# Biotechnological Strategies for diclofenac removal from the environment: microorganisms and immobilized systems

# Estrategias biotecnológicas para la eliminación de diclofenaco del medio ambiente: microorganismos y sistemas inmovilizados

# Estratégias biotecnológicas para a remoção do diclofenac do ambiente: microrganismos e sistemas imobilizados

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*Keywords:* Diclofenac; Bioremediation; Microorganisms; Immobilized Systems; Environmental Toxicity; Laccase.

*Abstract*
Due to its persistence and insufficient removal in traditional wastewater treatment facilities, diclofenac, one of the most used non-steroidal anti-inflammatory drugs (NSAIDs) worldwide, has been regularly found in aquatic and terrestrial ecosystems. This makes it an emerging pollutant of concern. With emphasis on the employment of microbes and immobilized systems, this review paper assesses biotechnological methods for removing diclofenac from the environment. The drug's physicochemical properties, its global distribution in surface water, groundwater, and soils, and its toxicity to non-target organisms such as fish, birds, invertebrates, and plants are all covered. Its effects range from oxidative stress and renal failure to genotoxicity and behavioral changes. Biodegradation mediated by bacteria (e.g., Rhodococcus ruber, Pseudomonas moorei) and fungi (e.g., Trametes spp.) shows potential for the compound's breakdown via mechanisms such as hydroxylation and aromatic ring cleavage, despite the efficiency and cost limitations of physicochemical methods. The use of immobilized biocatalysts, such as laccases in nanofibers, biochar, or membranes, and microbial cells in biofilters, is particularly noteworthy because it increases degrader stability, reusability, and resistance to toxicity. The creation of bioremediation systems based on immobilized preparations has been found to be an environmentally safe, effective, and promising alternative for reducing diclofenac-induced environmental contamination.

Palabras clave: Diclofenaco; Biorremediación; Microorganismos; Sistemas Inmovilizados; Toxicidad Ambiental; Lacasa.

*Resumen*
Debido a su persistencia y a su eliminación insuficiente en las instalaciones tradicionales de tratamiento de aguas residuales, el diclofenaco, uno de los medicamentos antiinflamatorios no esteroideos (AINEs) más utilizados en todo el mundo, se ha detectado con regularidad en ecosistemas acuáticos y terrestres. Esto lo convierte en un contaminante emergente de preocupación. Este artículo de revisión evalúa métodos biotecnológicos para eliminar el diclofenaco del medio ambiente, con énfasis en el uso de microbios y sistemas inmovilizados. Se cubren las propiedades fisicoquímicas del fármaco, su distribución global en aguas superficiales, subterráneas y suelos, y su toxicidad para organismos no objetivo, como peces, aves, invertebrados y plantas. Sus efectos van desde el estrés oxidativo y la insuficiencia renal hasta la genotoxicidad y los cambios de comportamiento. La biodegradación mediada por bacterias (ej. Rhodococcus ruber, Pseudomonas moorei) y hongos (ej. Trametes spp.) muestra potencial para la descomposición del compuesto a través de mecanismos como la hidroxilación y la escisión de anillos aromáticos, a pesar de las limitaciones de eficiencia y costo de los métodos fisicoquímicos. El uso de biocatalizadores inmovilizados, como lacasas en nanofibras, biocarbón o membranas, y células microbianas en biofiltros, es particularmente notable, ya que aumenta la estabilidad, reutilización y resistencia a la toxicidad de los degradadores. Se concluye que la creación de sistemas de biorremediación basados en preparaciones inmovilizadas es un sustituto eficaz, seguro para el medio ambiente y prometedor para reducir la contaminación ambiental inducida por el diclofenaco.

Palavras-chave: Diclofenaco; Biorremediação; Microrganismos; Sistemas Imobilizados; Toxicidade Ambiental; Lacase.

*Resumo*
Devido à sua persistência e à remoção insuficiente em instalações tradicionais de tratamento de águas residuais, o diclofenaco, um dos anti-inflamatórios não esteroides (AINEs) mais comumente utilizados em todo o mundo, tem sido encontrado com frequência em ecossistemas aquáticos e terrestres. Isso o torna um poluente emergente de preocupação. Este artigo de revisão avalia métodos biotecnológicos para remover o diclofenaco do ambiente, com ênfase no emprego de micróbios e de sistemas imobilizados. São abordadas as propriedades físico-químicas do fármaco, sua distribuição global em águas superficiais, águas subterrâneas e solos, e sua toxicidade para organismos não-alvo, como peixes, aves, invertebrados e plantas. Seus efeitos variam de estresse oxidativo e insuficiência renal a genotoxicidade e alterações comportamentais. A biodegradação mediada por bactérias (ex.: Rhodococcus ruber, Pseudomonas moorei) e fungos (ex.: Trametes spp.) mostra potencial para a degradação do composto por meio de mecanismos como hidroxilação e clivagem de anéis aromáticos, apesar das limitações de eficiência e custo dos métodos fisicoquímicos. O uso de biocatalisadores imobilizados, como lacases em nanofibras, biocarvão ou membranas, e células microbianas em biofiltros, é particularmente notável, pois aumenta a estabilidade, a reutilização e a resistência à toxicidade dos biocatalisadores. Conclui-se que a criação de sistemas de biorremediação baseados em preparações imobilizadas é um substituto eficaz, seguro para o meio ambiente e promissor para reduzir a contaminação ambiental induzida pelo diclofenaco.

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## 1. Introduction

Pain is a major global public health issue; over 100 million adults in the US and about 27% of adults in Europe suffer from chronic pain. The mechanism of action of nonsteroidal anti-inflammatory medications (NSAIDs), which were discovered more than a century ago, is based on the inhibition of cyclooxygenase (COX) isoenzymes (Georgin et al., 2022; Georgin, Franco, Meili et al., 2023; Georgin, Franco, Ramos et al., 2023). They remain an essential component of the pharmacological management of both acute and chronic pain and inflammation (El Messaoudi, N., Miyah, Y., Benjelloun et al., 2024; Georgin, Meili & Franco, 2023; Ighalo et al., 2024). They are the cornerstone of treatment for osteoarthritis and other chronic pain problems, and they are crucial for the management of acute pain during the perioperative phase (Monteiro-Steagall Steagall & Lascelles, 2013). Physicians frequently like them because of their long history of therapeutic use, strong efficacy, and low risk of dependence (Brune, Renner & Tiegs, 2015). Both the manufacture and consumption of these drugs have steadily increased in recent years. There are currently over 50 different kinds of NSAIDs on the global market, and one of the most well-known and widely used medications in this category is diclofenac. Since NSAIDs are distributed under several brand names and are frequently accessible without a prescription, it is challenging to determine the precise amount used worldwide. However, excluding veterinary use, it is estimated that 940 tons of diclofenac are consumed annually worldwide in capsule, suppository, tablet, intravenous solution, and ointment form (Drummond, Vinh, Guzik & Sobey, 2019).

The increasing difficulty of identifying active pharmaceutical chemicals (PhACs) in drinking water, surface water, and groundwater has led to the search for more effective and efficient wastewater bioremediation techniques (Ciğeroğlu et al., 2024; El Messaoudi, Miyah, Singh et al., 2024). Natural water circulation patterns, hydrological links across ecosystems, and the buildup of different toxins released into the environment over decades all contribute to the issue. Due to the potential for the total or partial destruction of hazardous compounds or their conversion into less toxic compounds, the microbial degradation of PhACs has been the focus of numerous investigations recently (Aouaini et al., 2024; Franco, Georgin, Netto, da Boit Martinello & Silva, 2022; Georgin et al., 2021). The efficiency of the biodegradation process drastically declines due to the unfavorable environmental conditions under which it occurs. To enable the immobilization of biocatalysts, appropriate techniques are sought. This increases the catalytic activity of biocatalysts, increases the degradability of contaminants, and extends their shelf life. Additionally, it enhances the likelihood that microbial cells will survive and adapt to changes in their surroundings, such as the concentration of harmful substances (Du et al., 2022; Zhou, Zhang & Cai, 2021).

There is an urgent need to explore more reliable and environmentally safe remediation technologies, given the persistence of diclofenac in the environment and the limitations of conventional wastewater treatment plants, which frequently result in the release of active and toxic compounds into ecosystems. One important tactic for mitigating microbial sensitivity to the drug's toxicity and extending the duration of its breakdown is the immobilization of biocatalysts. In this regard, the current study aims to assess diclofenac's toxicity to non-target organisms, gather data on its detrimental effects on biodiversity, and examine the viability of employing immobilized preparations for the drug's biodegradation. To offer a critical perspective on workable biotechnological solutions to reduce environmental contamination and advance the sustainability of water resources, special attention is given to technologies based on immobilized laccases and on microorganisms with enhanced decomposition capabilities.

## 2. Methodology

The goal of this study, described as a systematic and narrative literature review, was to assess biotechnological methods for removing diclofenac from the environment, with a focus on microbial and immobilized systems. The following methodological procedures were used in the elaboration of the article. Using combinations of keywords like "diclofenac," "bioremediation," "microorganisms", "immobilized systems, "" laccase, "environmental toxicity," "biodegradation," and "non-steroidal anti-inflammatory drugs," a thorough search was conducted in both national and international scientific databases for the first bibliographic survey. To represent the state of the art in remediation methods, articles published over the past several decades were considered. The second step is the selection criteria, wherein studies that provided experimental data on the following topics were chosen: (i) diclofenac concentration and distribution in various environmental matrices (water, soil, sediment); (ii) toxic effects on non-target organisms (aquatic, terrestrial, and birds); (iii) degradation efficiency by isolated bacteria and fungi; and (iv) performance of enzymes and cells immobilized on various supports (nanofibers, biochar, membranes, alginate).

The third stage involved the organization and critical analysis of the data along three main axes: (1) characterization of the pollutant (compilation of the physicochemical properties of diclofenac and its global distribution, as presented in Tables 1 and 2); (2) toxicity assessment (synthesis of acute and chronic effects in different species, including mechanisms of cellular toxicity and genotoxicity, consolidated in Table 3); and (3) removal strategies (comparison between biodegradation

by free microorganisms and immobilization matrices, analyzing enzymatic mechanisms (laccases, cytochrome P450), metabolic pathways, and removal efficiency in various immobilization matrices (Table 4). Lastly, the present drawbacks of physicochemical versus biological approaches were examined, and prospects for the practical use of immobilized biocatalysts to achieve economic sustainability, efficiency, and stability were suggested. The integration of these data enabled a thorough understanding of the potential of bioremediation as a viable alternative to reduce diclofenac-related environmental contamination.

## 3. Methods for detecting diclofenac in the environment

Diclofenac is present in the environment at low concentrations, typically in the nanogram to microgram per liter (ng/L to μg/L) range in surface and groundwater, making its precise detection and quantification in environmental matrices a major analytical challenge (Sharma et al., 2026). Representative sample, pretreatment and extraction, chromatographic separation, sensitive detection, and thorough data validation are the sequential and complementary phases that make up the entire analytical process in order to get beyond these restrictions. Preconcentration and analyte extraction from the complex matrix are crucial first steps (Solayman et al., 2025). For water, methods such as solid-phase extraction (SPE) with reversed-phase cartridges (C18), ion exchangers, or molecularly imprinted materials (MIPs) that give diclofenac selectivity are frequently used. Liquid-liquid extraction (LLE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) are commonly used for solid matrices, such as sediments, soils, and biological tissues. Solid-phase microextraction (SPME) and dispersive solid-phase extraction (d-SPE), two miniaturized and environmentally friendly methods that reduce organic solvent consumption and analysis time while maintaining recovery efficiencies above 80% under ideal conditions, have gained popularity in recent years (Chinnawat et al., 2026).

Following extraction, chromatographic methods, in conjunction with high-sensitivity detectors, are primarily used for the separation and detection of diclofenac. The gold standard for diclofenac analysis is high-performance liquid chromatography (HPLC) and its development, ultra-high-performance liquid chromatography (UPLC). This is because the molecule's polarity and thermolability prevent it from volatilizing for gas chromatography without first derivatization. Diclofenac can be distinguished from matrix interferents and its metabolites, such as 4'-hydroxy-diclofenac, thanks to the remarkable sensitivity (detection limits below 1 ng/L), structural selectivity, and confirmation capability provided by mass spectrometry (MS), particularly in a triple quadrupole (MS/MS) configuration (Tran, Ha, Nguyen & Tran, 2025). It is also possible to use gas chromatography coupled with mass spectrometry (GC-MS), however in order to make diclofenac volatile, a chemical derivatization step is needed. This increases the method's complexity and raises the possibility of analytical losses. Despite this, GC-MS is nevertheless helpful for routine analysis when approved techniques are available or in labs with inadequate infrastructure. In addition to chromatographic approaches, spectroscopic techniques such as molecular fluorescence and UV-Vis spectrophotometry have been investigated for rapid and inexpensive detection, particularly when combined with nanomaterials (metallic nanoparticles, quantum dots) that enhance the analytical signal. Nevertheless, these techniques typically have lower selectivity and greater detection limits, making them more appropriate for initial screening than for final quantification in complicated matrices (Alezra et al., 2026).

With its speed, portability, and lower cost per sample, immunoassays such as ELISA (Enzyme-Linked Immunosorbent Assay) offer a promising alternative for large-scale surveillance. Diclofenac can be detected in water at concentrations between 10 and 50 ng/L using commercial tests based on specific antibodies. However, their use as a single approach for regulatory purposes is limited by the potential for cross-reactions with structurally related chemicals and the requirement for validation against reference procedures (Martínez-Rodríguez, Martínez-Rodríguez, Hernández-Perales, del Pilar González-Muñoz & Avila-Rodriguez, 2025). New developments in environmental drug detection include biosensors based on aptamers, immobilized enzymes, or entire cells connected to optical or electrochemical transducers. These gadgets can enable real-time detection, field portability, and integration with the Internet of Things (IoT) systems for ongoing surveillance. Biosensors for diclofenac have already demonstrated competitive detection limits (below 1 ng/L under optimal conditions) and good specificity, although most applications remain in the laboratory development stage (Zwane, Kuvarega, Mamba, Feleni, 2025). To guarantee data trustworthiness, methodological validation is crucial, regardless of the approach selected. International guidelines (e.g., ISO, EPA, ICH) require a thorough evaluation of parameters such as limit of detection (LOD), limit of quantification (LOQ), accuracy (recovery in fortified matrices), precision (repeatability and reproducibility), linearity, and selectivity. Additionally, matrix blanks, participation in laboratory intercomparison programs, and internal isotopic standards (such as diclofenac-d4) for loss correction and matrix effects should all be part of analytical quality control (Rakhymbay et al., 2026).

The matrix effect, which can suppress or increase the analytical signal in mass spectrometry, is a recurrent problem in the environmental detection of diclofenac, particularly in raw sewage samples or sediments rich in organic matter. Fundamental techniques for reducing these interferents include sample dilution, two-step SPE for further purification, and the use of internal isotopic standards (Van Do et al., 2025). The stability of the analyte during storage is another

crucial factor. If samples are not properly kept (e.g., acidification, refrigeration, analysis within 48 hours), diclofenac may suffer photolytic or biological degradation. In conclusion, a stratified analytical approach is necessary for the accurate detection of diclofenac in the environment: high-resolution confirmatory methods (LC-MS/MS) for precise quantification and regulatory compliance, followed by quick screening techniques (immunoassays, biosensors) to identify critical areas (Pietrini et al., 2025). To make monitoring of emerging pollutants more frequent, accessible, and geographically comprehensive, future trends indicate the combination of pretreatment automation, artificial intelligence for data processing, and smaller analytical platforms. The generation of consistent data to support public policies, assess the efficacy of remediation technologies, and safeguard ecosystems and human health from the effects of diclofenac and other medicines in the environment can only be feasible with reliable and proven procedures (Garg, Alattar, Sabouni & Ghommem, 2025).

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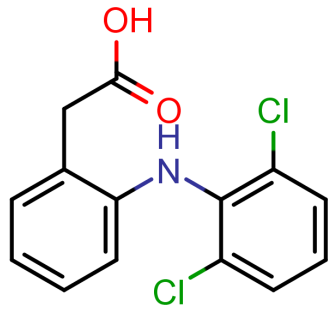
## 5. Diclofenac: properties and environmental distribution

One of the most popular NSAIDs is diclofenac [2-(2,6-dichloroanilino) phenylacetic acid], which inhibits cyclooxygenase, an enzyme that makes prostanoids. Table 1 summarizes the physicochemical characteristics of diclofenac sodium. Diclofenac is quickly and fully absorbed in the colon following oral administration, where it is hydroxylated and glucuronidated. Its oxidation to 4'-hydroxylated and 5'-hydroxylated derivatives is catalyzed by the cytochrome P450 protein family enzymes CYP3A4 and CYP2C9, whereas glucuronidation is catalyzed by UDP-glucuronosyltransferase-2B7 (UGT2B70). 65% of the oxidized metabolites are eliminated by the kidneys. The remainder is eliminated in the bile as acylglucuronide. Chemically unstable diclofenac acylglucuronides can be epimerized by acyl migration to 2-O-glucuronide, 3-O-glucuronide, or 4-O-glucuronide, particularly in bile's alkaline environment (Krasniqi, Dimovski, Domjanović, Bilić & Božina, 2016). After consumption, some diclofenac is not metabolized, and the sewage system releases it into the wastewater treatment facility either unaltered or slightly modified. Diclofenac's maximum concentrations in wastewater are thought to range from 0.01 µg/L to 510 µg/L. Even though modern oxidation procedures can remove diclofenac with up to 80% effectiveness, the limitations of physicochemical methods often preclude their use (Chen et al., 2014; Navrozidou et al., 2019).

Table 1. Main physical and chemical properties of diclofenac sodium.

| Property | Value/Description |
|---|---|
| Chemical name | 2-[2-(2,6-dichloroaniline) phenylacetate sodium |
| Molecular formula | $C_{14}H_{10}Cl_2NNaO_2$ |
| Chemical structure |   |
| Molar mass | 318.13 g/mol |
| CAS Number | 15307-79-6 |
| Density | 0.781 g/cm³ |
| Solubility in water | Slightly soluble (~50 mg/mL) |
| pKa | ~4 (a 25 °C) |
| Partition coefficient (Log P) | ~4.5 (for diclofenac acid) |

Note: chemical structure done with Marvinsketch from Chemdraw

Environmentally safe biological approaches are substitutes for harsh chemical methods. Nevertheless, diclofenac, a hydrophobic chlorinated derivative with electron-donating and electron-withdrawing groups that shows log < 3.2 at pH 8, is not biologically degradable (Żur, Piński, Wojcieszyńska, Smułek, & Guzik, 2020). In biological treatment plants, treatment efficiency ranges from 21% to 40%, with values as high as 80% reported (Moreira et al., 2018). Diclofenac

and its derivatives end up in the water because wastewater treatment facilities are not very effective at degrading this medication (Navrozidou et al., 2019). Around the world, diclofenac can be found in drinking water, surface water, groundwater, and soil (Table 2) (Ali, Sydnes, Alarif, Al-lihaibi & Aly, 2019; Biel-Maeso, Corada-Fernández & Lara-Martín, 2018; Ebele, Abou-Elwafa Abdallah & Harrad, 2017; Scheurell, Franke, Shah & Hühnerfuss, 2009).

Table 2. Diclofenac concentration in the surrounding environment.

| Sources | Country | Concentration | Reference |
|---|---|---|---|
| Soil: Jerez de la Frontera | Spain | 5.06 ng/g | (Biel-Maeso et al., 2018) there is the risk of potential accumulation of contaminants of emerging concern (CECs |
| Sawan River | Pakistan (Rawalpindi-Islamabad) | 62 µg/L | (Hanif et al., 2020)their continuous addition in the environment may pose hazardous effects. Among these, diclofenac (DCF |
| Ling Stream | Pakistan (Rawalpindi-Islamabad) | 23 µ/l | (Hanif et al., 2020) their continuous addition in the environment may pose hazardous effects. Among these, diclofenac (DCF |
| Sediments Ebro Delta region | Spain (Catalonia) | 6.8–7.5 ng/g | (Baranowska & Kowalski, 2011) |
| Gumrah Kas | Pakistan (Rawalpindi-Islamabad) | 14 µg/L | (Hanif et al., 2020) their continuous addition in the environment may pose hazardous effects. Among these, diclofenac (DCF |
| Vltava river | The Czech Republic (Prague) | 0.104 µg/L | (Baranowska & Kowalski, 2011) |
| Korang River | Pakistan (Rawalpindi-Islamabad) | 28 µg/L | (Hanif et al., 2020) their continuous addition in the environment may pose hazardous effects. Among these, diclofenac (DCF |
| Seawater | Portugal | 30.6 ng/L | (Baranowska & Kowalski, 2011) |
| Malir River | Pakistan (Karachi) | 0.08–0.3 µg/L | (Scheurell et al., 2009) |
| Odra river | Poland (Wrocław) | 0.429 µg/L | (Baranowska & Kowalski, 2011) |
| Swiss lakes | Switzerland | 1–12 ng/L | (Ebele et al., 2017) |
| Beiyun River | China | 1.8–1300 ng/L | (Yang et al., 2017) |
| Wisła river | Poland (Skoczów) | 74 ng/L | (Baranowska & Kowalski, 2011) |
| Groundwater survey | USA (Montana) | 46 ng/L | (Lapworth et al., 2012) |
| Mbokodweni river | South Africa (KwaZulu-Natal) | 0.9–5.3 µg/L | (Madikizela, Tavengwa & Chimuka, 2017) |
| Huangpu River | China | 13.6 ng/L | (Cherik, Benali & Louhab, 2015) |
| Umgeni River | South Africa (KwaZulu-Natal) | 10 µg/L | (Lorenzo et al., 2021) |
| Stream | Antarctica (Seymour/Marambio Island) | 77 ng/L | (González-Alonso et al., 2017) |
| Natural waters | Brazil (Rio de Janeiro) | 0.01–0.06 mg/L | (Lapworth et al., 2012) |
| Kaveri river | India | 103 ng/L | (Shanmugam, Sampath, Selvaraj, Larsson & Ramaswamy, 2014) |
| Tejo estuary | Portugal | 51.8 ng/L | (Reis-santos et al., 2018) |
| Wörthsee lake | Germany | 10–15 ng/L | (Huebner, Weber, Niessner, Boujday & Knopp, 2015) it has raised increased public concern on potential long-term impact on human health and wildlife. The importance of DCF has been emphasized by the European Union recently by including this pharmaceutical in the first watch list of priority hazardous substances in order to gather Union-wide monitoring data. Rapid and cheap methods of analysis are therefore required for fresh and wastewater monitoring with high sample load. Here, for the first time, well-characterized monoclonal antibodies (mAbs |
| Warta river | Poland (Częstochowa) | 0.277 µg/L | (Baranowska & Kowalski, 2011) |
| Mississippi river | USA (Louisiana) | 22–107 ng/L | (Lapworth et al., 2012) |
| Stream | Antarctica (Fildes Peninsula) | 84 ng/L | (González-Alonso et al., 2017) |
| Isar River | Germany | 9–13 ng/L | (Huebner et al., 2015) it has raised increased public concern on potential long-term impact on human health and wildlife. The importance of DCF has been emphasized by the European Union recently by including this pharmaceutical in the first watch list of priority hazardous substances in order to gather Union-wide monitoring data. Rapid and cheap methods of analysis are therefore required for fresh and wastewater monitoring with high sample load. Here, for the first time, well-characterized monoclonal antibodies (mAbs) |
| Aabach river | Switzerland | 11–310 ng/L | (Lapworth et al., 2012) |
| Danube river | Hungary (Budapest) | 7–90 ng/L | (Lapworth et al., 2012) |
| Red Sea | Saudi Arabia | 26.9 ng/L | (Ali et al., 2019) |

The average concentration in rivers worldwide is estimated at 0.02 ± 0.72 µg/L, and in extreme cases, freshwater concentrations have even been reported in the µg/L range (Fu et al., 2020). Additionally, depending on the climate, diclofenac has been detected in sewage sludge at concentrations as high as 87 ng/g and in soils at concentrations as high as 5.6 ng/g (Biel-Maeso et al., 2018). To remove this chemical from water, novel, effective, and financially feasible technologies must be developed, including those based on immobilized microbes.

## 6. Toxicity of diclofenac to non-target organisms

The near-total extinction of three vulture species in the Indian subcontinent was the most sad consequence of diclofenac's impact on non-target creatures (Naidoo, Wolter, Cuthbert & Duncan, 2009; Swan et al., 2006). This medication was widely used in India and Pakistan in the 1990s to treat infectious illnesses and trauma-induced inflammation in cattle and buffalo. Vultures that consumed the corpses of diclofenac-treated animals developed kidney failure and died as a result of the drug building up in their systems (Chen et al., 2014). The buildup of uric acid crystals in the visceral organs (visceral fundus) caused nephrotoxicity in vultures. A thorough investigation (Swan et al., 2006) found impaired renal architecture and markedly elevated uric acid and alanine aminotransferase levels. However, because of the low drug concentration found in the environment, consequences like those mentioned above are rare. Research is currently examining the possibility of negative consequences for aquatic organisms exposed to low concentrations of diclofenac over extended periods (Table 3) (Sathishkumar, Anu, Meena, & Palanisami, 2020).

Table 3. Diclofenac's toxicity to non-target creatures.

| Exposition time/ Concentration (mg/L) | Organism | Effect | Reference |
|---|---|---|---|
| 28 days/0.0046 | *Gasterosteus aculeatus* | Jaw lesions and renal hematopoietic hyperplasia | (Näslund, Asker, Fick, Larsson & Norrgren, 2020) |
| 35 days/0.001 | *Cirrhinus mrigala* | Reduction in the amounts of triiodothyronine and thyroxine | (Saravanan, Hur, Arul, & Ramesh, 2014) |
| 4 days/0.001 | *Oryzias latipes* | p53 gene expression induction | (Parolini, Binelli, & Provini, 2011) 6-dichlorophenyl |
| 6 months/0.00382 | *Dreissena polymorpha* | High genotoxicity, impacts on immunity, and high death rates | (Joachim et al., 2021) |
| 21 days/0.271 | *Gasterosteus aculeatus* | Death-LOEC | (Näslund et al., 2020) |
| 36–58 h/0.25/kg | *Gyps bengalensis* | Death from visceral gout and renal failure | (Peters, A., Crane, M., Merrington, G., & Ryan, 2022) |
| 96 h/0.48 ± 0.05 | *Danio rerio* | Death-LC50 | (Feito, Valcárcel & Catalá, 2012) |
| -/0.09 ± 0.02 | *Danio rerio* | EC50 for teratogenicity | (Feito et al., 2012) |
| 90 min/0.00003 | *Danio rerio* | Reduced lipid peroxidation in the zebrafish embryo | (Feito et al., 2012) |
| 25 days/0.1 | *Salmo trutta f. fario* | Hepatocytes with irregular shapes, vesiculations, degenerating nuclei, and no glycogen storage | (Schwarz, Schmieg, Scheurer, Köhler & Triebskorn, 2017) |
| 96 h/1 | *Lithobates catesbeianus* | Oedema, slowed growth, and axial abnormalities in the tail and notochord are examples of induction malformations. | (Cardoso-vera et al., 2017) |
| 96 h/1 | *Xenopus laevis* | Oedema, slowed growth, and axial abnormalities in the tail and notochord are examples of induction malformations. | (Cardoso-vera et al., 2017) |
| 96 h/0.001 | *Danio rerio* | Decreased gill cell viability | (Parolini, 2020b) antipyretic and anti-inflammatory properties to cure pain and inflammation in human and veterinary therapy. After use, NSAIDs are excreted in their native form or as metabolites, entering the aquatic ecosystems. A number of monitoring surveys has detected the presence of different NSAIDs in freshwater ecosystems in the ng/L - µg/L concentration range. Although the concentrations of NSAIDs in surface waters are low, the high biological activity of these molecules may confer them a potential toxicity towards non-target aquatic organisms. The present review aims at summarizing toxicity, in terms of both acute and chronic toxicity, induced by the main NSAIDs detected in surface waters worldwide, namely acetylsalicylic acid (ASA |

| Exposition time/ Concentration (mg/L) | Organism | Effect | Reference |
|---|---|---|---|
| | | | (Parolini, 2020b) antipyretic and anti-inflammatory properties to cure pain and inflammation in human and veterinary therapy. After use, NSAIDs are excreted in their native form or as metabolites, entering the aquatic ecosystems. A number of monitoring surveys has detected the presence of different NSAIDs in freshwater ecosystems in the ng/L - µg/L concentration range. Although the concentrations of NSAIDs in surface waters are low, the high biological activity of these molecules may confer them a potential toxicity towards non-target aquatic organisms. The present review aims at summarizing toxicity, in terms of both acute and chronic toxicity, induced by the main NSAIDs detected in surface waters worldwide, namely acetylsalicylic acid (ASA |
| 48 h/0.01 | *Danio rerio* | Decreased digestive cell viability | |
| 10 days/0.0001 | *Lemna minor* | Reduced photosynthetic pigment concentration, elevated reactive nitrogen and oxygen species in roots, elevated lipid peroxidation, and disruption of membrane integrity | (Babula & Kummerová, 2016) |
| | | | (Parolini, 2020b) antipyretic and anti-inflammatory properties to cure pain and inflammation in human and veterinary therapy. After use, NSAIDs are excreted in their native form or as metabolites, entering the aquatic ecosystems. A number of monitoring surveys has detected the presence of different NSAIDs in freshwater ecosystems in the ng/L - µg/L concentration range. Although the concentrations of NSAIDs in surface waters are low, the high biological activity of these molecules may confer them a potential toxicity towards non-target aquatic organisms. The present review aims at summarizing toxicity, in terms of both acute and chronic toxicity, induced by the main NSAIDs detected in surface waters worldwide, namely acetylsalicylic acid (ASA |
| 48 h/0.001 | *Danio rerio* | Decreased hemocyte viability | |
| 24 h/216 | *Gammarus pulex* | Death-LC50 | (Fu et al., 2020) |
| | | | (Parolini, 2020b) antipyretic and anti-inflammatory properties to cure pain and inflammation in human and veterinary therapy. After use, NSAIDs are excreted in their native form or as metabolites, entering the aquatic ecosystems. A number of monitoring surveys has detected the presence of different NSAIDs in freshwater ecosystems in the ng/L - µg/L concentration range. Although the concentrations of NSAIDs in surface waters are low, the high biological activity of these molecules may confer them a potential toxicity towards non-target aquatic organisms. The present review aims at summarizing toxicity, in terms of both acute and chronic toxicity, induced by the main NSAIDs detected in surface waters worldwide, namely acetylsalicylic acid (ASA |
| -/0.06 | *Dreissena polymorpha* | Fragmentation of DNA | |
| -/0.001 | *Oncorhynchus mykiss* | Changes in the liver, kidney, and gills' cytology | (Fu et al., 2020) |

| Exposition time/ Concentration (mg/L) | Organism | Effect | Reference |
|---|---|---|---|
| 21 days/2 | *Danio magna* | Death-LC50 | (Parolini, 2020b) antipyretic and anti-inflammatory properties to cure pain and inflammation in human and veterinary therapy. After use, NSAIDs are excreted in their native form or as metabolites, entering the aquatic ecosystems. A number of monitoring surveys has detected the presence of different NSAIDs in freshwater ecosystems in the ng/L - µg/L concentration range. Although the concentrations of NSAIDs in surface waters are low, the high biological activity of these molecules may confer them a potential toxicity towards non-target aquatic organisms. The present review aims at summarizing toxicity, in terms of both acute and chronic toxicity, induced by the main NSAIDs detected in surface waters worldwide, namely acetylsalicylic acid (ASA |
| -/0.5 | *Danio magna* | Decrease in the production of eggs | (Parolini, 2020b) antipyretic and anti-inflammatory properties to cure pain and inflammation in human and veterinary therapy. After use, NSAIDs are excreted in their native form or as metabolites, entering the aquatic ecosystems. A number of monitoring surveys has detected the presence of different NSAIDs in freshwater ecosystems in the ng/L - µg/L concentration range. Although the concentrations of NSAIDs in surface waters are low, the high biological activity of these molecules may confer them a potential toxicity towards non-target aquatic organisms. The present review aims at summarizing toxicity, in terms of both acute and chronic toxicity, induced by the main NSAIDs detected in surface waters worldwide, namely acetylsalicylic acid (ASA |
| 15 days/0.25 | *Mytillus galloprovincialis* | High levels of catalase activity and lipid peroxidation in the digestive gland, as well as the induction of superoxide dismutase and glutathione reductase in the gills | (Gonzalez-rey & Bebianno, 2014) |
| 1 h/0.25 | *Dreissena polymorpha* | Lysosomal membrane destabilization | (Parolini, 2020b) antipyretic and anti-inflammatory properties to cure pain and inflammation in human and veterinary therapy. After use, NSAIDs are excreted in their native form or as metabolites, entering the aquatic ecosystems. A number of monitoring surveys has detected the presence of different NSAIDs in freshwater ecosystems in the ng/L - µg/L concentration range. Although the concentrations of NSAIDs in surface waters are low, the high biological activity of these molecules may confer them a potential toxicity towards non-target aquatic organisms. The present review aims at summarizing toxicity, in terms of both acute and chronic toxicity, induced by the main NSAIDs detected in surface waters worldwide, namely acetylsalicylic acid (ASA |
| 24 h/175 | *Hyalella azteca* | Death-LC50 | (Fu et al., 2020) |
| 96 h/25.12 | *Clarias gariepinus* | LC50 mortality | (Ajima, Ogo, Audu, & Ugwoegbu, 2015) |

Diclofenac's bioaccumulation in living organisms, particularly aquatic microbes, is driven by its physicochemical properties, particularly the water/n-octanol partition coefficient (log Kow, 4.51) (Żur et al., 2020). Studies using species such as brown trout (Salmo trutta fario), common carp (Cyprinus carpio), longfin smelt (Gasterosteus aculeatus), and rainbow trout (Oncorhynchus mykiss) have shown the harmful effects of diclofenac, even at low µg/L doses (Fu et al., 2020). Up to doses of 100 µg/L, brown trout embryos exposed to diclofenac did not exhibit any signs of embryotoxicity. A NOEC of 500 µg/L is used for teratogenicity, development, hatching, and mortality. The embryonic and larval stages of Oncorhynchus mykiss, Danio rerio, and Cyprinus carpio exhibited similar outcomes, with notable effects occurring only at doses above 1 mg/L. When exposed to diclofenac, juvenile brown trout reacted far more sensitively than larvae.

At diclofenac concentrations in the μg/L range, there is a concerning rise in mortality. Longlines subjected to diclofenac showed a concentration-dependent increase in mortality, which became significant at 320 μg/L. Spinel-tricuspid showed a comparable effect at 271 μg/L diclofenac (Näslund et al., 2020). Diclofenac's EC50 for carp was found to be 71 mg/L in acute toxicity testing conducted on mature fish (Schwarz et al., 2017).

The growth curves of the rotifer populations *Moina macrocopa* and *Plationus patulous* were similarly changed by exposure to increasing diclofenac concentrations, ranging from 2 to 32 mg/L. This resulted in a decrease in organism density with increasing drug concentration and a decrease in daily population growth (Parolini, 2020a). After exposure to diclofenac for 96 hours, Danio rerio showed signs of teratogenicity (EC50: 90 ± 20 μg/L) and mortality (LC50: 480 ± 50 μg/L). LOEC and NOEC values at 8000 and 4000 μg/L, respectively, were determined using chronic toxicity bioassays on the viability of *Danio rerio* embryos exposed to diclofenac for ten days (Feito et al., 2012). Diclofenac's long-term toxicity in *Daphnia magna* was also investigated at the molecular and biochemical levels. Individual mortality rose following a 24-hour exposure to high diclofenac concentrations (486 mg/L). After 21 days of exposure, 50% of *D. magna* died after exposure to 2 mg/L of diclofenac, and at a dosage of 0.5 mg/L, egg production was significantly reduced. The expression of several genes linked to detoxification, growth, development, and reproduction was significantly altered after a 96-hour exposure to 50 μg/L of diclofenac. After 24 hours of exposure, their expression was suppressed, and after 48 hours, overexpression was seen (Parolini, 2020a).

In a long-term freshwater mesocosm experiment, researchers investigated the detrimental impacts of diclofenac on primary producers and consumers (Joachim et al., 2021). The investigation lasted six months, and the effective concentrations were 0.041 μg/L, 0.44 μg/L, and 3.82 μg/L. This experiment, designed to mimic environmental conditions, showed that diclofenac was more hazardous to non-target species than in the lab. The biovolume of macrophytes (*Callitriche platycarpa* and *Nasturtium officinale*) dramatically dropped during the course of the six-month exposure period. At every concentration tested, *Dreissena polymorpha* showed severe genotoxicity, decreased immunity, and high mortality. Additionally, the *Gasterosteus aculeatus* population structure changed at the highest concentration. The percentage of adult fish increased after a month of exposure, while the overall fish stock and juvenile percentage declined. As a result, the F1 generation's duration and frequency distributions changed overall compared to the control (Joachim et al., 2021).

When evaluating the harmful effects of diclofenac on non-target organisms, the toxicity of diclofenac biotransformation intermediates is essential to consider. Research on this phenomenon has focused on two important aquatic invertebrates: *Hyalella azteca* with *Gammarus pulex* (Fu et al., 2020). Diclofenac was transformed into a number of oxidation products and conjugates in both species, including the diclofenac methyl ester and the taurine-diclofenac conjugate. It was shown that the bioconcentration factor of these intermediates was significantly higher than that of the original medication. Additionally, for both species, diclofenac methyl ester exhibited greater acute toxicity than diclofenac alone, consistent with its higher bioconcentration potential. For *H. azteca*, the $LC_{50}$ of diclofenac was 216 mg/L, whereas the $LC_{50}$ of diclofenac methyl ester was just 0.53 mg/L. This indicates a 430-fold increase in acute toxicity when compared to diclofenac alone. Because of its somewhat reduced hydrophobicity, the diclofenac-taurine combination was less harmful to *H. azteca* than its parent compound (Fu et al., 2020). Additionally, it was found that the two most commonly observed hydroxylated derivatives of diclofenac, 5-OH-diclofenac and 4′-OH-diclofenac, can be further oxidized to reactive benzoquinone imines that interact with the protein's nucleophilic groups to form adducts (Żur et al., 2020). These results unequivocally demonstrate that a more thorough analysis of the toxicity of medications' biotransformation products is essential when studying the toxicity of pharmaceuticals in non-target organisms (Fu et al., 2020).

Diclofenac's toxicity mechanism can be better understood through histological investigation. Severe tissue responses and lesions, particularly in the liver, were more common in fish exposed to this medication. Researchers found lesions in the tricuspid longline jaw and renal hematopoietic hyperplasia at the lowest tested concentration of 4.6 μg/L (Näslund et al., 2020). It is crucial to stress that this is a concentration found in the surroundings. Reduced glycogen levels and macrophage infiltration have been found in rainbow trout liver ultrastructural investigations (Schwarz et al., 2017). Additionally, it has been demonstrated that even low environmental concentrations of diclofenac (1 μg/L) cause cellular alterations in rainbow trout's liver, kidneys, and gills that lower kidney and gill function (Parolini et al., 2011) 6-dichlorophenyl. Another in vitro study examined the toxicity of diclofenac in three distinct zebra mussel cell types: hemocytes, gill cells, and digestive gland cells, at concentrations ranging from 0.001 mg/L, 0.01 mg/L, 0.1 mg/L, 1 mg/L, and 10 mg/L. Even at the lowest dose used, a notable decrease in the viability of gill cells treated with diclofenac was observed after 96 hours of exposure. Additionally, after 48 hours of exposure to 0.01 mg/L diclofenac, the vitality of digestive cells was dramatically reduced, whereas hemocyte viability declined at 0.001 mg/L (Parolini, 2020a).

It is unexpected that diclofenac appears to affect interindividual connections in exposed fish. With an LOEC of 10 μg/L, the proportion of people displaying violent behavior rose dramatically as diclofenac concentration climbed. Additionally, behavioral alterations in African catfish (*Rhamdia quelen*) exposed to 25 mg/L of diclofenac were noted.

These included unsteady swimming, loss of balance, and respiratory failure, but there were no indications of hostility. This could be a result of the animals' diminished ability or inclination to defend themselves due to their debilitated state. For instance, after being exposed to diclofenac, the spiny fish showed a larger percentage of skin ulcers and were leaving food behind. Animals with open wounds are susceptible to possibly fatal outcomes due to pathogen infections. However, altering fish density may increase diclofenac-induced mortality and lead to increased aggressive behavior. The finding that diclofenac causes ocular perforation in rainbow trout is another explanation for the heightened aggression seen in young fish. Panic reactions triggered by vision impairment may cause behavioral abnormalities in young brown trout (Ajima et al., 2015; Schwarz et al., 2017).

Research on carp has demonstrated a notable rise in lipid peroxidation and hydroperoxide content at a diclofenac dose of 7.1 mg/L (Schwarz et al., 2017). Zebrafish embryos exposed to 30 ng/L of diclofenac for 90 minutes showed decreased levels of lipid peroxidation (LPO). Diclofenac was harmful to *Perna perna* mussels at ambient concentrations (ng/L), resulting in increased cyclooxygenase activity, reduced lysosomal membrane integrity, elevated oxidative stress, and DNA damage. Lipid peroxidation levels were increased in zebra mussels treated with 1 µg/L of diclofenac. Nevertheless, the mussel experienced oxidative stress. Despite being subjected to 0.25 µg/L of diclofenac, *Mytilus galloprovincialis* (Sathishkumar, Meena et al., 2020). Additionally, higher levels of enzymes, including catalase, superoxide dismutase, and glutathione transferase, as well as lipid peroxidation, were seen in zebrafish in response to oxidative stress caused by diclofenac and its photolysis products (Diniz et al., 2015). In microorganism cultures, diclofenac also causes oxidative stress. Increased catalase and superoxide dismutase activity, as well as the production of lipid peroxidation products, have all been shown. This process was accompanied by changes in the biological membrane and cell surface. *Pseudomonas moorei* KB4 strain treatment to diclofenac resulted in a decrease in zeta potential and an increase in cell wall hydrophobicity, according to multivariate analysis. Additionally, changes in the membrane fatty acid composition (including the emergence of a branched cyclopropane-17:0 cyclo and 19:0 anteiso fatty acid) were found to cause considerable membrane stiffness (Marchlewicz, Pi, Guzik, Wojcieszy & Zur, 2021). Diclofenac, as well as its metabolites, can induce oxidative stress in cells of non-target organisms.

In mouse liver cells, researchers assessed the toxicity of diclofenac intermediates produced during the drug's breakdown by *Enterobacter cloacae* isolated from an organic molecule (Aissaoui, Sifour, Ouled-Haddar, Benguedouar & Lahouel, 2017). Diclofenac at therapeutic concentrations and its metabolites have been shown to affect oxidative stress parameters, including reduced glutathione reserves, lipid peroxidation, and disruptions in hepatic detoxification enzymes, such as glutathione S-transferase, catalase, and superoxide dismutase. Nonetheless, these researchers caution that exposure to ambient concentrations of diclofenac and its metabolites did not have a detrimental effect on oxidative stress measures in mouse cells (Aissaoui et al., 2017).

The immunotoxicity of Diclofenac in snails (*Lymnaea stagnalis*) was assessed. For three days, they were exposed to ambient (1–10 µg/L) and therapeutic (100–1000 µg/L) concentrations of diclofenac. Diclofenac has a major impact on cochlear hemocytes' immunological function. An increase in NADPH oxidase activity, primarily following medication usage at a dose of 1000 µg/L, confirms that this result is typical of an inflammatory response (Parolini, 2020a). It was also demonstrated that the concentration of diclofenac affected the expression of hepatic C7 genes. The complement system, which is a component of the innate immune system, includes the C7 protein. Together with other complement component proteins, it creates a membrane assault complex that causes foreign cells to lyse. The arachidonic acid pathway links complement components, explaining how NSAID exposure affects C7 (Näslund et al., 2020). In turn, hemocytes from Dreissena polymorpha were exposed to 60 µg/L, 126 µg/L, and 250 µg/L of diclofenac for 60 minutes to assess the drug's cytogenotoxicity in vitro. Only exposure to 250 µg/L of diclofenac showed a substantial cytotoxic effect in the instability of lysosomal membranes, whereas exposure to all tested dosages resulted in primary genetic changes and persistent DNA damage (Parolini, 2020b) antipyretic and anti-inflammatory properties to cure pain and inflammation in human and veterinary therapy. After use, NSAIDs are excreted in their native form or as metabolites, entering the aquatic ecosystems. A number of monitoring surveys has detected the presence of different NSAIDs in freshwater ecosystems in the ng/L - µg/L concentration range. Although the concentrations of NSAIDs in surface waters are low, the high biological activity of these molecules may confer them a potential toxicity towards non-target aquatic organisms. The present review aims at summarizing toxicity, in terms of both acute and chronic toxicity, induced by the main NSAIDs detected in surface waters worldwide, namely acetylsalicylic acid (ASA.

Additionally, the mussel showed increased DNA fragmentation. *Mytilus galloprovincialis* following exposure to a quantity of 2.5 µg/L in the environment (Sathishkumar, Meena et al., 2020). However, after exposure to 1 µg/L of diclofenac for 4 days, Oryzias latipes showed increased p53 gene expression. Because its product is essential for cell cycle arrest, apoptosis, and DNA repair, the p53 gene is a significant biomarker in studies of carcinogenicity and DNA damage (Parolini et al., 2011) 6-dichlorophenyl. Studies on diclofenac's toxicity have not produced definitive findings, and other reports suggest that aquatic species are not at risk from diclofenac at ambient quantities. According to research, fish

have a very low bioconcentration of diclofenac, consistent with the drug's molecular characteristics, including a pKa of 3.99–4.16 (Memmert et al., 2013). Additionally, these authors show that concentrations above 320 µg/L reduce zebrafish growth, despite the NOEC being estimated at 10 µg/L (Aissaoui et al., 2017). Nonetheless, the findings of this study appear to be supported by the number of reports of diclofenac's negative effects on non-target organisms (Näslund et al., 2020). These authors recommend substituting naproxen, a drug with comparable efficacy in pain management, with diclofenac where therapeutically viable to lower the environmental risk associated with diclofenac toxicity (Georgin et al., 2021). While the effects of naproxen and diclofenac on fish are similar, naproxen poses fewer environmental risks and hazards than diclofenac because toxic effects manifest at greater naproxen concentrations (Näslund et al., 2020).

Plants are also vulnerable to the harmful effects of diclofenac due to the rising concentrations of NSAIDs in the soil. Diclofenac sensitivity differs by species, according to research on the stress response it causes in two crops, tomato and maize. The tomato was more susceptible, exhibiting reduced maximum quantum efficiency of PSII, reduced PSII activity, growth inhibition, and decreased photosynthetic pigment concentrations. Diclofenac, however, had no influence on the amount of photosynthetic pigments or the growth of maize. Diclofenac did, however, affect the PSII photosystem's quantum efficiency. Oxidative stress was also observed in both plants, as evidenced by an increase in hydrogen peroxide levels. As a result, the plants produced phenolic compounds as a defense mechanism (Figure 1) (Siemieniuk, Ludynia & Rudnicka, 2021). The green line shows the degree of control over the parameters shown; the data are displayed as a percentage of the control. FW stands for fresh weight, DW for dry weight, SWR for shoot weight ratio, RWR for root weight ratio, SLR for shoot length ratio, RLR for root length ratio, WC for water content, and R:S for root/shoot ratio. An asterisk indicates values that, according to the unpaired Student's t-test, differ significantly from the control ($p < 0.05$). (A) Zea mays L., seven days following diclofenac addition; (B) Zea mays L., 14 days following diclofenac addition; (C) Solanum lycopersicum L., seven days following diclofenac administration; (D) Solanum lycopersicum L., seven days following diclofenac administration. The SLR and RLR ratios were delineated with a dashed line, and the % values were written numerically in order to preserve graph D's clarity. Similarly, diclofenac has been shown to lower assimilation coefficients and stomatal conductance to water vapor in beans (Copolovici et al., 2017). Additionally, monoterpene concentrations (camphene, α-pinene, and 3-carene) were found to have increased. According to the authors' hypothesis, diclofenac may similarly disrupt the methylerythritol phosphate pathway in plastids.

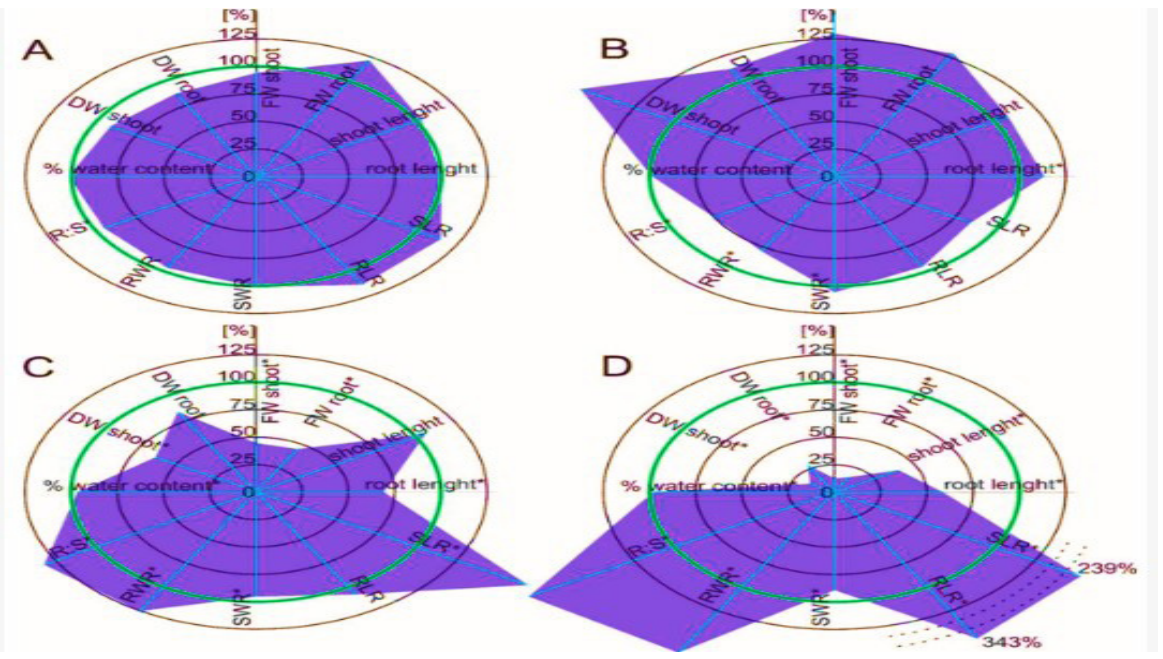



Figure 1. A radar graph showing how certain plant growth factors react to diclofenac. Reproduced from Siemieniuk et al., 2021, under the CC BY 4.0 license.

## 7. Diclofenac biodegradation by fungi and bacteria

There are currently very few known bacteria and fungi that can break down diclofenac. Furthermore, because of the development of difficult-to-degrade intermediates, only partial breakdown of this medication is often reported in the literature. Fungi that can degrade diclofenac include *T. polyzona*, *Trametes trogii*, *Aspergillus niger*, *Yarrowia lipolytica*, *Mucor circinelloides*, *Phanerochaete chrysosporium*, *Rhizopus microspore*, and *Trichoderma longibrachiatum* (Blanco-Orta, el at., 2023). Among the microorganisms that break down diclofenac, strains that stand out include *Bacillus subtilis* and *Raoultella sp.* DD4, *Rhodococcus ruber*, *Brevibacillus laterosporus*, *Alcaligenes faecalis*, *Labrys portucalensis* F11, *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Klebsiella sp.* KSC, *Pseudomonas moorei* KB4, and *Proteus mirabilis* were described (Domaradzka, Guzik, Hupert-Kocurek, & Wojcieszyńska, 2016; Grandclément et al., 2020; Ivshina, Tyumina, Kuzmina, & Vikhareva, 2019; Murshid & Dhakshinamoorthy, 2019; Stylianou, Hapeshi, Vasquez & Fatta-kassinos, 2018). Laccase, manganese peroxidase, and the cytochrome P450 enzyme system hydroxylate diclofenac,

most commonly producing intermediates such as 5-hydroxydiclofenac, 4-hydroxydiclofenac, 4,5-dihydroxydiclofenac, and 3-hydroxydiclofenac (Nosek & Zhao, 2024).

Additionally, diclofenac was broken down by the ligninolytic fungi *M. circinelloides*, *T. polyzona*, and *T. longibrachiatum* into intermediates: 2,4-dichlorobenzoic acid, 2,6-dichlorobenzoic acid adducts, and 3,5-dichlorobenzoic acid, which verified the cleavage of the CN link in the drug structure. These intermediates vanished from the culture after 10 days of incubation, suggesting that ring cleavage was the next step in their disintegration. The fungi's drug tolerance and manganese peroxidase activity were positively correlated. However, no such relationship was seen in terms of drug degradation efficiency, indicating that these strains' process of diclofenac degradation is more intricate (Kasonga, Coetzee, Kamika & Momba, 2021). The arrangement of aerated batch flasks (ABF) for assessing the elimination of certain pharmaceutical chemicals by separately isolated native fungal strains from South Africa is depicted in Figure 2. Samples were taken every day from day zero to day five, and then on days seven, ten, and fourteen. The experiments were conducted in duplicate (n = 2) during a 14-day period. Before adding to the cartridge column, the collected samples (1 mL) were diluted to 10 mL by adding 9 mL of pH 2.5 distilled water (Kasonga et al., 2021).

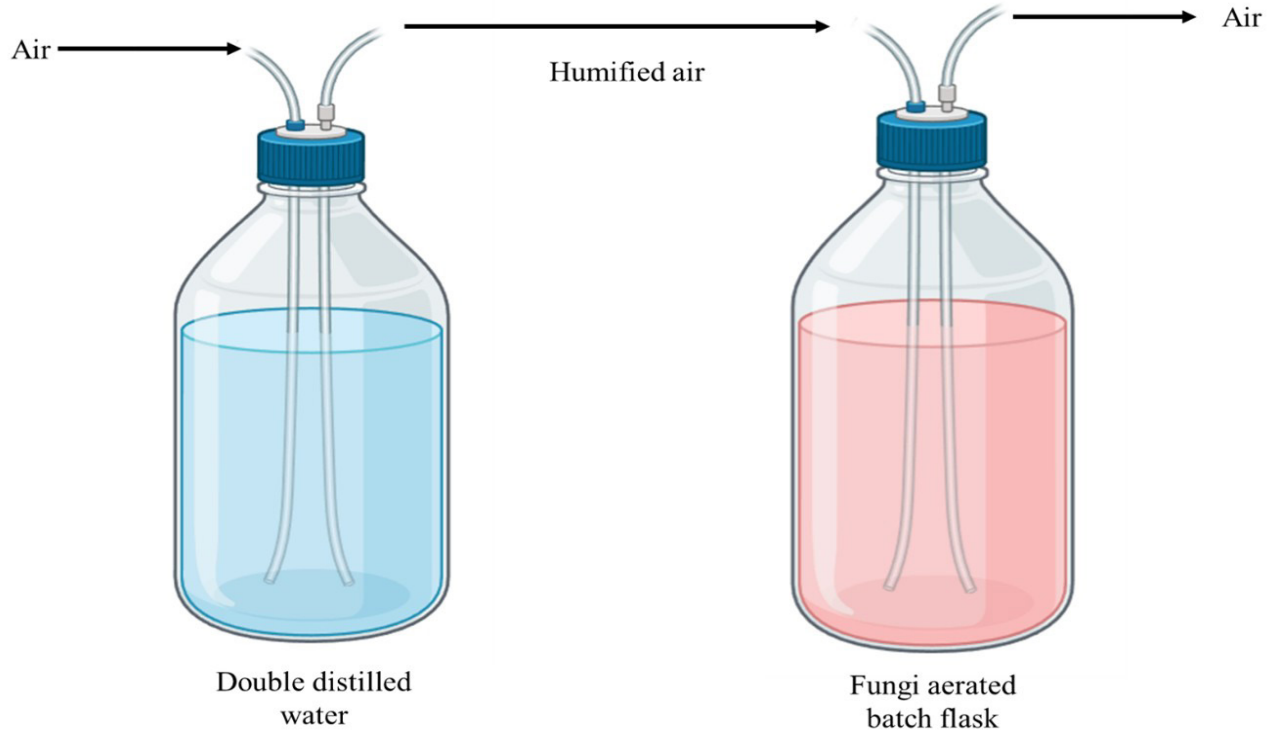



Figure 2. Setting up continuous aeration batch flasks (ABFs).

It has also been demonstrated that diclofenac metabolites, which are frequently found in fungi, break down during bacterial breakdown. Diclofenac was broken down into 4'-hydroxydiclofenac by cultures of *Bacillus* and *Brevibacillus*, among others (Grandclément et al., 2020). *Rhodococcus ruber* IEGM 346 and *Raoultella sp*. DD4 were the first bacterial strains known to degrade diclofenac (Domaradzka et al., 2016; Ivshina et al., 2019). *Raoultella sp*. DD4 showed strong tolerance to the harmful effects of diclofenac, reducing the concentration of the medication from 0.6 mg/L to undetectable levels within 28 days (Domaradzka et al., 2016). High concentrations of diclofenac (50 mg/L) can be degraded by the *Rhodococcus ruber* IEGM 346 strain. It was established that the aromatic ring of the diclofenac structure opens and the CN bond is disrupted during degradation. The IEGM 346 strain broke down this medication into sixteen intermediates. The mechanism outlined results in homogentisic acid via a sequence of oxidation reactions. Acetoacetic acid, fumaric acid, and 4,6,7-trioxooct-2-enedioic acid are the end products of further oxidation of this acid via a quinone derivative.

*Rhodococcus ruber* IEGM 346 adapts to high concentrations of this drug by altering the bacterial cell's potential, increasing its hydrophobicity and total lipid content, forming multicellular clusters, and changing its surface area-to-volume ratio (Ivshina et al., 2019). Additionally, researchers have shown that the *Labrys portucalensis* F11 strain hydroxylates diclofenac, producing benzoquinone imine as a crucial metabolite (Moreira et al., 2018). After that, the final product underwent hydroxylation and decarboxylation. The F11 strain completely degraded the medication, as evidenced by the stoichiometric release of chlorine and the lack of identified metabolites at the conclusion of the trials. Additionally, a sulfation reaction was reported for the first time during the bacterial breakdown of diclofenac, demonstrating the similarity between the metabolites produced during this process and the conjugates that develop during Phase II of diclofenac detoxification in mammals (Figure 3) (Navrozidou et al., 2019). For instance, it has been demonstrated that bacterial metabolism and drug detoxification routes in mammals are identical (Murshid & Dhakshinamoorthy, 2019). Diclofenac and glucuronic acid are conjugated to diclofenac 1-acyl-glucuronide by *Alcaligenes sp*. and *Staphylococcus sp*. with glucuronidase activity.

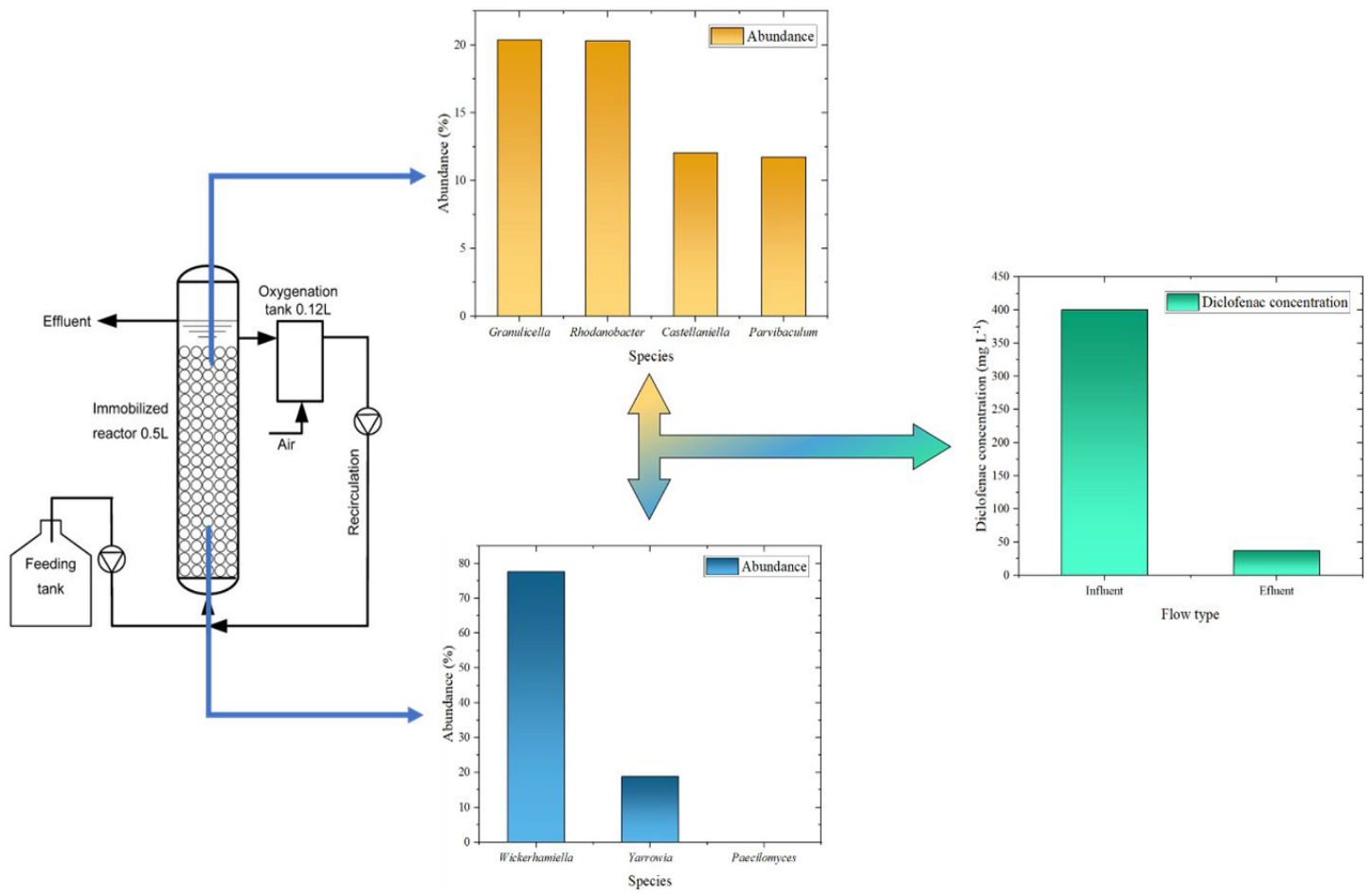



Figure 3. Diclofenac-degrading microbiota diversity and biodegradation capacity in an immobilized cell biofilter. Adapted and reproduced from Navrozidou et al., 2019, under the CC BY 4.0 license.

In a mono-substrate culture, researchers found that the *Pseudomonas moorei* KB4 strain could break down 0.5 mg/L of diclofenac (Żur, Marchlewicz, Piński, Guzik & Wojcieszyńska, 2021). In contrast, this strain degraded 1 mg/L of diclofenac in 12 days in a culture fed glucose and sodium acetate. Intermediates were identified as 4-OH-diclofenac and diclofenac-lactam. According to gene expression analysis, the cytochrome P450 system, aromatic ring cleavers and dihydroxylating agents, monooxygenase dioxygenases, and other genes encoding biotransformation enzymes were upregulated in the presence of diclofenac (Żur et al., 2021). Conversely, it was reported that the bacterial strain *Klebsiella sp.* KSC, isolated from animal husbandry soil, was able to biodegrade high concentrations of diclofenac. Diclofenac mineralization occurred 72 hours after KSC was exposed to 70 mg/L of the drug. Twelve diclofenac biotransformation metabolites were found in this instance, suggesting that dehydroxylation, hydroxylation, decarboxylation, and dechlorination are important stages in the compound's breakdown. Alcohol and ketones are produced as a result of these processes. There were derivatives that were monohydroxylated, dihydroxylated, trihydroxylated, and tetrahydroxylated. These molecules were produced by simultaneously adding hydroxyl groups to the parent chemical and removing the carboxyl group and two hydrogens from diclofenac. Furthermore, hydroxylation products were identified following the cleavage of the acetate group from the original substance's structure. It was also possible to identify the cyclization product between the nitrogen atom and the carboxyl group (Stylianou et al., 2018).

## 8. Diclofenac biodegradation in immobilized systems

Inadequate treatment, low efficiency, high cost, production of hazardous byproducts, and limited applicability to a range of organic compound concentrations in wastewater are some of the major drawbacks of conventional wastewater treatment methods for diclofenac, such as physical and chemical procedures. Therefore, developing an effective, affordable, and environmentally safe bioremediation method to offer superior remediation options in place of existing treatment technologies is challenging for environmental engineers and biotechnologists (Bilal, Rasheed, Nabeel, Iqbal & Zhao, 2019). The use of immobilized biopreparations has drawn more attention because diclofenac has been shown to be hazardous to microorganisms, particularly biodegradable bacteria (Table 4).

Table 4. Diclofenac biodegradation using matrix/immobilization methods.

| Pros and Cons of Matrix/Technology | Immobilisation Matrix/Technology | Microorganism/Enzyme | Reference |
|---|---|---|---|
| Biocompatibility, porosity, thin structure, and a large number of functional groupings | Poly(L-lactic acid)-co-poly(ε-caprolactone) electrospun nanofibers | Laccase (Trametes versicolor) | (Zdarta et al., 2019) |
| Water permeability, mechanical strength, selectivity, thermal resistance, specific surface area, and resistance to impurities | Multi-wall carbon nanotube-modified polyvinylidene chloride membrane | Laccase (Trametes hirsuta) | |
| Extremely successful when using an electron mediator in later cycles | Polyvinyl alcohol, silicon dioxide, and sodium alginate | Laccase (Sphingobacterium ksn-11) | (Masjoudi, Golgoli, Ghobadi, Sadeghzadeh & Mehdi, 2021) |
| Availability, porous structure, large specific surface area, and high adsorption capacity | Activated carbon in granules | Laccase | |
| Great efficacy, great storage stability, and high adsorption capacity | Biocarbon from pig dung | Laccase | (Neelkant, Shankar, Jayalakshmi & Sreeramulu, 2020) |
| Aggregation resistance | Nanoparticles of palladium | Microorganisms | |

Currently, adhesion to permanent or porous surfaces and so-called self-immobilization in granules are the most widely used techniques for immobilizing living microorganisms. The first approach is predicated on certain microbe species' innate propensity to form granules in submerged cultures. The second approach uses a secreted exopolysaccharide that functions as an adhesive, helping cells adhere to the support material. Additionally, encapsulation in pores or the use of chemical or physical traps in solids or porous matrices might result in immobilization on the support material. Because of their suitability for self-immobilization and the potential to attach another microbe to such aggregates or substances, creating self-immobilized biomixes, the use of mushroom granules has gained interest recently (Figure 4) (Skoronski, 2021).

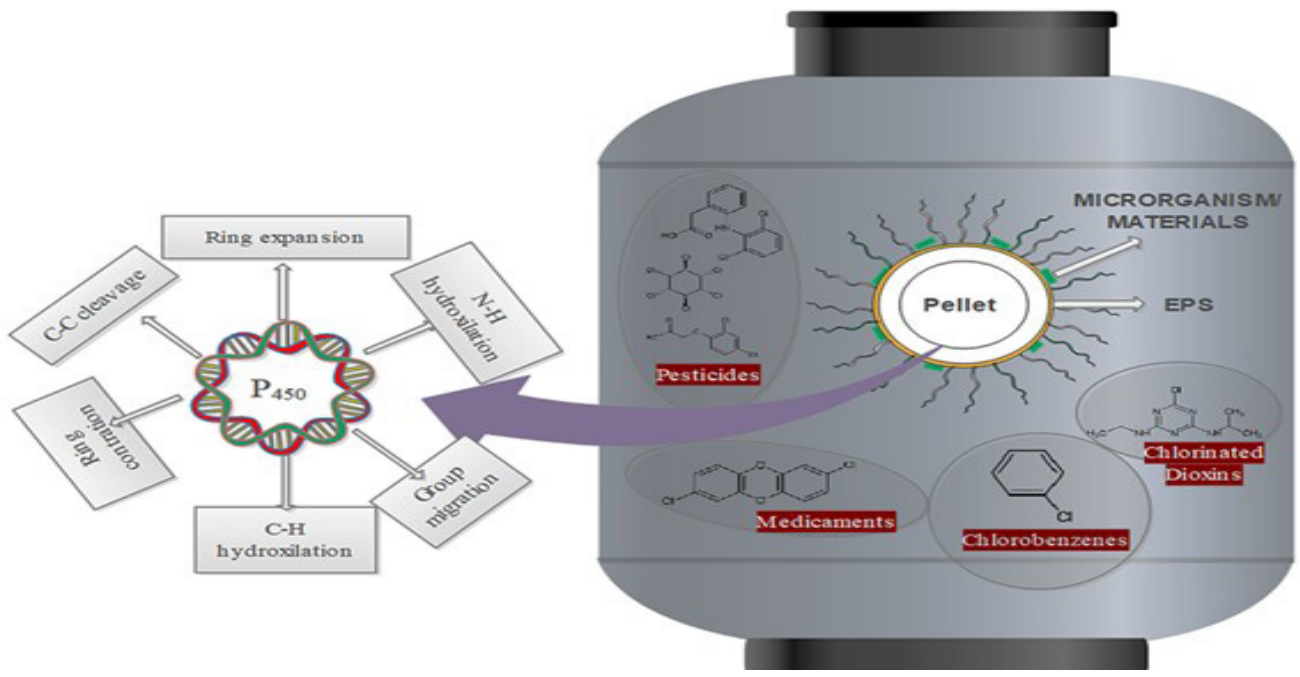



Figure 4. Immobilized mycelium-based pellets are used to eliminate organochlorine pollutants. Reproduced from Skoronski, 2021, under the CC BY 4.0 license.

Biodegradation and biotransformation techniques have employed both complete microorganism cells and specific oxidizing enzymes (Domaradzka et al., 2016; Zdarta et al., 2019; Żur et al., 2021). The bioreactor built on a biofilter with immobilized activated sludge microorganisms illustrates the use of immobilized microorganisms in the diclofenac

breakdown process. The developed system's efficiency in eliminating diclofenac was 97% over a two-month adaptation period. The primary bacterial taxa in the biofilter were determined using Illumina sequencing and included: *Rhodanobacter terrae, Granulicella pectinivorans, Parvibaculum lavamentivorans, Castellaniella denitrificans, Bryocella elongata*, *Bordetella petrii* and *Rhodopseudomonas palustris*. The primary fungal taxon in the immobilized cell biofilter was *Wickerhamiella*, suggesting that it plays a major role in the breakdown of diclofenac in activated sludge systems. The reactor's effective operation was made possible by the vast diversity of microorganisms. The method did, however, result in a drop in pH, which had no effect on the degrading efficiency. Additionally, this design worked well when a variety of carbon sources were included (Navrozidou et al., 2019). Researchers suggest employing "biomixtures" to enhance microorganisms' ability to degrade organochlorine derivatives, such as diclofenac (Skoronski, 2021). The ability of bacteria and fungi to self-aggregate is used to create biopreparations. These authors note that further investigation is necessary to understand the interspecific interactions between bacterial and fungal granules, particularly because co-immobilization affects granule attachment and the potential for continued function. Such studies may help develop effective and affordable biodegradation methods for diclofenac and other recalcitrant chemicals (Sham, Ng, & Pan, 2025).

Data from the literature indicate that diclofenac can be broken down by both microorganisms and enzymes extracted from them. The role of enzymes in the biotransformation and breakdown of diclofenac, however, has not been well documented. A new study provided a more thorough description of the enzymatic breakdown pathway of diclofenac (Żur et al., 2020). The authors verified that aromatic ring-cleaving enzymes (homogentisate 1,2-dioxygenase, catechol 1,2-dioxygenase, and salicylate 1,2-dioxygenase) and hydroxylating mono- and dioxygenases were involved in the disintegration of the drug structure. Additionally, it was demonstrated that deaminase significantly affected the disintegration of diclofenac's dicyclic structure. Nevertheless, they did not suggest any immobilized enzyme-based purification method utilizing the previously described enzymes. The cytochrome P-450 system and laccase, which are responsible for hydroxylation and glucuronide formation, are additional enzymes that contribute to the breakdown of diclofenac (Fernández-fernández, Sanromán, & Moldes, 2013; Murshid & Dhakshinamoorthy, 2019; Żur et al., 2019). However, because of their low selectivity and high oxidizing capacity relative to diclofenac, only laccases have found broader use in immobilized enzyme-based systems for the transformation of diclofenac so far. For this enzyme, both conventional, straightforward supports like alginate and cutting-edge synthetic supports have been employed (Masjoudi et al., 2021; Neelkant et al., 2020; Quan et al., 2019; Zdarta et al., 2019). The enzyme produced by *Sphingobacterium ksn*-11, immobilized on sodium alginate-silicon dioxide-polyvinyl alcohol beads, illustrates how laccase is used in the biodegradation of diclofenac. After four hours of incubation, the immobilized laccase was shown to oxidize diclofenac to 4-OH diclofenac, and the preparation proved quite successful in subsequent cycles. Additionally, the transformation period was shortened to 90 minutes using an electron mediator (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (Neelkant et al., 2020).

Researchers used adsorption, encapsulation, and covalent bonding to immobilize laccase from Trametes versicolor onto electrospun poly(L-lactic acid)-co-poly(ε-caprolactone) (PLCL) nanofibers (Zdarta et al., 2019). The materials made by the electrospinning process have fine structures, with lengths up to several thousand meters and diameters between 100 nm and 1 μm. These materials are advantageous for enzyme immobilization due to properties such as porosity, biocompatibility, and the large number of functional groups on the nanofiber surfaces. Thus, under ideal conditions, more than 90% of 1 mg/L diclofenac was degraded. After the fifth cycle, the immobilized enzyme retained 40% efficiency and remained active in subsequent cycles. A comprehensive toxicity analysis of the biodegradation products was also conducted. Furthermore, it was demonstrated that the final solution was approximately 65% less hazardous than the initial diclofenac solution (Zdarta et al., 2019).

Conversely, five hours after the application of laccase immobilized by adsorption on swine dung biochar (BC-PM) at an initial concentration of 500 μM, full biodegradation of diclofenac (500 μg/L) was observed. BC-PM demonstrated the greatest laccase adsorption capacity among various biochar supports, including those made from almond shell (BC-AS) or pine wood (BC-PW). Following the introduction of biochar support, it was shown that the enzyme-binding capacity and, subsequently, the immobilization efficacy increased with the initial laccase concentration in the solution. The charcoal support's ability to bind enzymes was enhanced by pretreating it with citric acid. Additionally, it was discovered that homogenous monolayer adsorption is the primary mechanism of enzyme adsorption on biochar. The immobilized laccase demonstrated greater storage stability. The shelf life was found to be three times longer than that of free laccase (Lonappan, Liu, Rouissi, Kaur et al., 2018; Lonappan, Liu, Rouissi, Pourcel et al., 2018).

Laccase from *Trametes hirsuta*, which is immobilized by covalent bonding to MWCNT/PVDF membranes (multi-walled carbon nanotube-modified polyvinylidene chloride membranes), is another illustration of the enzymatic breakdown of diclofenac (Masjoudi et al., 2021). The resulting nanocomposite membrane's resistance to contamination is enhanced by polymeric ingredients. Nanomaterials can be applied to the membrane's surface or within its matrix. They have special

qualities, including greater mechanical strength and specific surface area, as well as physicochemical qualities such as water permeability, contamination resistance, selectivity, and thermal resistance. MWCNTs and other carbon-based nanomaterials are widely utilized as membrane reinforcing materials in wastewater treatment. Polymeric membranes do not interfere with wastewater treatment plant operations and are compatible with their equipment. There was an issue with the separation of nanoparticles during the purification stage of laccase immobilization on MWCNTs. This issue could be avoided by combining MWCNTs with PVDF membranes. The physical characteristics of PVDF are enhanced by the inclusion of MWCNTs. It makes laccases more effective by increasing the rate at which electrons are transferred from the substrate to the enzyme. A high activity of 4.47 U/cm² and an activity recovery of 38.31% were demonstrated by covalently immobilized laccase. 95% of diclofenac was degraded in 4 hours by using chemically immobilized laccase in the mini-membrane reactor through covalent attachment to MWCNT/PVDF membranes (Masjoudi et al., 2021).

Additionally, laccase is immobilized via physical adsorption onto granulated activated carbon (GAC) (Nguyen et al., 2016). Large specific surface area, high adsorption capacity, porous structure, and widespread market availability are characteristics of acetaminophen (AC). Because of these qualities, AC has great promise for immobilizing enzymes. It has been demonstrated that the laccase structure remains unaffected by adsorption on AC, allowing it to remain active. Micropollutants like diclofenac can be effectively adsorbed by AC. But as with all adsorbents, surface saturation diminishes AC's ability to absorb micropollutants over exposure time. Because of this issue, AC regeneration is required to keep the system operating. Thus, by pre-adsorbing laccase to AC, a regeneration method was created. The adsorption sites are released when the immobilized laccase breaks down the adsorbed micropollutants. Additionally, due to increased electron transfer between laccase and micropollutants, co-adsorption of laccase and micropollutants in CAG enhances biodegradation efficiency. Since about 65% of the carbon surface remains accessible to adsorbates following enzyme immobilization, laccase immobilization in CAG has little effect on micropollutant adsorption. Additionally, during biodegradation, sorption sites on the CAG surface are freed, restarting the sorption-degradation cycle. Consequently, the elimination of diclofenac becomes more effective with each cycle. Laccase keeps CAG from reaching full saturation, which is necessary for ongoing operation. Higher laccase loads, or "complete saturation," produced the best diclofenac degradation outcomes across all cycles. Some issues with using free laccase for the catalytic degradation of micropollutants are resolved by coupling laccase to CAG. Laccase's reusability and stability over a broad pH and temperature range were enhanced by immobilization, and the enzyme was more effective at eliminating micropollutants (Nguyen et al., 2016).

Palladium (Pd)-based catalysts have garnered more attention lately. They can catalyze several processes, including hydrodechlorination and denitrification. Chemical processes are typically used to manufacture palladium catalysts, which are subsequently immobilized on supports like silica. This keeps them from clumping together and makes recycling easier. Microbial reduction is a promising method for producing palladium nanoparticles (Quan et al., 2019). This method is environmentally beneficial because it uses less hazardous chemicals and does not need stabilizers or supports. Compared to those supported by traditional supports, nanoparticles supported on biomass show greater resistance to aggregation. Biogenic nanopalladium (Bio-Pd) can be produced in the cytoplasm and cell membranes of a variety of microorganisms, including pure strains and mixed bacterial cultures. Anaerobic granular sludge (AGS) is one type of Bio-Pd producer. A specific type of microbial aggregation known as AGS is composed of mixed microbial cultures with a heterogeneous, three-dimensional structure. The microbial metabolic role of AGS and the catalytic role of palladium are combined in the Pd-AGS biocatalyst. It has the advantage of initiating Pd autocatalysis using hydrogen or electron donors produced from organic molecules by microbial transformation and fermentation, since it is a heterogeneous catalyst comprising Pd nanoparticles and microbial granules. Numerous elements have been shown to affect Pd's catalytic activity during diclofenac bioremediation, including pH, hydrogen and electron donors, the remediation medium, and the immobilization vehicle. Compared to free Pd nanoparticles, Pd-AGS is more resistant to inactivation by chloride or sulfide because hydrogen is the most efficient electron donor. 96% of the diclofenac was broken down by the Pd-AGS system over four reduction cycles, and its catalytic activity was quickly restored after purification with water. Pd-AGS appears to be a practical and affordable alternative to traditional supported heterogeneous Pd catalysts or homogeneous Pd complexes (Quan et al., 2019).

## 9. Comparative analysis of diclofenac remediation technologies

A comparison of several methods for eliminating diclofenac from the environment paints a complicated picture in which environmental sustainability, economic viability, and technical efficiency frequently clash (Iula et al., 2026). Adsorption, which can reach 80–99% depending on the material employed, is closely followed by membrane technologies (nanofiltration and reverse osmosis) and advanced oxidation processes (AOPs) in terms of removal efficiency, with rates exceeding 90–95%. Free microorganisms exhibit the lowest removal rates, typically between 20 and 50%, while immobilized systems exhibit competitive efficiency, ranging from 60 to 95%. This is especially true in conventional

treatment plants where the retention time is insufficient for the proper degradation of this resistant compound (**Table 5**). However, removal efficiency is not a complete picture. The ultimate destination of the pollutants must be considered. In this regard, biological systems, both free and immobilized, offer a major benefit by facilitating the efficient mineralization of diclofenac into water and carbon dioxide (Nazim & Cegłowski, 2026). Adsorption and membrane methods, on the other hand, merely transfer contamination from one phase to another without eliminating it, posing disposal issues. Despite being effective at initial degradation, AOPs pose a unique risk since mineralization is inconsistent and frequently incomplete, producing degradation byproducts that may be more hazardous than the original diclofenac itself. This behavior has been reported in multiple investigations (Oladipo et al., 2025).

The economic aspect highlights notable distinctions between the technologies. Free microorganisms use the infrastructure already in place in treatment facilities to operate at little expenditures. Because the biocatalyst is reused, the average cost of immobilized systems is offset by the expenditure in immobilization matrix preparation. AOPs, on the other hand, demand substantial energy and chemicals, while nanofiltration and reverse osmosis membranes have the highest operating costs because they require high pressures and specialized maintenance, making them impractical for widespread use in impoverished nations (Obeso et al., 2023).

Another crucial element of differentiation is the creation of waste. Sludge produced by biological systems is typically biodegradable and can be broken down anaerobically, but it still needs to be treated. When composed of natural materials like alginate, depleted immobilization matrices are typically biodegradable. However, AOPs have the potential to produce chemical sludge and, more concerning, long-lasting hazardous consequences. While membranes produce a highly hazardous liquid concentrate containing diclofenac and other contaminants in high concentrations, which presents an additional treatment issue, adsorption produces saturated solids that need energy regeneration or appropriate final disposal. Treated effluents vary greatly in their toxicity. Because properly functioning biological systems encourage detoxification concurrent with degradation, they generate effluents with minimal toxicity (Olasupo, Mohammad & Suah, 2023). If AOPs are not optimized for full mineralization, there is a substantial risk because degradation intermediates, for diclofenac hydroxylated derivatives, might be more acutely hazardous than the parent drug. Only when the components do not reach saturation can adsorption and membranes provide low-toxicity effluents, necessitating continuous monitoring.

Adsorption and free-living microorganisms benefit from established and accessible technology in terms of scalability. Although they are subject to financial constraints, membranes may also be scaled. AOPs and immobilized systems are in an intermediate stage, with promising pilot applications but technological obstacles to widespread deployment. The cost-effective manufacture of matrices in large quantities, the avoidance of biofouling, and the preservation of the mechanical stability of immobilized particles in continuous flow reactors are among the difficulties associated with immobilized systems (Thanhmingliana & Tiwari, 2015) the B and LC clay samples are pillared with aluminium and modified with the HDTMA as to obtain inorgano–organo-modified clay hybrid materials (viz., BAH and LCAH solids. It is obvious that biological systems benefit from global sustainability. With their low carbon footprint, utilization of renewable resources, and adherence to green chemistry principles, immobilized microbes are the most sustainable technology. Despite their technical efficiency, AOPs and membranes have reduced sustainability due to their high energy consumption and waste generation. In summary, there is not a single perfect way to get rid of diclofenac (Sathishkumar et al., 2021). The particular context, contamination concentrations, the flow rate to be treated, the resources at hand, and local laws must be taken into account while selecting a technique. For large-scale wastewater treatment under varying concentrations, the ideal balance is achieved by either immobilized systems as a polishing phase or by optimized conventional biological systems. Despite the expense, membranes or AOPs might be justified for applications that require near-total removal, such as potable reuse. The most promising trend is hybrid systems, which maximize efficiency while reducing costs and environmental impacts by combining complementary technologies, such as pretreatment with mild AOPs followed by biodegradation using immobilized systems (Latif et al., 2023).

Table 5. Comparative analysis of strategies for removing diclofenac from the environment.

| Criterion | Free microorganisms | Immobilized systems (Bio/Enzymatic) | Advanced Oxidation Processes (AOPs) | Adsorption | Membranes (NF/RO) |
|---|---|---|---|---|---|
| Removal efficiency | Low to Medium (20-50%) | High (60-95%) | Very High (>90%) | High (80-99%) | Very High (>95%) |
| Mineralization | High (converts to $CO_2$/$H_2O$) | High | Variable (generates byproducts) | None (only separates) | None (only separates) |
| Operational cost | Low | Medium | High (Energy/ Chemicals) | Medium | Very high |
| Residue generation | Biological sludge | Spent matrix (biodegradable) | Chemical sludge/Toxic byproducts | Saturated solid | Toxic liquid concentrate |
| Effluent toxicity | Low (generally) | Low | Risk of toxic byproducts | Low (if not saturated) | Low (in permeate) |
| Scalability | High (conventional WWTPs) | Medium (under development) | Medium | High | High |
| Sustainability | High (Green Technology) | Very High | Low | Medium | Low (Energy-intensive) |

Legend: DCF (Diclofenac); AOPs (Advanced Oxidation Processes); NF/RO (Nanofiltration / Reverse Osmosis); WWTPs (Wastewater Treatment Plants).

## 10. Limitations and future prospects

The widespread use of biotechnological approaches for diclofenac elimination is currently hampered by a number of issues, despite notable advancements in this field. One of the primary obstacles is the drug's partial breakdown by several microbes and enzymes, which leads to the creation of biotransformation intermediates that occasionally show acute toxicity greater than that of the parent chemical, as was the case with diclofenac methyl ester. Furthermore, the saturation of adsorbent supports (such as activated carbon) over time may reduce the effectiveness of immobilized systems, necessitating intricate regeneration procedures. To maintain operational stability in continuous systems, there are additional technical challenges related to the separation of nanoparticles during purification and the need for a deeper understanding of interspecific interactions in biomixtures (bacteria and fungi). The cost of creating sophisticated immobilization matrices, such as nanofibers and nanocomposites, and their economic feasibility relative to traditional techniques, remains a significant barrier.

Future plans should concentrate on creating inexpensive, long-lasting immobilization matrices that can encourage diclofenac's full mineralization rather than merely its modification in order to get over these restrictions. To verify the effectiveness of bioreactors and biofilters outside controlled laboratory settings, pilot and real-world-scale research is crucial. By combining omics technologies (genomics and proteomics), it may be possible to fully understand the metabolic pathways and enzymes at play, enabling the development of more resilient strains. Furthermore, removal studies must include thorough ecotoxicological evaluations of treated effluents to ensure that degradation products do not continue to pose hazards to the environment. Ultimately, a possible method to boost removal efficiency and make diclofenac bioremediation a workable and sustainable alternative for wastewater treatment facilities is the integration of immobilized biological systems with complementing physicochemical processes (hybrid systems).

## 5. Conclusions

In conclusion, diclofenac has proven to be an emergent pollutant of considerable environmental danger, and the low removal effectiveness of traditional wastewater treatment plants exacerbates its persistence in aquatic and terrestrial ecosystems. This research demonstrated that the drug's toxicity affects a variety of non-target creatures, resulting in everything from oxidative stress and renal failure to genotoxic and behavioral changes, necessitating the urgent need for more potent remediation techniques. The examined results show that oxidative enzymes (mostly laccases) and biodegradation mediated by particular bacteria (such *Rhodococcus ruber* and *Pseudomonas moorei*) and ligninolytic fungi comprise viable metabolic routes for the breakdown of diclofenac. In contrast to free biocatalysts, the use of immobilized systems is a significant technological advancement. In addition to extending the degraders' operational stability and longevity, immobilization on supports such nanofibers, biochar, membranes, and activated carbon also boosts their resistance to drug toxicity and permits the reuse of biocatalysts in numerous cycles. As a result, biotechnology approaches based on immobilized preparations offer themselves as technically superior, economically feasible, and environmentally safe substitutes for conventional physicochemical techniques. A sustainable way to reduce diclofenac-related environmental contamination and safeguard human health and biodiversity from the harmful effects of this pharmaceutical pollutant is to integrate these technologies into current treatment procedures on a large scale.

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