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The influence of N-fertilizers on the biodegradation of N-heterocyclic pesticides by acclimated microbial communities in HMPBR

La influencia de los fertilizantes nitrogenados en la biodegradación de plaguicidas N-heterocíclicos por comunidades microbianas aclimatadas en HMPBR

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Abstract

The persistence of N-heterocyclic pesticides such as triazines, pyridines, pyrimidines, benzimidazoles, triazoles, and neonicotinoids, poses a significant threat to soil health, a problem often exacerbated by nitrogenated fertilizers that inhibit biodegradation by native microbiota. This study investigates the potential of acclimated microbial communities (MCs) to circumvent this inhibition and enhance soil bioremediation.

The research used two prototypes of horizontal multistage packed-bed biofilm reactors (HMPBRs) functioning as permeable reactive biobarriers. These reactors consist of four equalizer-oxygenator compartments (EOCs) designed to supply increasing loads of N-fertilizers while ensuring adequate oxygenation. Through convective transport, dissolved oxygen is delivered to the packed zones, maintaining a microaerophilic environment that sustains the microbial biofilm.

The experiments, conducted using gradient D-Stat cultures, utilized MCs specifically acclimated to atrazine and diazinon. Contrary to existing literature suggesting that alternative nitrogen sources hinder pesticide degradation, the results demonstrated that acclimated MCs maintain removal rates and efficiencies near 100%, even under elevated nitrogen loading rates. This study concludes that acclimated microbial communities are robust and suitable tools for the effective remediation of soil and water contaminated with N-heterocyclic pesticides, providing a viable solution for environments where synthetic fertilizers are extensively applied.

Keywords: Biofilm reactor, diazinon, atrazine, biodegradation, microbial community.

Resumen

La persistencia de plaguicidas N-heterocíclicos heterocíclicos tales como triazinas, piridinas, pirimidinas, benzimidazoles, triazoles y neonicotinoides impacta negativamente la salud del suelo, agravada por el hecho de que los fertilizantes nitrogenados suelen inhibir su biodegradación por parte de la microbiota autóctona. Este estudio investiga cómo el uso de comunidades microbianas (CM) aclimatadas puede eludir dicho efecto inhibitorio para mejorar la biorremediación de suelos.

La investigación empleó dos prototipos de reactores de biopelícula de lecho empacado multietapa horizontal (HMPBR), que actúan como biobarreras reactivas permeables. Estos reactores constan de cuatro compartimentos ecualizadores-oxigenadores (EOC) diseñados para suministrar cargas crecientes de fertilizantes nitrogenados. El oxígeno disuelto se transporta convectivamente hacia las zonas empacadas, manteniendo un entorno microaerofílico que favorece el crecimiento de la biopelícula.

Mediante cultivos D-Stat en gradiente, se evaluaron CM aclimatadas a atrazina y diazinón. Los resultados revelaron que, a diferencia de los informes previos sobre los efectos adversos de fuentes alternativas de nitrógeno, las CM aclimatadas mantienen altas tasas y eficiencias de remoción incluso bajo cargas elevadas de fertilizantes. Esta característica posiciona a las comunidades microbianas aclimatadas como herramientas idóneas para la remediación de suelos y aguas contaminadas, demostrando su eficacia en escenarios agrícolas de alta fertilización.

Palabras clave: Reactor de biopelícula, diazinon, atrazina, biodegradación, comunidad microbiana.

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1 Introduction

Soil microbiota is crucial to maintaining soil health, but agricultural practices have been shown to dramatically alter soil microorganisms (Jeyaseelan *et al*., 2024). Among agrarian practices, the use of pesticides is common. Nevertheless, pesticide exposure negatively influences the activity, abundance, and interactions of soil microbial species or groups (Sebiomo *et al*., 2011; Mukherjee, 2022; Wang *et al*., 2024). Although pesticide levels in soils eventually decay due to abiotic or biotic effects, their long persistence and concentration affect the population dynamics of soil microbiota, mainly due to the toxicity of some pesticides (Prashar & Shah, 2016); in addition, some heavy metals act synergistically with pesticides, enhancing their toxic effects on earthworms which are critical determinants in soil fertility (Bhardwaj *et al*., 2021). Thus, soil detoxification by microbial degradation of pollutants is vital to ensure soil health.

1.1 Effect of fertilizers on soil microbiota

In addition to their environmental effects, fertilizers and pesticides influence soil microbial properties. The combination of pesticides with fertilizers or heavy metals has a greater adverse impact on soil microorganisms than using a pesticide alone (Mohammadi Aria *et al*., 2024). In the case of microbial biodegradation of nitrogenous pesticides, organic or inorganic fertilizers such as urea, ammonium sulfate, ammonium nitrate, and manure could act as competitive nitrogen sources, retarding the N-pesticide biodegradation (Guo *et al*., 2024). The biodegradation of N-heterocyclic pesticides such as triazines, pyridines, pyrimidines, benzimidazoles, triazoles, and neonicotinoids, adsorbed on soil particles that are carried to surface water bodies by runoff or leaching from agricultural soils can be affected by the presence of ammonium, nitrate, and ureic fertilizers, which are the more bioavailable forms of nitrogen for microbial growth (Caracciolo *et al*., 2005; Zablotowicz *et al*., 2008; Kuypers *et al*., 2018;). Presumptively, by nitrogen-mediated repression, the bacterium *Pseudomonas* sp. shows a significant decrease in atrazine degradation rate when cultivated on nitrogen sources that support rapid growth (García-González *et al*., 2003). After application, pesticides also undergo numerous biochemical and physicochemical transformations, forming other metabolites that can be even more toxic to soil microbes (Mukherjee *et al*., 2022).

1.2 Environmental factors affecting diazinon biodegradation

Many microorganisms use organophosphorus compounds as carbon and nitrogen sources (Verma *et al*., 2014; Kumar *et al*., 2018). Organophosphorus (OP) pesticides are classified into distinct chemical subclasses based on the atoms attached to the central phosphorus core. Modifying these atoms changes the stability and toxicity of the OP molecule. By chemical group, the main OP pesticides are: Phosphates (P=O bond), Phosphorothioates (P=S bond), Phosphorodithioates (P=S bond + C-S-P bond), Phosphoramidates and Phosphoramidothioates (P-N bond). Diazinon pertains to the group of Phosphorothioates. It is a highly potent, broad-spectrum organophosphorus insecticide. While it remains chemically effective at pest eradication, its heavy environmental toll and toxicity profile have triggered sweeping global restrictions. Their biodegradation in nature depends on many factors; in agricultural soils, diazinon degradation is affected by pH, temperature, moisture, soil type, tillage, pesticide concentration, organic amendments (Schoen & Winterlin, 1987; Aggarwal *et al*., 2013; El-Ghany & Masmali, 2016), and metals such Zn^{2+}, Cd^{2+}, V^{3+}, and Mn^{2+} (Mohammadi-Aria *et al*., 2024).

Some authors have found that soil amendment with organic residues affects the dissipation of pesticides differently; for example, the removal of linuron is increased, while the levels of others, like diazinon, decrease in amended soil (Marín-Benito *et al*., 2014). Other authors studying the effects of tillage on microbial activity and diazinon degradation in the topsoil layers observed high diazinon mineralization rates. They attributed this to the higher microbial populations and activity found in non-tillage soils (Levanon *et al*., 1994). Although several studies on the impact of organic amendments, which can serve as nitrogen sources, on diazinon biodegradation in soils exist in the literature, the effect of inorganic nitrogen compounds is scarce or nonexistent.

1.3 Oxygen Levels Affecting Atrazine and Diazinon Biodegradation

Although atrazine and diazinon can be degraded in oxygen-limited environments, aerobic conditions generally provide higher biodegradation rates and efficiencies than anaerobic or anoxic conditions. Atrazine undergoes rapid microbial mineralization or hydrolytic dechlorination when oxygen is available. In oxygen-deprived environments, the absence of aerobic bacterial pathways (such as those utilizing the *atzA* or *trzN* genes) leaves only slow abiotic chemical reductions or highly specific, inefficient anaerobic pathways (Akinyi *et al*., 2026).

The half-life in aerobic conditions is about 49

days on average in typical non-sterile soils. Controlled studies in non-sterile anaerobic soils show an average half-life of 407 days. In Subsurface/Aquifer Deposits: Aerobic half-lives can be as fast as 38 days. In Subsurface/Boreal Aquifer Studies**:** Deep anaerobic conditions stretch the atrazine half-life out to 430 to 829 days (Talja *et al*., 2008).

Upflow Fixed Bed Bioreactors operating in sequential aerobic cycles regularly reach 75.2% to 81.6% atrazine removal. In comparison, standard anaerobic treatments (like Upflow Anaerobic Sludge Blanket, or UASB reactors) only achieve a stagnant 40% to 50% removal efficiency (Kaman-Malek *et al*., 2024).

The microbial degradation pathways are often more efficient and complete in the presence of oxygen (Abbasi *et al*., 2025). Under aerobic conditions, specialized bacteria convert atrazine to hydroxy atrazine, which is subsequently transformed through a series of steps into cyanuric acid (Solomon *et al*., 2013).

The efficiency of aerobic degradation is enhanced by a variety of enzymes involved in the biodegradation of diazinon, including hydrolases, acid phosphatases, laccases, cytochrome P450, and flavin monooxygenases (Wu *et al*., 2021). In summary, although both atrazine and diazinon can be degraded in the absence of oxygen, the metabolic pathways, efficiency, and rates of complete biodegradation are generally superior under aerobic conditions. For these reasons, the biofilm reactor was designed to assure aerobic, or at least microaerophilic conditions for the acclimated microbial communities.

1.4 Effect of competitive nitrogen compounds on atrazine biodegradation

Atrazine is primarily used as a nitrogen source by degrading strains, and the effect of competitive nitrogen sources on atrazine biodegradation has been observed in soil microbiota. Generally, nitrogen amendments reduce the removal rates of atrazine by soil microbial populations and pure bacterial cultures (García-González *et al*., 2003). Soil nitrogen amendments could be organic and inorganic. Organic options like compost, worm castings, or alfalfa pellets build long-term soil health and release nutrients slowly. Fast-acting inorganic options like urea or ammonium sulfate deliver immediate nitrogen to correct severe deficiencies but require precise application. However, Entry *et al*. (1993), in their study on the influence of nitrogen on atrazine mineralization in grassland soils, report that low nitrogen additions reduce atrazine removal, whereas higher additions increase it. An explanation for this effect is that, although an inorganic nitrogen source affects atrazine biodegradation, its impact is not related to the amount of nitrogen added to soils. This suggests that supplying more nitrogen to crop fields increases microbiota, which could theoretically mineralize atrazine. Other studies with microorganisms that degrade atrazine as the sole nitrogen source have shown marked differences in their responses to a competitive nitrogen source (Bichat *et al*., 1999).

1.5 Advantages of acclimatized native microbial communities over genetically engineered microorganisms for soil bioremediation

Genetically modified microorganisms capable of degrading hazardous compounds have been proposed for soil bioremediation, although concerns have arisen about their use. In recent decades, several questions have been raised concerning the advantages and disadvantages of genetically modified organisms (GMOs) and the possible ecological damage they may cause to native biodiversity (Rebello *et al*., 2021). Instead of using GMOs, acclimated microbial communities that are resistant to pesticides and can degrade them, even in the presence of fertilizers, could be beneficial for removing them from soil or water through bioremediation. This study suggests a novel approach to mitigating the adverse effects of nitrogen-based fertilizers on the biodegradation of nitrogenous pesticides in soils.

For these reasons, and because of their widespread use in agriculture and their significance as harmful agents for aquatic biota, soil microbiota, and human health, two significant N-heterocyclic pesticides were studied: atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) and diazinon (O,O-diethyl-O-[6-methyl-2-(1-methyl-ethyl)-4-pyrimidine] thiophosphate). The impact of the N-fertilizers urea, ammonium sulfate, and ammonium nitrate on their biodegradation rates was evaluated using specialized microbial communities acclimatized to diazinon and atrazine.

2 Materials and methods

2.1 Horizontal multistage packed-bed biofilm reactor

The present study assesses the impact of increasing fertilizer concentrations on the pesticide-degradative ability of immobilized microbiota in environmentally distinct microcosms within a horizontal multistage packed-bed biofilm reactor (HMPBR). The preservation of an aerobic environment in a fixed-bed biofilm reactor depends on how oxygen is supplied.

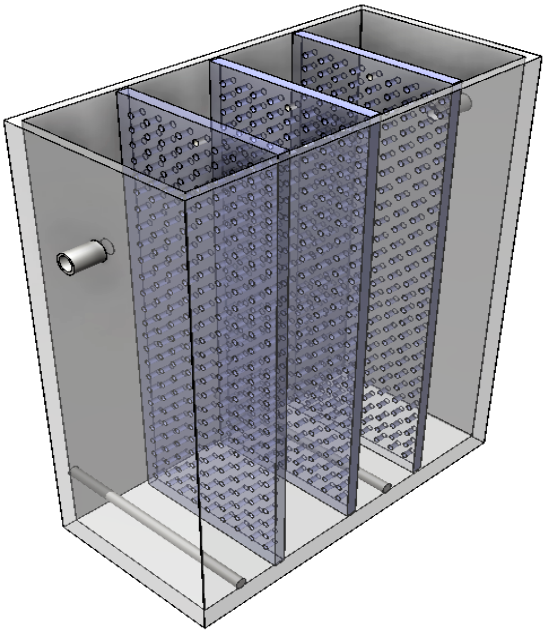

Fig. 1 Detail of HMPBR segment showing multiperforated plates separating equalizers from packed bed zones.

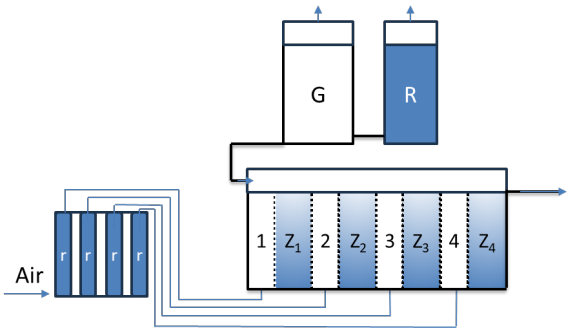

Fig. 2 HMPBR system setup showing the device generating a crescent gradient of N-fertilizer loading rate. The concentrated compound in the tank reservoir [R]. Gradient forming tank [G]. Equalizer-oxygenator compartments [1 to 4]. Packed bed zones separated by multiperforated plates, [Z_1 to Z_4]. Airflow rotameters [r].

Typical methods to provide oxygen in vertical fixed-bed bioreactors include sparging aerated liquid or injecting air into the fixed-bed reactor (Liu *et al.*, 2023). However, this study employed a different method to maintain an aerobic environment in a horizontal fixed-bed reactor. The HMPBR prototypes have gas-liquid equalizer-oxygenator compartments (EOCs) and packed-biphasic zones (solid-liquid). The reactor was designed for diffusive O_2 transfer in the EOCs and for convective oxygen transfer to the packed zones that host an attached microbial community (González-Cuna *et al.*, 2016). A scheme of an empty HMPBR section (**Fig. 1**) shows multi-perforated walls separating packed zones (Z_1 and Z_2) from aerated equalizers (EOC_1 and EOC_2). EOCs work as airlift pumps, delivering oxygenated liquid to the packed beds (Gómez-De Jesús *et al.*, 2009). The oxygen-exhausted liquid flowing from the packed zones is reaerated in the equalizers. Then, via the airlift effect, the reoxygenated liquid is recirculated axially and transversely to the packed zones. This design promotes convective oxygen transport to the attached microbial communities that degrade atrazine or diazinon.

2.2 *System setup and operation*

Two identical HMBPRs, A and B, were constructed to evaluate the effect of the fertilizer loading rate supplied to the bioreactor and the rate and efficiency of pesticide biodegradation. Each reactor has 5 equalizers, the first with a liquid volume of 0.135 L and the remaining four with 2.49 L each. The liquid contained within the four packed zones totaled 1.37 L, representing a total volume of 2.5 L. This volume served as the basis for calculating the volumetric feed rate (B_V) and removal rate (R_V) for each pesticide throughout the study. Once the oxygen transfer capacity was characterized, an air flow rate of 2.0 VVM was selected for each equalizer.

Reactors were filled with MSM, using atrazine (20 mg L^{-1}) or diazinon (30 mg L^{-1}). Then, they were respectively inoculated with the acclimated communities MCATZ and MCDZN, by placing colonized carrier materials in different compartments Z_1 to Z_4.

After batch cultivation for about two weeks, to promote the formation of microbial biofilm in the packed-bed zones, Reactor A had an attached microbial community (MCATZ) acclimated to atrazine, while Reactor B had an MCDZN acclimated to diazinon. Once that continuous operation was underway, the cascade of the HMPBR compartments represented four environmentally distinct zones, Z_1 to Z_4.

The environment of a zone depends on the liquid composition flowing from the previous one, which changes due to the biokinetic behavior of the microbial community. The system was designed to operate dynamically as a cascade of biofilm bioreactors, with back-mixing driven by transverse liquid recirculation across zones interconnected by multiperforated plates. The backward movement is accompanied by a corresponding forward movement, as occurs in typical tubular bioreactors.

The temporal behavior of the crescent fertilizer loading rates during operation of the HMPBRs was simulated and experimentally verified using the device shown in Fig. 2, which generates crescent reactant loading rate gradients while maintaining a constant volumetric flow rate (D-Stat system).

The dynamics of pesticide biodegradation was evaluated as a function of fertilizer concentration

at the HMPBRs, which held immobilized microbial communities able to degrade atrazine or diazinon.

2.3 Microorganisms

The source of the microbial communities (MCs) was activated sludge from the wastewater treatment plant "Cerro de la Estrella" in Mexico City, Mexico. The MCs were acclimatized through repeated batch cultivation on minimal salt media (MSM), using atrazine (20 mg L^{-1}) or diazinon (30 mg L^{-1}) as the carbon and nitrogen sources.

During the acclimatization period, porous material fragments were added to the flasks to promote the growth of biofilm-forming microorganisms. During the whole study, fragments of volcanic stone, known as tezontle, were used as a packed-bed biofilm support. The non-uniform rough particles of tezontle have a porous microstructure, and their fragment size was 4.8 ± 2.7 mm.

The concentrations of atrazine and diazinon were determined at the end of each batch, after replacing a portion of the liquid. The microbial community was considered acclimatized once removal efficiencies were above 90% and stable volumetric removal rates were achieved. The process took about 8 weeks to enrich the communities MC_{ATZ} and MC_{DZN} with microbial populations capable of efficiently degrading atrazine and diazinon, respectively.

2.4 Minimal Salt Media (MSM)

Two minimal salt media (MSM) compositions were used to reactivate the microbial communities that use atrazine or diazinon as the sole carbon and nitrogen source. The commercial pesticide was added separately, with each experiment using a specific active ingredient concentration.

The composition of the minimal saline medium used for microbial community reactivation on atrazine was, in g L^{-1}, K_2HPO_4, 1.60; KH_2PO_4, 0.40; $MgSO_4{\cdot}7H_2O$, 0.10; NaCl, 0.10; $CaCl_2$, 0.02, and for reactivation on diazinon was, in g $L^{-1,}$ K_2HPO_4, 0.40; KH_2PO_4, 0.10; $MgSO_4{\cdot}7H_2O$, 0.10; $FeCl_3$, 0.02; $MnSO_4$, 0.02; $ZnSO_4$, 0.02.

Standard Count Agar from Merck, USA, was used to quantify colony-forming units and morphologically differentiate the most abundant cultivable microorganisms.

2.5 Chemicals

2.5.1 Commercial and standard pesticides

The atrazine used in this work was the commercial formulation Gesaprim (Syngenta SA de CV, Mexico), containing 480 g L^{-1} of the active ingredient. Diazinon corresponds to the commercial formulation HORTA 25 (Agroquímica Tridente S.A. de CV, Mexico), which has 230 g L^{-1} of the active ingredient. Atrazine (99.0%) and diazinon (98.9%) standards were from ChemService, PA, USA.

2.5.2 Fertilizers

Urea of 98% purity (Sigma-Aldrich, Mexico), $(NH_4)_2SO_4$, and NH_4NO_3, technical grade (Merck, Mexico), were used.

2.6 Analytical methods

2.6.1 Dissolved oxygen monitoring in the HMPBR

During experiments, the dissolved oxygen concentration was regularly determined in the HMPBR compartments using a calibrated DO system (YSI-55, YSI, USA).

2.6.2 Determination of cultivable cells attached to the support material

The cultivable cell concentration was determined from support material fragments sampled from the HMPBR. Weighted fragments were collocated into Falcon tubes having a measured volume of sterile water. Tubes were shaken in a Vortex agitator to detach the adhered biofilm. The cell suspension was drained, and the porous fragments were rinsed three times with sterile water. The liquid collected was measured and recorded. Decimal solutions of the cell suspension were used for cell counting in Standard Count Agar plates. The dry weight of the rock fragments was determined, and the cell concentration was reported as CFU per gram of support material.

2.6.3 Determination of free cultivable cells

While sampling support material fragments, liquid samples were simultaneously taken from the HMPBR compartments. Decimal solutions of the liquid samples were used for cell counting in Standard Count Agar plates.

2.6.4 Spectrophotometric method for regular determination of atrazine and diazinon

The absorption spectrum and the absorption maxima at 221 nm for atrazine and 247 nm for diazinon were obtained using a Beckman DU 650 spectrophotometer.

2.6.5 HPLC determination of atrazine, cyanuric acid, and diazinon

Atrazine, cyanuric acid, and diazinon were determined using a Shimadzu chromatograph model SPD-10-A with an Agilent Zorbax SB-C-18 column. An isocratic mobile phase of 60% acetonitrile in water at a flow

Table 1. Forward and reverse primers were used to amplify the atrazine-degrading genes.

Primer	Primer sequence	Reference
trzN_f	CACCAGCACCTGTACGAAGG	Mulbry *et al*. (2002)
trzN_r	GATTCGAACCATTCCAAACG	Mulbry *et al*. (2002)
atzA_f	ATGGCGGTCTATGGTGAGGTG	*
atzA_r	ATCGCATTCCTTCAACTGTCA	*
atzB_f	TGGCGTCGATATTCTGGTGAGA	*
atzB_r	AGCAGGTTAGCGGCATCAAACT	*
atzC_f	AGCAGTGGAAGCAGTTTTAGA	*
atzC_r	ACACCAGGCATGACTCGTAGT	*
atzD_f	AATGGCTGCGTCAACGATTACA	*
atzD_r	AGGCGATCTTTGCTGGTGTCA	*

*This work

rate of 1.0 mL min^{-1} was used to determine atrazine and cyanuric acid. For diazinon, an aqueous solution of water and acetonitrile (45:55) at a flow rate of 1.0 mL min-1 was used as the mobile phase. Detection was performed at 221 nm and 247 nm for atrazine and diazinon, respectively. Cyanuric acid was evaluated at 213 nm.

2.6.6 *Detection of trzN and atz genes (A, B, C, D)*

To complement the evaluation of the impact of an added nitrogen source on the functional behavior of the microbial community that uses atrazine as a carbon and nitrogen source, the detection of the *trzN* and *atz* genes (A, B, C, D) coding for the synthesis of enzymes involved in atrazine biodegradation was carried out.

In addition to the kinetic evaluation of the culture, including the formation of atrazine degradation by-products, the genes encoding atrazine degradation dynamics were identified in the microbial community under different operating conditions of the HMPBRs.

For their detection, primers for amplification of the *trzN* and *atz* genes (A, B, C, D) present in DNA extracted from the microbial community were designed and evaluated. The sequences of the Forward (5' 3') and Reverse (3' 5') primers are presented in **Table 1.**

The Invitrogen Genomic DNA Mini Kit (Thermo Fisher Scientific, USA) was used to extract DNA from microbial communities according to the manufacturer's protocols. Gel electrophoresis substantiated the quality of the extracted DNA.

Amplification of the DNA fragments corresponding to each gene was performed by PCR using the Promega PCR kit reagent set. The reaction mixture was as follows: five μL of Go Taq Flexi buffer, 2.5 μL of 25 mM $MgCl_2$, 0.5 μL of 10 mM nucleotide mix, 0.125 μL GoTaq® DNA Polymerase (U μl^{-1}), two μL sample DNA, and 0.5 μL of each *atz* Forward and Reverse primers. The mixture was brought to a final volume of 25 μL with nuclease-free water. A Heal Force-GeneMate Series K960 thermal cycler was used with the following program: initial denaturation at 94 °C for 3 minutes, followed by 30 cycles of sample denaturation (94 °C for 30 seconds), primer annealing (58 °C for 1 minute), and DNA extension (72 °C for 2 minutes). PCR products were visualized on the ENDURO GDS II gel documenter from Labnet International after separation by agarose gel electrophoresis (1 %) with HydraGreen indicator from HydraGene and TAE 1X buffer.

2.6.7 *Estimation of the increase in the loading rate of a compound to an HMPBR holding a microbial community able to degrade atrazine or diazinon*

Equation [1] (Salazar-Huerta *et al*., 2019) was used to simulate the transient behavior of a nitrogenated fertilizer, Ng, in the concentration gradient generator of communicating vessels.

$$N_g = N_r + (N_{Go} - N_r)\, V_{Go}^{-\frac{Dr^2}{Dg^2}} \left(-Ft(1-\varphi_r) + V_{Go}\right)^{\frac{Dr^2}{Dg^2}} \quad (1)$$

The term ϕ_r defines the relative effect of tank diameters G and R on the transient profile of $N_g[t]$.

$$\varphi_r = \frac{{D_r}^2}{{D_g}^2 + {D_r}^2} \quad (2)$$

The flow F of liquid containing the nitrogen compound $N_g[t]$, deriving from the gradient generator system, was fed to the first equalizer-oxygenator of the HMPBR, where the concentration of the compound transiently varies $N_{eq}[t]$. This flow will feed the microbial community immobilized in the reactor's packed zones.

To estimate the transient behavior $N_{eq}[t]$, the balance equation [3], which defines the rate of change in the concentration of the nitrogenous material in the equalizer (Salazar-Huerta *et al*., 2019), was numerically solved using Mathematica 14.0 (Wolfram Research USA).

$$\left(\frac{dN_{eq}}{dt}\right)_{ac} = \frac{F\left(N_r + (N_{Go} - N_r)\,V_{Go}^{-\frac{Dr^2}{Dg^2}}\,(-Ft(1-\varphi r) + V_{Go})^{\frac{Dr^2}{Dg^2}} - N_{eq}\right)}{V_e} - q_N x \quad (3)$$

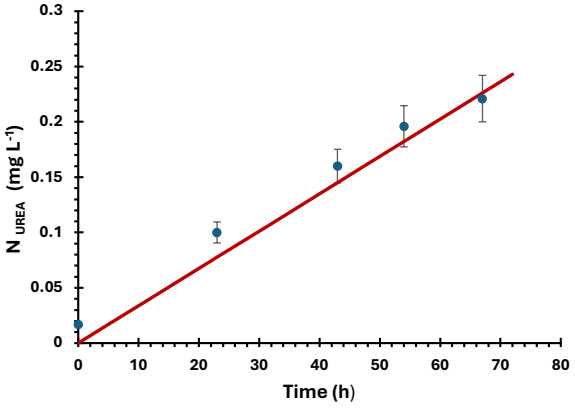

Fig. 3 Example of the theoretical and experimental profile of the transient variation of the total nitrogen concentration of urea as N-fertilizer in the first equalizer-oxygenator using the D-Stat system. The red line is obtained after numerically solving equation [5], and the blue dots are the experimental values obtained.

These equations were used to select the experimental system's most proper operating conditions N_r, N_{go}, V_{go}, and F. By simulation, the variation profiles of the nitrogen compounds, urea (NH_2CONH_2), $(NH_4)_2SO_4$, or NH_4NO_3, supplied to the HMPBRs, the variation in the overall volumetric rate of nitrogen compound supply $B_{V,N}[t] = F(N_{eq})/\Sigma V_L$, and the duration of each experiment were defined.

Fig. 3 shows an example of the theoretical and experimental profile of the transient variation of the total nitrogen concentration of urea as N-fertilizer in the equalizer-oxygenator using the D-Stat system. The red line is obtained after numerically solving equation [3] when the term $qN\ x$ is not significant, meaning there is no appreciable N-fertilizer consumption by suspended cells contaminating the EOC by detached cells from the packed bed zone. The dots represent the experimental values obtained in the EOC during urea gradient formation.

3 Results

3.1 Acclimatization of the microbial communities

The MCs were acclimatized through successive changes in the minimal salt medium (MSM) with varying concentrations of atrazine and diazinon as the sole carbon and nitrogen sources to select microorganisms capable of consuming these compounds. Every 24 h, the concentration of atrazine and diazinon was evaluated until stable volumetric removal rates were achieved. After twelve successive MSM changes, the removal efficiencies of acclimated communities were 93.6% and 94.7% for atrazine and diazinon, respectively. The final acclimatization occurred once microbial communities MC_{DZN} and MC_{ATZ} were implanted in the HMPBRs.

3.2 Characterization of the horizontal multistage packed-bed biofilm reactor

3.2.1 Determination of oxygen transfer coefficient in equalizer-oxygenator compartments

In aerobic systems, the oxygen transfer coefficient k_La affects the rate at which oxygen is incorporated into the liquid medium. Oxygen can be a limiting factor for microorganism growth, so its transport rate in the equalizers is essential. For the characterization of aerobic bioreactors, the oxygen transfer coefficient was evaluated using the gas-displacement method (Bandyopadhyay *et al.*, 1967) at different air supply rates in equalizers (**Table 2**).

Oxygen reaches the attached biomass in the packed zones through the convective transport of aerated liquid flowing from equalizers. The dissolved O_2 (DO) is then transported by the liquid currents created by the airlift effect between the equalizer (acting as the riser) and the packed-bed zones (acting as the downcomer). The O_2-exhausted liquid returning at any height from the packed zones is reaerated into equalizers, effectively closing the airlift loop (González-Cuna *et al.*, 2016).

3.2.2 The dependence of dissolved oxygen (c_L) on liquid flow rate (F) and oxygen transfer rate (k_La) in HMPBR equalizers in continuous operation

Because the microbial communities used in this study were isolated and acclimated in oxic conditions, the behavior of diffusive and convective O_2 transfer rates was analyzed to ensure the oxic environment in the HMPBR reactors was maintained. The predicted behavior of dissolved oxygen (DO) in response to a range of k_La values and liquid flow rate (F) in an equalizer-oxygenator compartment, along with the DO rate of convective transport to the packed bed zones ($B_{V,DO}$), enabled the selection of adequate HMPBR operating conditions.

Table 2 Oxygen transfer coefficients in HMPBR equalizers at different air flow rates.

EOC	V_e (L)	Q_{air} = 1.0 (L min^{-1}) k_La (h^{-1})	Q_{air} = 1.5 (L min^{-1}) k_La (h^{-1})	Q_{air} = 2.0 (L min^{-1}) k_La (h^{-1})
1	0.135	14.06±0.89	20.81±0.42	30.28±0.27
2 to 4	0.294	11.94±2.38	18.77±1.49	27.97±1.50

The study by Ambriz-Mexicano *et al*. (2022), which considers two factors for oxygen transport in equalizers, the liquid flow rate (F) and the O_2 transfer coefficient (k_La), served as the basis for estimating the variation in dissolved O_2 concentration (c_L). Since suspended cells were detected in the EOC, their impact on c_L levels was also considered in the oxygen balance equation.

The oxygen accumulation profile is obtained from the balance of EOCs of volume V_e, considering three factors: (*i*) diffusive transport $(dc_L/dt)_{dif} = k_La(c_s\text{-}c_L)$, (*ii*) convective transport $(dc_L/\mathrm{dt})_{\mathrm{conv}} = F(c_{Li}\text{-}c_L)$, caused by the liquid flow entering to equalizer, with a concentration c_{Li} of dissolved oxygen, and leaving it with a concentration c_L, and *iii*) the oxygen consumed at a specific rate (q_{O2}) if a portion of free cells (x), detached from the adjacent packed bed zones, contaminate equalizers $(dc_L/dt)_{cons} = q_{O2}x$.

$$\left(\frac{dc_L}{d_{\theta RH}}\right)_{ac} = k_La(c_s - c_L) - \frac{F(c_{Li} - c_L)}{V_e} - q_{O2}x \quad (4)$$

$$c_L = \frac{-\theta RH\left(k_La + \frac{F}{V_e}\right)(-c_{Li}\,F + c_{Lo}\,F + c_{Lo}k_La\,V_e - c_s\,k_La\,V_e + q_{O2}x\,V_e + \theta RH(k_La + \frac{F}{V_e})(c_{Li}\,F + c_s\,k_La\,V_e - q_{O2}x\,V_e))}{F + k_La\,V_e} \quad (5)$$

The oxygen balance equation [6] is solved to obtain the function $c_L = f(k_La, F, q_{O2}x)$, which enables the estimation of the EOC oxygen concentration c_L (equation [7]). The term (θ_{RH}) in equations [4] and [5] stands for the hydraulic retention time in the equalizer $\theta_{RH} = V_e/F$.

As mentioned, the dissolved oxygen concentration depends on the oxygen transfer coefficient k_La and the liquid residence time in the equalizer (θ_{RH}). The predicted values in the equalizers at k_La values ranging from 10 to 35 h^{-1} and at RH values ranging from 0.9 to 5.88 hours are sufficient to maintain predicted DO levels up to 5.2 mg L^{-1} across the different equalizers. The convective DO transport rate from EOCs ($B_{V,DO} = F\ c_L/V_L = c_L/\theta_{RH}$) to the reactor's packed zones. In the HMPBR, the experimental DO levels were above 5.0 mg L^{-1} in the EOCs and 3.5 mg L^{-1} in the packed zones, indicating an oxic environment in the bioreactor.

3.3 *Use of atrazine and diazinon by the microbial communities*

MC_{ATZ} and MC_{DZN} *in continuously operated HMPBRs*

In the first stage of the work, porous rock was used as support material to favor biofilm growth within the system, and air was supplied at a flow rate of 1.0 L min^{-1} to maintain dissolved O_2 levels in the equalizer above 3.5 mg O_2 L^{-1}. The packed zones were inoculated with the previously acclimated microbial communities. To enable microbial colonization, the reactors were maintained in batch culture for seven days until the pesticide concentration decreased. Continuous operation was then initiated, maintaining a constant flow rate until the complete removal of atrazine and diazinon was achieved. This condition indicates that communities can use these pesticides

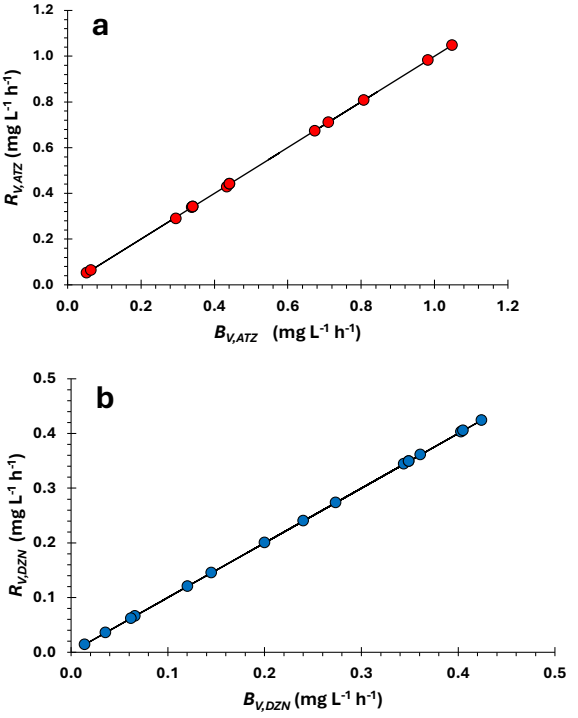

Fig. 4. Removal capability of microbial communities MC_{ATZ} and MC_{DZN} to use atrazine (**a**) or diazinon (**b**) as carbon and nitrogen sources at different loading rates. The continuous line stands for 100% atrazine removal efficiency.

Table 3. Average cell counting of cultivable cells grown in the HMPBRs supplied with different fertilizers and diazinon (MC_{DZN}) or atrazine (MC_{ATZ}) after D-Stat experiments.

Fertilizer	MC_{DZN} (CFU $\times 10^{10}$/Reactor)	MC_{ATZ} (CFU $\times 10^{10}$/Reactor)
Urea	2.502 ± 0.296	8.186 ± 0.876
$(NH_4)_2SO_4$	2.428 ± 0.273	1.444 ± 0.147
NH_4NO_3	0.477 ± 0.048	0.6058 ± 0.071

as sources of carbon and nitrogen. The flow rate and pesticide concentration were varied in the medium fed to the reactors; thus, they were operated at different volumetric loading rates of diazinon or atrazine ($B_V=FS_r/\Sigma V_L$).

In the absence of alternative nitrogen sources, atrazine and diazinon are efficiently used as the sole carbon and nitrogen sources. The microbial communities' response to the variation of B_V is shown in **Fig. 4**. It can be observed that the kinetic behavior of both communities was not affected by the increase in pesticide loading, as the volumetric removal rates (R_V = F(S_r - s)/ΣV_L) increase proportionally with the volumetric loading rates. The S_r values ranged from 3.0 to 19.0 mg L^{-1} for atrazine and 3.0 to 8.5 mg L^{-1} for diazinon. The F values used ranged from 46 to 110 mL h^{-1}.

3.4 Effect of nitrogenated fertilizers on the microbial communities MC_ATZ and MC_DZN during D-Stat operation

The impact of crescent loading rates of urea, ammonia, and nitrates on microbial community behavior was evaluated in several ways.

A) By measuring the average cultivable cell counting (CFU) grown in the HMPBRs supplied with the different fertilizers and diazinon (MC_{DZN}) or atrazine (MC_{ATZ}) after a D-Stat operation.

When urea, a nitrogen and carbon source, was supplied, the highest levels of cultivable cells (x) in the reactor were obtained, compared to those reached when $(NH_4)_2SO_4$ or NH_4NO_3 were provided (**Table 3**).

B) By measuring the changes in volumetric removal rates (R_V) of atrazine, diazinon, and N-fertilizers along gradient loading charges of N-fertilizers in D-Stat experiments and comparing the effect of crescent loading rates of fertilizers on the specific uptake rates q_{ATZ} and q_{DZN} by MC_{ATZ} and MC_{DZN} during D-Stat operation.

As shown in **Figures 5** to **7**, despite increasing the supply rates of any of the N-fertilizers — urea (**Fig. 5**), ammonium (**Fig. 6**), or nitrates (**Fig. 7**) — the removal rates of atrazine or diazinon by the acclimated MC_{DZN} and MC_{ATZ} communities remained unchanged, showing no preference for alternative nitrogen sources that are easier to assimilate than N-heterocyclic pesticides, as has been

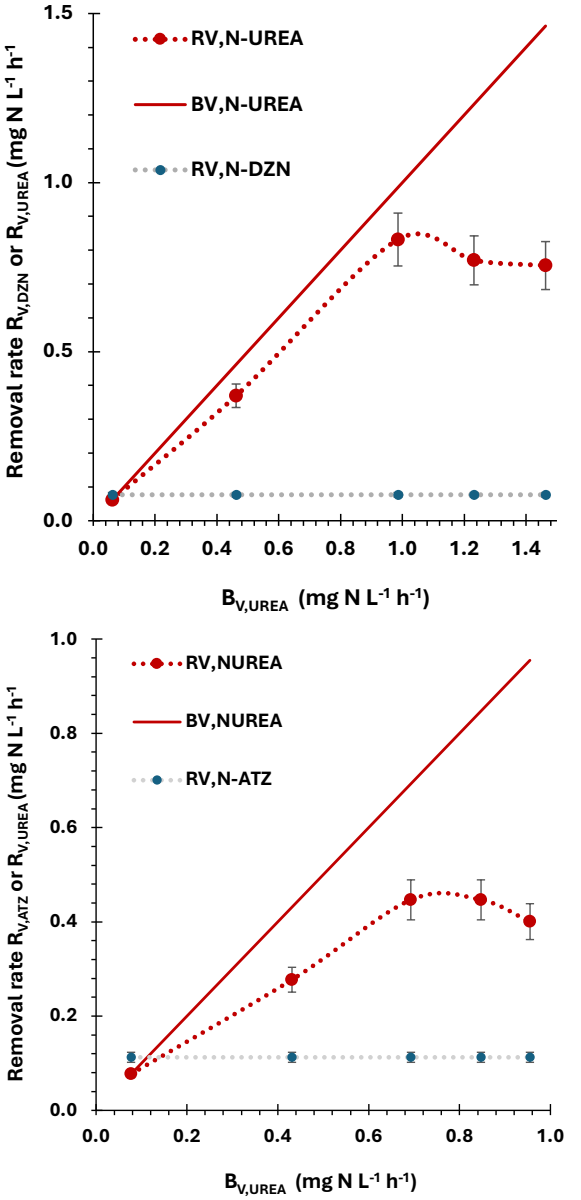

Fig. 5. Nitrogen consumption rates of urea, diazinon, and atrazine by microbial consortia MC_{DZN} (left) and MC_{ATZ} (right), during a gradient loading rate of the N-fertilizer.

reported with indigenous soil bacteria (Sims, 2006) or with axenic bacterial cultures (García-González *et al.*, 2003). However, other effects of N-fertilizers on microbial communities were observed in the D-Stat experiments.

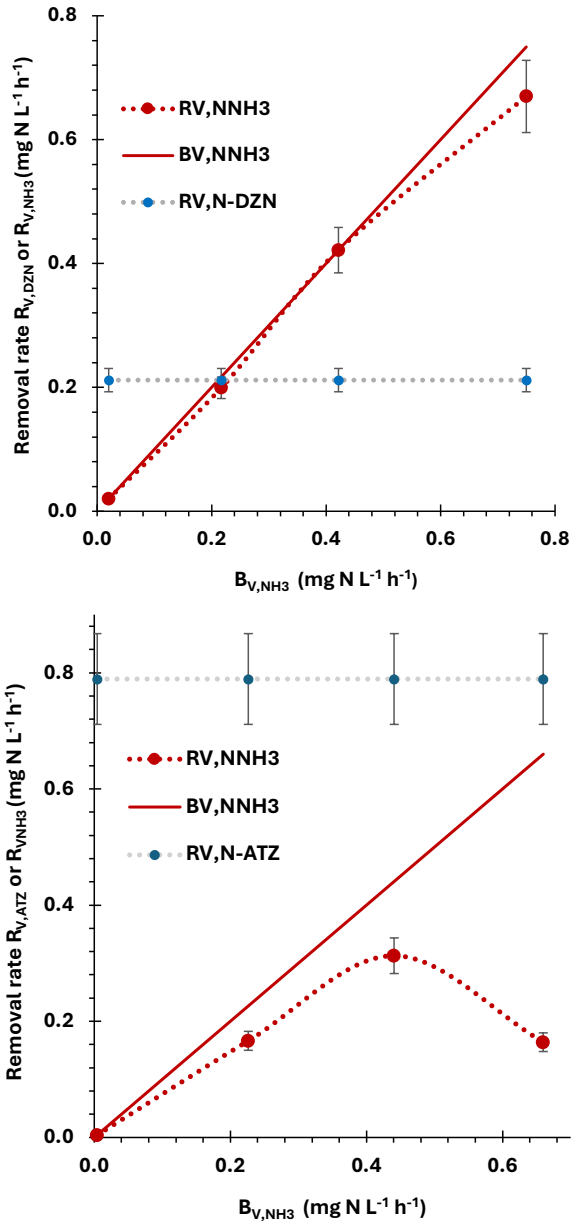

Fig. 6. Nitrogen consumption rates of ammonium, diazinon, and atrazine by microbial consortia MC_{DZN} (left) and MC_{ATZ} (right), during a gradient loading rate of $(NH_4)_2SO_4$.

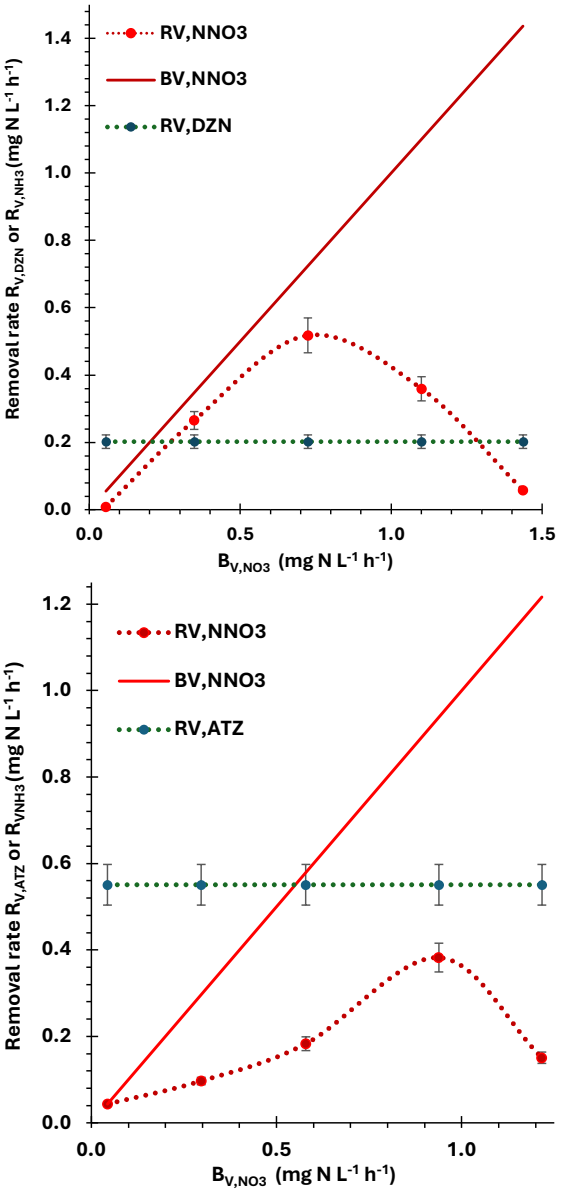

Fig. 7. Nitrogen consumption rates of ammonium nitrate, diazinon, and atrazine by microbial consortia MC_{DZN} (left) and MC_{ATZ} (right), during a gradient loading rate of the N-fertilizer.

Since the volumetric biodegradation rate of a compound depends on the reactive cell mass concentration, it is convenient to evaluate the MCs removal ability in terms of specific removal rates (degradation rate per unit cell mass), which, as shown in **Table 4,** varies notably depending on the source of N-fertilizer, particularly urea, since it is an additional carbon source. Therefore, the degradation capacity of pesticides by acclimatized microbial communities was quantified by evaluating the impact of each fertilizer on the specific degradation rate (q) of each pesticide. These rates were expressed as pesticide degradation rates per unit cell mass (q_{dzn} and q_{atz}) or, based on their respective nitrogen content, qN_{dzn} and qN_{atz}.

In both cases, notable effects of each fertilizer on the microbial communities were observed, mainly due to the comparatively high diazinon degradation rate of the microbial community MC_{DZN} in the presence of NH_4NO_3. The impact of this alternative nitrogen source on a carbendazim-degrading strain of *Bacillus velezensis*, has been reported, attributing its effect to the up-regulation of two carbendazim-degrading genes (Song *et al*., 2024).

C) In the case of the MC_{atz} community, by examining the presence of the genes involved in the atrazine catabolism *atz*-(*A, B, C, D*) and *trzN*, and measuring the accumulation of cyanuric acid in the HMPBR.

Table 4. Atrazine and diazinon specific uptake rates q_{ATZ}, q_{DZN}, *and* qN_{ATZ}, qN_{DZN}, expressed in terms of the pesticides' nitrogen content, for the corresponding microbial communities MC_{ATZ} *and* MC_{DZN}.

Fertilizer	q_{ATZ} mg ATZ $(CFU*10^{10})^{-1}$ h^{-1}	q_{DZN} mg DZN $(CFU*10^{10})^{-1}$ h^{-1}	q_{NATZ} mg N_{ATZ} $(CFU*10^{10})^{-1}$ h^{-1}	q_{NDZN} mg N_{DZN} $(CFU*10^{10})^{-1}$ h^{-1}
Urea	0.350 ± 0.037	0.322 ± 0.038	0.114 ± 0.012	0.029 ± 0.003
$(NH_4)_2SO_4$	2.313 ± 0.235	0.943 ± 0.106	0.754 ± 0.077	0.087 ± 0.001
NH_4NO_3	2.787 ± 0.326	4.578 ± 0.460	0.296 ± 0.034	0.421 ± 0.042

Table 5: Effect of N-fertilizer loading rate on the presence of *atz* genes in the MC_{ATZ} acclimated microbial community able to degrade atrazine.

Fertilizer	Peak loading rate (mg N L^{-1} h^{-1})	trzN	atzA	atzB	atzC	atzD
MSM	-	-	+	+	+	+
Urea	0.954	-	+	+	+	-
MSM	-	+	-	+	+	+
$(NH_2)_2$ SO_4	0.659	+	-	+	+	-
MSM	-	+	-	+	+	+
NH_4 NO_3	1.216	+	-	+	+	+

In bacteria of the genus *Pseudomonas*, the atrazine-degradation pathway is encoded by the *atzA*, *atzB,* atzC, and *atzDEF* operon. The expression of this operon is regulated by the availability of nitrogen and the presence of cyanuric acid (Govantes *et al*., 2010). In other bacteria, such as *Nocardioides* (Mulbry *et al*., 2002) and *Arthrobacter*(Li *et al*., 2020), the first gene involved in the atrazine-degradation pathway is the *trzN* gene. For some *Pseudomonas* strains, the transcription/expression of *atz*(*A, B, C*) genes is inducible, while for others it is constitutive (Sharma *et al*., 2018).

A microbial community is taxonomically and functionally complex, encompassing a great diversity of bacterial species and metabolic functions (Alvarado *et al*., 2024). Thus, in the case of MC_{atz}, various species are expected to have the whole train of genes involved in atrazine catabolism. The *atz-(A ,B, C, D)* genes and the *trzN* gene, which, like *atzA*, encode a triazine hydrolase (Mulbry *et al*., 2002), are distributed among distinct microbial species that constitute the community; however, some of these species will be predominant depending on environmental conditions.

In the case of the microbial community MC_{TZ}, the N-fertilizers impacted it in diverse ways. Two closely related effects are shown in **Table 5,** which presents the *atz* genes detected in the microbial community at peak supply rates for different N-fertilizers. **Figure 8** illustrates the accumulation of cyanuric acid, whose CN-hydrolase is encoded by the *atzD* gene. This gene was not detected when urea or $(NH_4)_2SO_4$ was supplied to the reactor. On the contrary, when the N-fertilizer supplied was NH_4NO_3, this gene was detected; thus, no accumulation of cyanuric acid was observed in the reactor. These facts are consistent with other authors' results, suggesting that atrazine degradation genes are up-regulated by atrazine but repressed by simple nitrogen sources (Sharma *et al*., 2018).

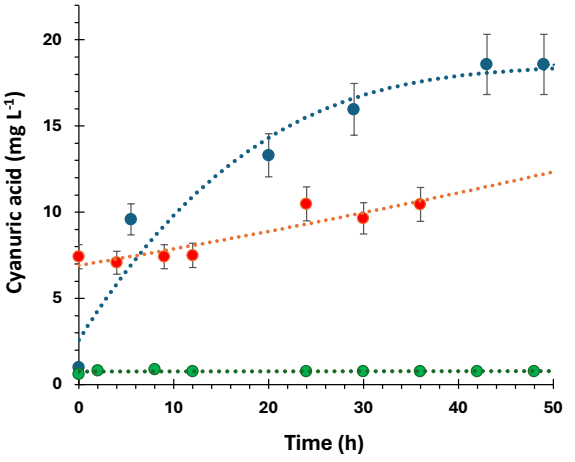

Fig. 8 Transient accumulation of cyanuric acid during a gradient supply of nitrogenated fertilizers to the MC_{ATZ}. Urea, blue points; $(NH4)_2SO_4$, red points; NH_4NO_3 green points.

4 Discussion

Although nitrogen and organic matter can be removed by using different types of packing materials and packed bed reactors (Muñoz-Sánchez & Reyes-Mazzoco., 2012; Serrano-Meza *et al*., 2024), or immobilized native microbial consortium can be

successfully applied in packed bed bioreactors in wastewater treatments processes (Houbron *et al*., 2021; García-Uitz *et al*., 2024), the findings of this study demonstrate that acclimated microbial communities can sustain robust degradation of N-heterocyclic pesticides even under high nitrogen fertilizer loading, challenging the conventional view that nitrogen repression inherently limits such bioremediation processes.

This persistence can be attributed to physiological and ecological adaptations within the biofilms, which reduced their sensitivity to the inhibitory effects of alternative nitrogen sources. The genetic and enzymatic machinery underlying this resilience is particularly well characterized in the context of atrazine degradation. The acclimated communities within the high-mass portable biofilm reactor (HMPBR) harbor the *atz* and *trz* gene clusters, which encode a cascade of enzymes including atrazine chlorohydrolase (AtzA/TrzN), hydroxyatrazine hydrolase (AtzB), and N-isopropylammelide isopropylaminohydrolase (AtzC), culminating in the enzymes (AtzD–F) responsible for cyanuric acid mineralization. The presence and expression of these genes within the biofilm provide a clear mechanistic basis for the sustained atrazine removal observed even at elevated fertilizer concentrations. Similarly, the observed degradation of diazinon is consistent with the known metabolic capabilities of several bacterial genera present in the community, such as *Pseudomonas*, *Serratia*, and *Ochrobactrum*, while other members like *Burkholderia* and *Sphingobium* are recognized for their roles in degrading organophosphorus pesticides through analogous enzymatic pathways.

The operational success of this bioremediation strategy depends on HMPBR design, which ensures an oxic environment crucial for aerobic degradation pathways. The microbial communities, isolated and acclimated under oxic conditions, benefit from diffusive and convective oxygen transfer. The reactor's configuration, with equalizer-oxygenators between packed zones, was based on predicted dissolved oxygen (DO) behavior and the DO-limited rate of convective transport, thereby ensuring that the immobilized communities are not oxygen-limited. Using this system, the study investigated the impact of different nitrogen fertilizers on degradation efficiency. While the acclimated communities efficiently removed pesticides across a wide range of loading rates for urea, ammonium sulfate, and ammonium nitrate, the completeness of biodegradation was fertilizer-dependent. A critical observation was the accumulation of cyanuric acid, a stable intermediate, when atrazine was combined with either urea or ammonium sulfate. In contrast, when atrazine was combined with ammonium nitrate, the study observed near-complete mineralization, suggesting a shift in community function that prevents the bottleneck at the cyanuric acid ring-cleavage step.

This fertilizer-specific output indicates that the functional structure of the acclimated community is not static but changes in response to the nitrogen source provided. The results suggest that different nitrogen fertilizers select distinct metabolic profiles within the biofilm, influencing downstream degradation pathways and determining whether intermediate compounds accumulate or are fully biodegraded. Importantly, the community's ability to restructure itself functionally allowed it to maintain high degradation rates for both atrazine and diazinon, even as nitrogen loading increased. This study thus represents an integration of bioprocess engineering and microbial ecology. By combining the advanced reactor design (HMPBR) with robust, acclimated natural communities, we have demonstrated that specialized biofilms can overcome the inhibitory effects of agricultural fertilizers challenge previously considered difficult due to nitrogen-mediated repression. Collectively, these results support the conclusion that this approach offers an environmentally sustainable and adaptable strategy for the bioremediation of pesticide-contaminated soils and waters in intensive agricultural settings.

Conclusions

In conclusion, this work provides evidence that acclimated microbial communities, when supported within a properly engineered HMPBR, offer a viable and robust solution for the bioremediation of N-heterocyclic pesticides under the challenging conditions of intensive nitrogen fertilization. The sustained degradation activity, underpinned by key genetic pathways and community-level functional plasticity, demonstrates that the negative effects of nitrogen repression can be effectively mitigated. A particularly significant finding is that the choice of nitrogen fertilizer is critical, as it dictates the extent of pesticide mineralization: ammonium nitrate promotes complete breakdown, whereas urea and ammonium sulfate lead to the accumulation of recalcitrant intermediates. This research underscores the importance of integrating microbial ecology with bioprocess engineering to develop effective bioremediation technologies. Together, these findings establish that fertilizer choice and reactor design jointly determine whether pesticide removal is partial or complete, strengthening the case for this approach as a sustainable and adaptable strategy. Future work should focus on optimizing the HMPBR design and operational parameters to further enhance community stability and performance, and on validating these

findings at a larger, field-relevant scale, with a specific focus on managing fertilizer types to ensure complete detoxification.

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Nomenclature and abbreviations

$B_{V,N}$	Volumetric N-fertilizer loading rate [g L^{-1} h^{-1}]
$B_{V,O2} = Fc_L/V_L$	Convective dissolved oxygen transfer rate [g L^{-1} h^{-1}]
c_s	Oxygen concentration at the gas-liquid interface [g_{O2} L^{-1}]
c_L	Dissolved oxygen concentration in equalizers (DO) [g_{O2} L^{-1}]
D_r	Internal diameter of reservoir tank R in the gradient generator system [cm]
D_g	Internal diameter of gradient tank G in the gradient generator system [cm]
D-Stat System	A dynamic feeding method of the HMPBR
EOC	Equalizer-oxygenator compartment
F	Liquid flow rate[L h^{-1}]
HMPBR	Horizontal-multistage-packed-bed biofilm reactor
k_La	Oxygen transfer coefficient in equalizer [h^{-1}]
MSM	Minimal Salts Medium
N_g	N concentration in tank G of the gradient generator system [mg L^{-1}]
N_r	N concentration in reservoir tank R [mg L^{-1}]
N_{go}	Starting N concentration in the gradient tank G
$q_Nx = (dC_L/dt)_{cons}$	Volumetric consumption rate of N fertilizer [g_N L^{-1} h^{-1}]
$q_{O2}x = (dC_L/dt)_{cons}$	Volumetric consumption rate of dissolved oxygen [g_{O2} L^{-1} h^{-1}]
t	time [h]
V_e	Equalizer volume [L]
V_{Go}	Initial volume of liquid in tank G [L]
V_L	Liquid volume in the *HMPBRReferences*
V_{Ro}	Initial volume of liquid in tank R [L]
$\varphi r = Dr^2/(Dg^2+Dr^2)$	Geometry factor for the gradient generator system
$\theta_{RH} = V_e/F$	Hydraulic residence time in equalizer [h]

References

Abbasi, S., Devers-Lamrani, M., Martin-Laurent, F., Michel, C., Romdhane, S., Rouard, N., & Spor, A. (2025). Assessing the efficiency and the side effects of atrazine-degrading biocomposites amended to atrazine-contaminated soil. *FEMS Microbiology Ecology, 101*(7), Article fiaf071. https://doi.org/10.1093/femsec/fiaf071

Aggarwal, V., Deng, X., Tuli, A., & Goh, K. S. (2013). Diazinon—chemistry and environmental fate: A California perspective. In D. Whitacre (Ed.), *Reviews of Environmental Contamination and Toxicology* (Vol. 223, pp. 107–140). Springer. https://doi.org/10.1007/978-1-4614-5577-6_6

Akinyi, T., Diaz-Reck, D., Raj, A., & Bernstein, A. (2026). Abundance of atrazine degrading genes *trzN* versus *atzA* in soil cultures and in situ degradation rates. *Pest Management Science*. Advance online publication. https://doi.org/10.1002/ps.71068

Alvarado-Gutiérrez, M. L., Ruiz-Ordaz, N., Galíndez-Mayer, J., Santoyo-Tepole, F., García-Mena, J., Nirmalkar, K., & Curiel-Quesada, E. (2024). Dynamic and structural response of a multispecies biofilm to environmental perturbations induced by the continuous increase of benzimidazole fungicides in a permeable reactive biobarrier. *Journal of Environmental Health Science and Engineering, 22*, 329–344. https://doi.org/10.1007/s40201-024-00903-3

Ambriz-Mexicano, I., Gonzalez-Juarez, S., Ruiz-Ordaz, N., Galindez-Mayer, J., Santoyo-Tepole, F., Juarez-Ramirez, C., & Galar-Martinez, M. (2022). Integrated adsorption and biological removal of the emerging contaminants ibuprofen, naproxen, atrazine, diazinon, and

carbaryl in a horizontal tubular bioreactor. *Bioprocess and Biosystems Engineering, 45*(9), 1547–1557. https://doi.org/10.1007/s00449-022-02764-2

Bandyopadhyay, B., Humphrey, A. E., & Taguchi, H. (1967). Dynamic measurement of the volumetric oxygen transfer coefficient in fermentation systems. *Biotechnology and Bioengineering, 9*(4), 533–544. https://doi.org/10.1002/bit.260090408

Bhardwaj, L., Pandey, J., & Dubey, S. K. (2021). Effects of herbicides on soil enzymes and their regulatory factors in agroecosystem: A review. In S. K. Dubey & S. K. Verma (Eds.), *Plant, Soil and Microbes in Tropical Ecosystems* (pp. 89–115). Springer. https://doi.org/10.1007/978-981-16-3364-5_5

Bichat, F., Sims, G. K., & Mulvaney, R. L. (1999). Microbial utilization of heterocyclic nitrogen from atrazine. *Soil Science Society of America Journal, 63*(1), 100–110. https://doi.org/10.2136/sssaj1999.03615995006300010016x

Caracciolo, A. B., Giuliano, G., Grenni, P., Cremisini, C., Ciccoli, R., & Ubaldi, C. (2005). Effect of urea on degradation of terbuthylazine in soil. *Environmental Toxicology and Chemistry, 24*(5), 1035–1040. https://doi.org/10.1897/04-253R.1

El-Ghany, A., & Masmali, I. A. (2016). Fungal biodegradation of organophosphorus insecticides and their impact on soil microbial population. *Journal of Plant Pathology & Microbiology, 7*(5), 1000349. https://doi.org/10.4172/2157-7471.1000349

Entry, J. A., Mattson, K. G., & Emmingham, W. H. (1993). The influence of nitrogen on atrazine and 2,4-dichlorophenoxyacetic acid mineralization in grassland soils. *Biology and Fertility of Soils, 16*(3), 179–182. https://doi.org/10.1007/BF00361404

García-González, V., Govantes, F., Shaw, L. J., Burns, R. G., & Santero, E. (2003). Nitrogen control of atrazine utilization in *Pseudomonas* sp. strain ADP. *Applied and Environmental Microbiology, 69*(12), 6987–6993. https://doi.org/10.1128/AEM.69.12.6987-6993.2003

García-Uitz, K., Cruz, J. C., León-Pech, M. G., Moreno-Andrade, I., Giácoman-Vallejos, G., & Ponce-Caballero, C. (2024). Isolation and characterization of microbial diversity in phenanthrene-degrading consortia in a pollution zone in Ciudad del Carmen, Mexico. Revista Mexicana de Ingeniería Química, 23(2), Bio24241. https://doi.org/10.24275/rmiq/Bio24241

Gómez-De Jesús, A., Romano-Baez, F. J., Leyva-Amezcua, L., Juárez-Ramírez, C., Ruiz-Ordaz, N., & Galíndez-Mayer, J. (2009). Biodegradation of 2,4,6-trichlorophenol in a packed-bed biofilm reactor equipped with an internal net draft tube riser for aeration and liquid circulation. *Journal of Hazardous Materials, 161*(2-3), 1140–1149. https://doi.org/10.1016/j.jhazmat.2008.04.077

González Brambila, M. M., León Santiesteban, H. H., Trejo Aguilar, G. M., Colín Luna, J. A., García Martínez, J. C., & Reyes Chilpa, R. (2024). Biodegradation of atrazine: A review of its metabolic pathways. *International Journal of Chemical Reactor Engineering, 22*(11), 1265–1288. https://doi.org/10.1515/ijcre-2024-0241

González-Cuna, S., Galíndez-Mayer, J., Ruiz-Ordaz, N., Murugesan, S., Piña-Escobedo, A., García-Mena, J., Lima-Martínez, E., & Santoyo-Tepole, F. (2016). Aerobic biofilm reactor for treating a commercial formulation of the herbicides 2,4-D and dicamba: Biodegradation kinetics and biofilm bacterial diversity. *International Biodeterioration & Biodegradation, 107*, 123–131. https://doi.org/10.1016/j.ibiod.2015.11.014

Govantes, F., García-González, V., Porrúa, O., Isabel Platero, A., Jiménez-Fernández, A., & Santero, E. (2010). Regulation of the atrazine-degradative genes in *Pseudomonas* sp. strain ADP. *FEMS Microbiology Letters, 310*(1), 1–8. https://doi.org/10.1111/j.1574-6968.2010.01991.x

Guo, Q., Zhai, W., Li, P., Xiong, Y., Li, H., Liu, X., Zhou, Z., Li, B., Wang, P., & Liu, D. (2024). Nitrogen fertilizer-domesticated microbes change the persistence and metabolic profile of atrazine in soil. *Journal of Hazardous Materials, 469*, 133974. https://doi.org/10.1016/j.jhazmat.2024.133974

Houbron, E., Cruz-Carmona, E., Ponciano-Rosas, A., Rustrián-Portilla, E., & Canul-Chan, M. (2021). Motor oil wastewater treatment in a packed bed bioreactor using immobilized native microbial consortium. *Revista Mexicana de Ingeniería Química, 20*(2), 761–773. https://doi.org/10.24275/rmiq/IA2271

Jeyaseelan, A., Murugesan, K., Thayanithi, S., & Palanisamy, S. B. (2024). A review of the impact of herbicides and insecticides on the microbial communities. *Environmental Research, 245*, 118020. https://doi.org/10.1016/j.envres.2023.118020

Kaman-Malek, S., Dabestani Rahmatabad, A., & Borghei, S. M. (2024). Biological Atrazine removal from low-strength wastewater by moving bed biofilm reactor and upflow fixed bed bioreactor: Performance and kinetic analysis. *Research Square*. https://doi.org/10.21203/rs.3.rs-4707102/v1

Köninger, J., Labouyrie, M., Ballabio, C. *et al.* (2026). Pesticide residues alter taxonomic and functional biodiversity in soils. *Nature* 650, 367–373 https://doi.org/10.1038/s41586-025-09991-z

Kumar, S., Kaushik, G., Dar, M. A., Nimesh, S., Lopez-Chuken, U. J., & Villarreal-Chiu, J. F. (2018). Microbial degradation of organophosphate pesticides: A review. *Pedosphere, 28*(2), 190–208. https://doi.org/10.1016/S1002-0160(18)60017-7

Kuypers, M. M., Marchant, H. K., & Kartal, B. (2018). The microbial nitrogen-cycling network. *Nature Reviews Microbiology, 16*, 263–276. https://doi.org/10.1038/nrmicro.2018.9

Levanon, D., Meisinger, J. J., Codling, E. E., & Starr, J. L. (1994). Impact of tillage on microbial activity and the fate of pesticides in the upper soil. *Water, Air, and Soil Pollution, 72*(1–4), 179–189. https://doi.org/10.1007/BF01257123

Li, M. R., Zhan, F. D., Chen, J. J., Zu, Y. Q., & Li, Y. (2020). Atrazine degradation pathway and genes of *Arthrobacter* sp. FM326. *Polish Journal of Environmental Studies, 29*(5), 3683–3689. https://doi.org/10.15244/pjoes/115326

Liu, S., Gao, P. F., Li, F. H., Wang, L., Dai, Y., & Fu, M. (2023). A review of the recent progress in biotrickling filters: packing materials, gases, microorganisms, and CFD. *Environmental Science and Pollution Research, 30*, 125398–125416. https://doi.org/10.1007/s11356-023-31004-7

Marín-Benito, J. M., Herrero-Hernández, E., Andrades, M. S., Sánchez-Martín, M. J., & Rodríguez-Cruz, M. S. (2014). Effect of different organic amendments on the dissipation of linuron, diazinon, and myclobutanil in an agricultural soil incubated for different periods. *Science of the Total Environment, 476-477*, 611–621. https://doi.org/10.1016/j.scitotenv.2014.01.052

Mohammadi Aria, M., Nourbakhsh, F., & Jafari, M. T. (2024). Potentially toxic elements influence diazinon degradation rate in soil. *Water, Air, & Soil Pollution, 235*, Article 278. https://doi.org/10.1007/s11270-024-07094-5

Mukherjee, S. (2022). Impact of pesticides and fertilizers on soil microbial diversity. In *Current Topics in Soil Science* (pp. 417–440). Springer. https://doi.org/10.1007/978-3-030-92669-4_20

Mulbry, W. W., Zhu, H., Nour, S. M., & Topp, E. (2002). The triazine hydrolase gene trzN from *Nocardioides* sp. strain C190: Cloning and construction of gene-specific primers. *FEMS Microbiology Letters, 206*(1), 75–79. https://doi.org/10.1016/S0378-1097(01)00517-1

Muñoz-Sánchez, T. J., & Reyes-Mazzoco, R. (2012). Organic matter and nitrogen removal capacity of a new packing for trickling filters. Revista Mexicana de Ingeniería Química, 11(2), 279–286. https://rmiq.org/iqfvp/Vol11No2.html

Prashar, P., & Shah, S. (2016). Impact of fertilizers and pesticides on soil microflora in agriculture. In E. Lichtfouse (Ed.), *Sustainable Agriculture Reviews* (Vol. 19, pp. 331–361). Springer. https://doi.org/10.1007/978-3-319-26777-7_8

Rebello, S., Nathan, V. K., Sindhu, R., Binod, P., Awasthi, M. K., & Pandey, A. (2021). Bioengineered microbes for soil health restoration: present status and future. *Bioengineered, 12*(2), 12839–12853. https://doi.org/10.1080/21655979.2021.2004645

Rostami, S., Jafari, S., Moeini, Z., Jaskulak, M., Keshtgar, L., Badeenezhad, A., Azhdarpoor, A., Rostami, M., Zorena, K., Dehghani, M. (2021). Current methods and technologies for degradation of atrazine in contaminated soil and water: A review. *Environmental Technology & Innovation, 24*, Article 102019. https://doi.org/10.1016/j.eti.2021.102019

Salazar-Huerta, M. A., Ruiz-Ordaz, N., Galíndez-Mayer, J., García-Mena, J., & Juárez-Ramírez, C. (2019). Simulation and experimental

validation of a gradient feeding system for fast assessment of the kinetic behavior of a microbial consortium in a tubular biofilm reactor. *Bioprocess and Biosystems Engineering, 42*(1), 17–27. https://doi.org/10.1007/s00449-018-2009-x

Schoen, S. R., & Winterlin, W. L. (1987). The effects of various soil factors and amendments on the degradation of pesticide mixtures. *Journal of Environmental Science and Health, Part B, 22*(3), 347–377. https://doi.org/10.1080/03601238709372561

Sebiomo, A., Ogundero, V. W., & Bankole, S. A. (2011). Effect of four herbicides on microbial population, soil organic matter, and dehydrogenase activity. *African Journal of Biotechnology, 10*(5), 770–778. https://doi.org/10.4314/ajb.v10i5

Serrano-Meza, A., Vigueras-Cortes, J. M., & Allen, C. D. (2024). Municipal wastewater treatment in a hybrid biofiltration system packed with agave fiber. *Revista Mexicana de Ingeniería Química, 23*(3), IA24347. https://doi.org/10.24275/rmiq/IA24347

Sharma, A., Kalyani, P., Trivedi, V. D., Kapley, A., & Phale, P. S. (2019). Nitrogen-dependent induction of atrazine degradation pathway in *Pseudomonas* sp. strain AKN5. *FEMS Microbiology Letters, 366*(1), Article fny277. https://doi.org/10.1093/femsle/fny277

Sims, G. K. (2006). Nitrogen starvation promotes biodegradation of N-heterocyclic compounds in soil. *Soil Biology and Biochemistry, 38*(8), 2478–2480. https://doi.org/10.1016/j.soilbio.2006.01.006

Solomon, R. D., Kumar, A., & Satheeja Santhi, V. (2013). Atrazine biodegradation efficiency, metabolite detection, and trzD gene expression by enrichment bacterial cultures from agricultural soil. *Journal of Zhejiang University SCIENCE B, 14*(12), 1162–1172. https://doi.org/10.1631/jzus.B1300001

Song, S., & Hwang, C. W. (2024). Microbial degradation of the benzimidazole fungicide carbendazim by *Bacillus velezensis* HY-3479. *International Microbiology, 27*, 797–805. https://doi.org/10.1007/s10123-023-00427-0

Talja, K. M., Kaukonen, S., Kilpi-Koski, J., Malin, I., Kairesalo, T., Romantschuk, M., Tuominen, J., & Kontro, M. H. (2008). Atrazine and terbutryn degradation in deposits from groundwater environment within the boreal region in Lahti, Finland. *Journal of Agricultural and Food Chemistry*, 56(24), 11962–11968. https://doi.org/10.1021/jf802528a

Verma, J. P., Jaiswal, D. K., & Sagar, R. (2014). Pesticide relevance and their microbial degradation: a-state-of-art. *Reviews in Environmental Science and Bio/Technology, 13*, 429–466. https://doi.org/10.1007/s11157-014-9341-7

Wackett, L. P., Sadowsky, M. J., Martinez, B., & Shapir, N. (2002). Biodegradation of atrazine and related *s*-triazine compounds: From enzymes to field studies. *Applied Microbiology and Biotechnology, 58*(1), 39–45. https://doi.org/10.1007/s00253-001-0862-y

Wang, H., Ren, W., Xu, Y., Wang, X., Ma, J., Sun, Y., Hu, W., Chen, S., Dai, S., Song, J., Jia, J., & Teng, Y. (2024). Long-term herbicide residues affect soil multifunctionality and the soil microbial community. *Ecotoxicology and Environmental Safety, 283*, 116783. https://doi.org/10.1016/j.ecoenv.2024.116783

Wu, X., Li, J., Zhou, Z., Lin, Z., Pang, S., Bhatt, P., Mishra, S., & Chen, S. (2021). Environmental occurrence, toxicity concerns, and degradation of diazinon using a microbial system. *Frontiers in Microbiology, 12*, Article 751155. https://doi.org/10.3389/fmicb.2021.751155

Zablotowicz, R. M., Krutz, L. J., Weaver, M. A., Accinelli, C., & Reddy, K. N. (2008). Glufosinate and ammonium sulfate inhibit atrazine degradation in adapted soils. *Biology and Fertility of Soils, 45*, 19–26. https://doi.org/10.1007/s00374-008-0299-x