catalysis: frontiers in carbon-based energy conversion and utilization. Catalysts. https://doi.org/10.3390/catal15050477

A.S. Felsot, Enhanced Biodegradation of Insecticides in Soil: Implications for Agroecosystems, (n.d.) 24.

Gar Alalm M, Tawfik A, Ookawara S (2015) Comparison of solar TiO2 photocatalysis and solar photo-Fenton for treatment of pesticides industry wastewater: operational conditions, kinetics, and costs. J Water Process Eng 8:55–63. https://doi.org/10.1016/j.jwpe.2015.09.007

Gomez-Herrero E, Tobajas M, Polo A, Rodriguez JJ, Mohedano AF (2019) Removal of imidazolium-based ionic liquid by coupling Fenton and biological oxidation. J Hazard Mater 365:289–296. https://doi.org/10.1016/j.jhazmat.2018.10.097

He Z-W, Yang C-X, Tang C-C, Liu W-Z, Zhou A-J, Ren Y-X, Wang A-J (2021) Response of anaerobic digestion of waste activated sludge to residual ferric ions. Bioresour Technol 322:124536. https://doi.org/10.1016/j.biortech.2020.124536

Hodges BC, Cates EL, Kim J-H (2018) Challenges and prospects of advanced oxidation water treatment processes using catalytic nanomaterials. Nat Nanotechnol 13(8):642–650. https://doi.org/10.1038/s41565-018-0216-x

Huang D, Hu C, Zeng G, Cheng M, Xu P, Gong X, Wang R, Xue W (2017) Combination of Fenton processes and biotreatment for wastewater treatment and soil remediation. Sci Total Environ 574:1599–1610. https://doi.org/10.1016/j.scitotenv.2016.08.199

Kazemizad L, Ghaffari Y, Kermani M, Farzadkia M, Hajizadeh A (2016) Investigation of photo–fenton–like process efficiency in diazinon pesticide removal from aqueous solutions. J Saf Environ Health Res 1(1):17–22. https://doi.org/10.22053/jsehr.2016.33383

Kouloumbos VN, Tsipi DF, Hiskia AE, Nikolic D, van Breemen RB (2003) Identification of photocatalytic degradation products of diazinon in TiO2 aqueous suspensions using GC/MS/MS and LC/MS with quadrupole time-of-flight mass spectrometry. J Am Soc Mass Spectrom 14:803–817. https://doi.org/10.1016/S1044-0305(03)00333-7

Latif M, Fatima M, Asif M, Naz I, Naeem T, Rana AS, Atamurotov F, Alshehri NA, Abdujabbarov A, Aziz MH, Shaheen F (2025) Tetracycline (TC-HCl) antibiotic photodegradation using $MgFe_2O_4$/MXene/NiO nanocomposites as potent photocatalysts for environmental remediation. Mat Lett 399:139032. https://doi.org/10.1016/j.matlet.2025.139032

Li W, Liu Y, Duan J, van Leeuwen J, Saint CP (2015) UV and UV/H2O2 treatment of diazinon and its influence on disinfection byproduct formation following chlorination. Chem Eng J 274:39–49. https://doi.org/10.1016/j.cej.2015.03.130

Li B, Wang X, Khurshid A, Saleem SF (2025) Environmental governance, green finance, and mitigation technologies: pathways to carbon neutrality in European industrial economies. Int J Environ Sci Technol. https://doi.org/10.1007/s13762-025-06608-w

Li S, Xie Q, Yang M, Wu N, Lian Y, Fang C (2026) Degradation of leachate and high concentration emerging pollutant tetracycline through electro oxidation. J Environ Sci 159:142–153. https://doi.org/10.1016/j.jes.2025.03.059

Martínez F, Molina R, Rodríguez I, Pariente MI, Segura Y, Melero JA (2018) Techno-economical assessment of coupling Fenton/biological processes for the treatment of a pharmaceutical wastewater. J Environ Chem Eng 6:485–494. https://doi.org/10.1016/j.jece.2017.12.008

Mirsoleimani-azizi SM, Setoodeh P, Samimi F, Shadmehr J, Hamedi N, Rahimpour MR (2018) Diazinon removal from aqueous media by mesoporous MIL-101(Cr) in a continuous fixed-bed system. J Environ Chem Eng 6:4653–4664. https://doi.org/10.1016/j.jece.2018.06.067

Mousset E, Loh WH, Lim WS, Jarry L, Wang Z, Lefebvre O (2021) Cost comparison of advanced oxidation processes for wastewater treatment using accumulated oxygen-equivalent criteria. Water Res 200:117234. https://doi.org/10.1016/j.watres.2021.117234

Moyes RB, Reynolds J, Breakwell DP (2009) Differential Staining of Bacteria: Gram Stain. In: R. Coico, T. Kowalik, J. Quarles, B. Stevenson, R. Taylor (Eds.), Curr. Protoc. Microbiol., John Wiley & Sons, Inc., Hoboken, NJ, USA. https://doi.org/10.1002/9780471729259.mca03cs15.

Nazir A, Rasool AT, Chen C, Mukhitdinov O, Jumanazarov D, Sun D (2026) Novel Z-scheme AgI@$PbBiO_2Br$ heterojunction for efficient photodegradation of organic pollutants and bacteria inactivation: DFT simulation, explore active radicals, and mechanism insight. Materials Science in Semiconductor Processing 202:110128. https://doi.org/10.1016/j.mssp.2025.110128

Nguyen DD, Jeon B-H, Jeung JH, Rene ER, Banu JR, Ravindran B, Vu CM, Ngo HH, Guo W, Chang SW (2019) Thermophilic anaerobic digestion of model organic wastes: evaluation of biomethane production and multiple kinetic models analysis. Bioresour Technol 280:269–276. https://doi.org/10.1016/j.biortech.2019.02.033

Ni ZL, Ma JS, Liu Y, Li BH, Nazarov AA, Li H, Yuan ZP, Ling ZC, Wang XX (2025) Numerical analysis of ultrasonic spot welding of Cu/Cu joints. J Mater Eng Perform 34:20624–20635. https://doi.org/10.1007/s11665-025-10733-5

Ochoa-Herrera V, León G, Banihani Q, Field JA, Sierra-Alvarez R (2011) Toxicity of copper(II) ions to microorganisms in biological wastewater treatment systems. Sci Total Environ 412:380–385. https://doi.org/10.1016/j.scitotenv.2011.09.072

Olalla F, José M (2018) Combination of advanced oxidation processes with biological treatment for the remediation of water polluted with herbicides, Universitat Autònoma de Barcelona, 2007. https://ddd.uab.cat/record/38273

Peck M, Badrick T (2017) A review of contemporary practice and proficiency with gram staining in anatomical pathology laboratories. J Histotechnol 40:54–61. https://doi.org/10.1080/01478885.2017.1327474

Pera-Titus M, Garcia-Molina V, Banos MA, Giménez J, Esplugas S (2004) Degradation of chlorophenols by means of advanced oxidation processes: a general review. Appl. Catal. B Environ. 47:219–256. https://doi.org/10.1016/j.apcatb.2003.09.010

Pirsaheb M, Dargahi A, Hazrati S, Fazlzadehdavil M (2014) Removal of diazinon and 2,4-dichlorophenoxyacetic acid (2,4-D) from aqueous solutions by granular-activated carbon. Desalin Water Treat 52:4350–4355. https://doi.org/10.1080/19443994.2013.801787

Poirier L, Brun L, Jacquet P, Lepolard C, Armstrong N, Torre C, Daudé D, Ghigo E, Chabrière E (2017) Enzymatic degradation of organophosphorus insecticides decreases toxicity in planarians and enhances survival. Sci Rep. https://doi.org/10.1038/s41598-017-15209-8

Pordel MA, Maleki A, Ghanbari R, Rezaee R, Khamforoush M, Daraei H, Athar SD, Shahmoradi B, Safari M , Hossein Ziaee A, Lalhmunsiama, Lee S.-M (2019) Evaluation of the effect of electrospun nanofibrous membrane on removal of diazinon from aqueous solutions, React Funct Polym 139 85–91. https://doi.org/10.1016/j.reactfunctpolym.2019.03.017.

Pourali P, Rashtbari Y, Behzad A, Ahmadfazeli A, Poureshgh Y, Dargahi A (2023) Loading of zinc oxide nanoparticles from green synthesis on the low cost and eco-friendly activated carbon and its application for diazinon removal: isotherm, kinetics and retrieval study. Appl Water Sci 13:101. https://doi.org/10.1007/s13201-023-01871-z

Qi H, Huang Q, Hung Y-C (2018) Effectiveness of electrolyzed oxidizing water treatment in removing pesticide residues and its effect on produce quality. Food Chem 239:561–568. https://doi.org/10.1016/j.foodchem.2017.06.144

Qin X, Cui H, Zhou Q (2023) Physisorption behaviors of organochlorine pesticides on the InP_3 monolayer from theoretical insight. ACS Omega 8:32168–32175. https://doi.org/10.1021/acsomega.3c04665

Samarghandi MR, Nemattollahi D, Asgari G, Shokoohi R, Ansari A, Dargahi A (2019) Electrochemical process for 2,4-D herbicide removal from aqueous solutions using stainless steel 316 and graphite anodes: optimization using response surface methodology. Sep Sci Technol 54(4):478–493. https://doi.org/10.1080/01496395.2018.1512618

Samarghandi MR, Rahmani A, Khazaei M, Dargahi A, Bahiraei A, Shabanloo A (2024) Degradation and mineralization of diazinon pesticide by G/PbO_2 anodic oxidation process. Case Stud Chem Environ Eng 9:100685. https://doi.org/10.1016/j.cscee.2024.100685

Shemer H, Linden K (2006) Degradation and by-product formation of diazinon in water during UV and UV/H2O2 treatment. J Hazard Mater 136:553–559. https://doi.org/10.1016/j.jhazmat.2005.12.028

Sonwani RK, Giri BS, Geed SR, Sharma A, Singh RS, .Rai BN (2018) Combination of UV-Fenton oxidation process with biological technique for treatment of polycyclic aromatic hydrocarbons using Pseudomonas pseudoalcaligenes NRSS3 isolated from petroleum contaminated site, INDIAN J EXP BIOL 10.

Sun Y, Wang M, Liang L, Sun C, Wang X, Wang Z, Zhang Y (2022) Continuously feeding fenton sludge into anaerobic digesters: Iron species change and operating stability. Water Res 226:119283. https://doi.org/10.1016/j.watres.2022.119283

E Team (2019) Gram Staining : Principle, Procedure, Interpretation and Animation, LaboratoryInfo.Com. https://laboratoryinfo.com/gram-staining-principle-procedure-interpretation-and-animation/ (accessed May 25, 2019).

Wang C, Shih Y (2015) Degradation and detoxification of diazinon by sono-Fenton and sono-Fenton-like processes. Sep Purif Technol 140:6–12. https://doi.org/10.1016/j.seppur.2014.11.005

Wang C-K, Shih Y-H (2016) Facilitated ultrasonic irradiation in the degradation of diazinon insecticide. Sustain Environ Res 26:110–116. https://doi.org/10.1016/j.serj.2016.04.003

Wang N, Zheng T, Zhang G, Wang P (2016) A review on fenton-like processes for organic wastewater treatment. J Environ Chem Eng 4:762–787. https://doi.org/10.1016/j.jece.2015.12.016

Wang X, Gong Y, Sun C, Wang Z, Sun Y, Yu Q, Zhang Y (2024) New insights into inhibition of high Fe(III) content on anaerobic digestion of waste-activated sludge. Sci Total Environ 916:170147. https://doi.org/10.1016/j.scitotenv.2024.170147

Wei J, Fan P, Huang Y, Zeng H, Jiang R, Wu Z, Zhang Y, Hu Z (2024) (±)-Hypandrone A, a pair of polycyclic polyprenylated acylphloroglucinol enantiomers with a caged 7/6/5/6/6 pentacyclic skeleton from *Hypericum androsaemum*. Org Chem Front 11:3459–3464. https://doi.org/10.1039/D4QO00444B

Yan H, Yang B, Zhou X, Qiu X, Zhu D, Wu H, Li M, Long Q, Xia Y, Chen J, Li Y, Qiu T (2022) Adsorption mechanism of hydrated $Lu(OH)^{2+}$ and $Al(OH)^{2+}$ ions on the surface of kaolinite. Powder Technol 407:117611. https://doi.org/10.1016/j.powtec.2022.117611

Zhang Y, Zhang W, Liao X, Zhang J, Hou Y, Xiao Z, Chen F, Hu X (2010) Degradation of diazinon in apple juice by ultrasonic treatment. Ultrason Sonochem 17:662–668. https://doi.org/10.1016/j.ultsonch.2009.11.007

Zhang Y, Hou Y, Chen F, Xiao Z, Zhang J, Hu X (2011) The degradation of chlorpyrifos and diazinon in aqueous solution by ultrasonic irradiation: effect of parameters and degradation pathway. Chemosphere 82:1109–1115. https://doi.org/10.1016/j.chemosphere.2010.11.081

Zhang L, Su F, Wang N, Liu S, Yang M, Wang Y-Z, Huo D, Zhao T (2019) Biodegradability enhancement of hydrolyzed polyacrylamide wastewater by a combined Fenton-SBR treatment process. Bioresour Technol 278:99–107. https://doi.org/10.1016/j.biortech.2019.01.074

Zhang T, Zheng Y, Chen T, Gu Y, Gong Y, Wang D, Li Z, Du Y, Zhang L, Gao J (2025) Biomaterials mediated 3R (remove-remodel-repair) strategy: holistic management of *Helicobacter pylori* infection. J Nanobiotechnol 23:475. https://doi.org/10.1186/s12951-025-03455-2

Zhou H, Guo J, Zhu G, Xu H, Tang X, Luo X (2024) Flotation behavior and mechanism of smithsonite under the system of bidentate ligand sulfide sodium thiocyanate. Sep Purif Technol 334:126086. https://doi.org/10.1016/j.seppur.2023.126086

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