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Eco-Friendly Remediation to Mitigate the Toxic Effects of Emerging Contaminant Diclofenac

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Abstract*: Diclofenac (DCF), a non-steroidal anti-inflammatory drug has turned into an anthropogenic pollutant of emerging concern. Chronic exposure to DCF not only draws special attention to health issues for aquatic organisms, arthropods, and higher plants but also leads to serious damage to human beings. Its persistence in the ecosystem and synergistic interactions with existing pollutants may lead to proliferation of newly emerging contaminants which further disrupt the balance of nature. This comprehensive review highlights the toxicity effects of DCF and its metabolites. To counter this critical environmental imbalance, bioremediation is exploited for effective environmental clean-up. It is a fast, simple, cost effective and eco –friendly approach that explores microbial communities for mitigating the harmful effects of such toxic contaminants to control the levels of pollution. The practice of bioremediation approach using beneficial microorganisms in DCF degradation is important as it generates less hazardous compounds by maintaining the environment stability. Microorganisms adapt in the extreme conditions to transform DCF into innocuous intermediate metabolic products. These beneficial bioremediators can be used in the biological treatment of waste water thereby providing cleaner water resources to reduce the levels of water pollution, which is a great concern worldwide.

Keywords* : Bioremediation, Degradation metabolites, Diclofenac, Toxicity effects.

I. INTRODUCTION

With the accidental discovery of Penicillin in 1928 by Alexander Fleming, many natural, synthetic and semi-synthetic antibiotics have been produced for improving survival conditions of humans, plants and animals [1]. Now in this fast-paced life, with pollution, health problems are increasing on a daily basis. To overcome these issues, new pharmaceutical compounds are being manufactured and introduced into the market. But the extensive use of such pharmaceutical compounds and their improper disposal in the environment is harmful for aquatic as well as terrestrial domains [2]. Their untreated disposal not only affects the target population but also influences the non- target population with high toxicity [3]. One such compound of emerging concern that requires prompt monitoring is Diclofenac (DCF). It was included in the previous Watch List of EU Decision 2015/495 and considered as a "contaminant of emerging concern" that requires monitoring to reduce its harmful effects on the environment [4].

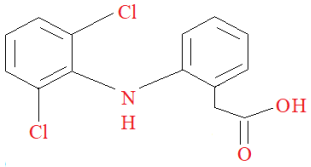

Fig. 1 Schematic chemical structure of DCF.

Chemical Formula - $C_{14}H_{11}Cl_2NO_2$
Molecular Mass- 296.148 g mol^{-1}
IUPAC ID: 2-[2-(2,6-dichloroanilino)phenyl]acetic acid
Water solubility- 0.00482 mg mL^{-1} [5]

II. LITERATURE REVIEW

DCF is one of the well-known and broadly prescribed non-steroidal anti-inflammatory drug (Fig. 1). It has been extensively used as a pain reliever to reduce inflammation in disorders like rheumatoid arthritis, ankylosing spondylitis, dysmenorrhoea ,etc. [6]. Due to its global consumption of 940tons per year , residual DCF is reported to persist in seawater, drinking water, surface water, soil, sediments, and sludge in different nations worldwide [7], [8]. General sources of DCF introduction are pharmaceutical waste, livestock, domestic drainage, inefficiently treated wastewater, disposal of expired medicines, etc. [9]. Recently, DCF has been categorized as a profound pollutant of emerging concern among other 100 pharmaceutical compounds to review its eco-toxicological impact in the aquatic environment of China [10]. The DCF removal efficiencies from Waste Water Treatment Plants (WWTP) lie in the range of 20-40% [11]. Due to its incomplete degradation, its residues like 4′-hydroxy-DCF, 5-hydroxy-DCF and *p*-benzoquinone imine of 5-hydroxy-DCF have been reported to persist in the drinking water, wastewater, soil, food web that pose a threat to human life [12]. Various remediation strategies for DCF removal have emerged like advanced oxidation, membrane-based technologies but these may lead to the production of intermediate compounds that are more toxic than the parent compound. These processes are eco unsafe and cost-ineffective [13], [14].

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Hence, to minimize the post-treatment contamination moieties it is suggested that an effective, safe and environmentally friendly approach be employed for the future work that will be focused on microbial or enzymatic remediation. As microorganisms can adapt to and possibly transform pollutants into innocuous products, these biological techniques are preferred over chemical treatment [15].

III. OBJECTIVES

- Study the toxicity effects of DCF.
- Focus on bioremediation approach for DCF degradation.
- Identify highly efficient microbial strains for transforming DCF into innocuous intermediate compounds.

IV. TOXICITY EFFECTS OF DCF

The disruption in the balance of the ecosystem by DCF was firstly reported by declining of the population of Gyps vultures in Asian countries due to the ingestion of DCF-medicated domestic animals [16]. As compared to other pharmaceutical compounds like carbamazepine and metoprolol, it has the highest toxicity effect in the fish liver, kidney, and gills [14]. After exposure to 30 ng L^{-1} DCF for 90 min, a decrease in the level of lipid peroxidation was observed in zebra fish embryo [17]. Toxicity effects have also been reported in Japanese rice fish (*Oryzias latipes*), Nile tilapia (*Oreochromis niloticus*) and starfish (*Asterias rubens*) [3], [18]. It has been reported to cause cardiac toxicity [19], hepatotoxicity effects on mammals [20]. In the case of plants, the phytotoxic effect of DCF was observed when chlorophyll alterations occurred in the duckweed *Lemna minor* upon 100 µg L^{-1} DCF exposure [11]. Bartha et al. [21] reported the rise in the activity of glutathione S transferase (GST) of the plant *T. latifolia* in both roots and shoots exposed to DCF. Recently, it has also been reported to induce photosynthetic damage in the fern *Azolla filiculoides* and lichen *Xanthoria parietina* [22]. Additionally, it has been indicated to reduce the survival and reproduction rates of arthropod *Folsomia candida* and declared to be highly toxic to non-target soil invertebrates [23]. It has also shown drug-induced oxidative stress effects in the mussel *P. perna*, even at low concentrations (ng L^{-1}) [24]. Hence, DCF has deleterious effects on aquatic animals, arthropods, plants, and mammals.

Soil microorganisms play a crucial role in maintaining organic matter turnover and release of nutrients in the soil. They ensure soil fertility and also act as biocontrol agents by inhibiting the growth of pathogens for maintaining homeostasis of soil [25]. But, when DCF percolates the soil even below minimum inhibitory concentration (MIC), it promotes genetic changes in genome of bacteria. The changes arise in the form of antibiotic-resistant genes (ARGs) that give rise to resistant bacterial community. ARGs and mobile genetic elements like plasmids, transposons, and genomic islands, are transferred among microbial population, even between distantly related bacterial species [3], [17]. It may also affect the abundance of soil microorganisms, overall microbial and enzymatic activity, carbon mineralization and nitrogen cycling which impacts the functional, structural and genetic diversity of microbes [26]–[28]. Eventually, bacteria that represents reservoir of resistant genes can transfer these ARGs to the bacterial species that colonize in human body [18], [29]. The development of this resistance represents serious health risks and a worldwide danger to the human population as DCF accumulates in the crops and food [30]. Therefore, chronic exposure even at ng to µg levels of DCF can lead to an emerging ecological problem affecting different environmental matrices (Fig.2). Therefore, an effective approach needs to be developed to detoxify the hazardous effects of this compound and its metabolites, mitigating the risks associated with the stability of the environment.

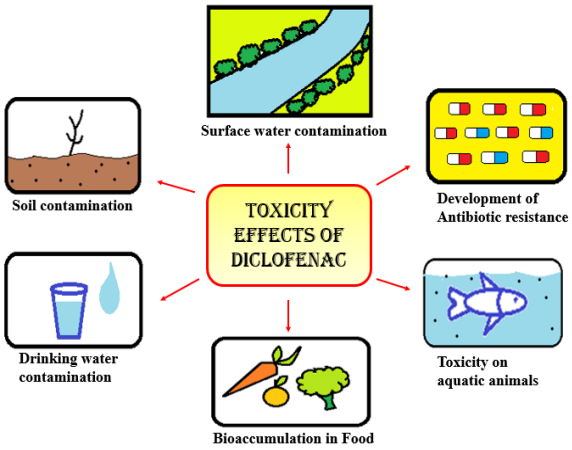


Fig. 2. Toxicity effects of DCF in different environmental matrices.

V. BIOREMEDIATION OF DCF

Unfortunately, industrialization has led to a negative impact on the environment with the release of untreated harmful recalcitrant compounds like DCF. Therefore, effective remedial approaches need to be practiced for reducing the hazardous impact of DCF. Although, various techniques like adsorption, activated sludge treatments, membrane bioreactors have been established for the removal of DCF and its metabolites [6], [13], [31], [32]. But chemical and physical approaches of remediation are not enough to reduce the effect of pollution as they are costly and generate recalcitrant compounds. In order to employ a fast, simple, cost-effective and eco-friendly approach bioremediation is being explored for effective DCF removal [33]. Bioremediation can play a crucial role in cleaning the sites of contamination. Biodegradation can be achieved by biotic (microorganisms) or abiotic (sorption, hydrolysis, photolysis, redox reaction) processes [34]. For meeting up the eventual goal of bioremediation, it is necessary to understand the environmental factors that influence microbial cellular processes. Microbial communities, gene expressions, behaviour in stressed conditions due to toxic compounds need to be apprehended thoroughly [35].

A. Mechanism of biodegradation

Microbes are reliable bioremediators as they neutralize the pollutants effectively for environmental clean-up. They are present naturally in soil and water and play a major role in fundamental degradation processes by indulging in metabolic or co-metabolic pathways [36]. As bacteria encounter DCF, it gets adsorbed on the bacterial surface through biosorption and facilitated transport across the cell membrane through the process of diffusion. Inside the cell, DCF is attacked and degraded into less toxic products by the action of different naturally occurring enzymes secreted by the bacteria. The specific enzyme activity of these enzymes signifies the response of bacteria to the stress caused by the pollutant. Presence of toxic compounds might affect the general activity of enzymes that might trigger the metabolic degradation of toxicants, increasing the abundance of microbes and enzyme production [25]. Hence, the accumulation of these toxins is prevented and they are eradicated from the ecosystem [37], [38].

Other approach of microbial adaptation to extreme environmental conditions is attainment of ARGs. Microbial populations attain ARG in order to degrade pollutants [39]. This resistance is attributed to the increase in the minimum inhibitory concentration of the pollutants that can be tolerated by microbes. As bacterial cells possess different molecular hierarchy (DNA, RNA proteins), under stressed conditions, various kinds of resistant genes (ARG) are activated which become an integral part of microbial machinery (Fig. 3). With the uptake of pollutant (DCF), bacteria produces modifying enzymes that transfer different functional groups onto the pollutants. The process of modification is carried out by acetylation, phosphorylation, ribosylation, adenylation, nucleotidylation, and glycosylation in the presence of ATP as a co-substrate [40]. Bacteria may also produce degrading enzymes that directly degrade DCF into its metabolites. Contrary to this, bacteria can escape the target interaction through bypass or efflux of DCF from the cell [41]. Hence, DCF gets degraded or modified into innocuous products by the action of enzymes secreted by the microorganism.

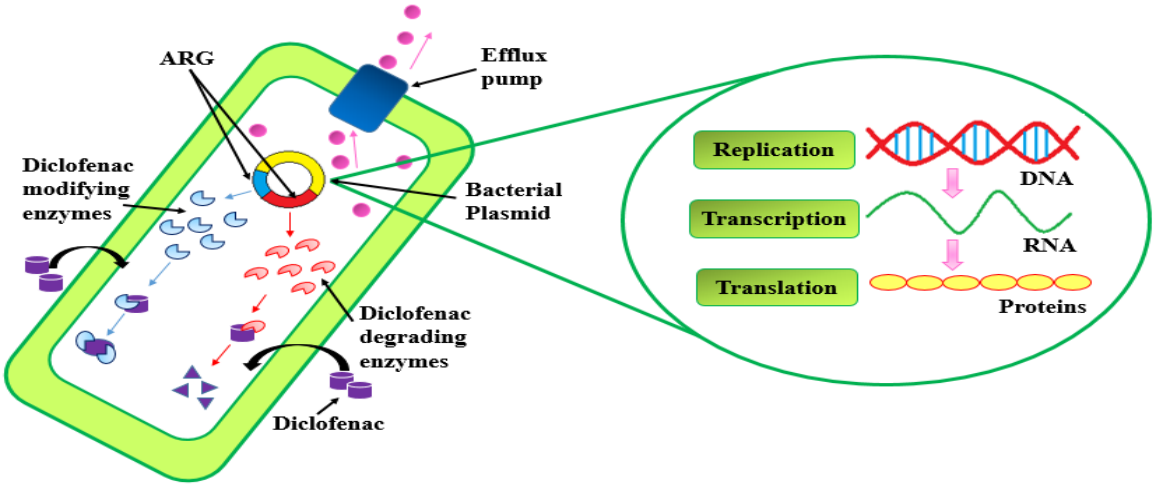


Fig. 3. Mechanism of microbial degradation of DCF.

VI. EFFICIENT DCF DEGRADING STRAINS

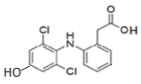
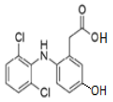
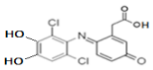
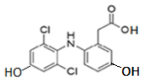
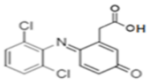
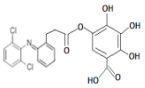
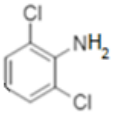
Central role of microorganisms in DCF degradation or transformation has been confirmed by many studies carried out in sterile and non-sterile environment. In the majority of a few published reports affirming the DCF degradation, the microorganism responsible for the process of degradation has not been identified [42], [43]. A bacterial consortium of *Alcaligenes faecelis*, *Staphylococcus aureus*, *Staphylococcus haemolyticus* and *Proteus mirabilis*, have been reported to degrade 150 mg L^{-1} DCF within 120 h with the help of monooxygenase and glucuronidase enzyme [44]. Quintana et al. [108] has reported the use of fresh activated sludge as inoculum for degradation of 20 mg L^{-1} DCF used as a sole carbon source, but no transformation was observed for 28 days under aerobic conditions. Even with the addition of co-metabolite, degradation was not effective. Fatehifar et al. [45] has reported DCF degradation up to 66% using biomass from Moving Bed Biofilm Reactor (MBBR). However, the microorganism responsible for the degradation was not identified and the removal pathway of DCF and its metabolic products were not assessed. The study of Moreira et al. [15] stated the degradation of 34 µM of DCF in 30 days by *L. portucalensis F11* along with the addition of 5.9 mM acetate as a co-metabolite. Bouju et al. [46] reported that 40% of DCF was transformed during 25 days using MBBR biomass and identified two metabolic end products of the biotransformation. Palyzova et al. [47] mentioned the biotransformation of (1.0 g L^{-1}) DCF by chemically modifying *Roultella sp. KDF8* strain. *Brevibacterium D4*, an isolate from the WWTP has also been reported to degrade 35% of 10 mg L^{-1} of DCF when used as sole carbon source and 90% degradation was achieved only with periodic feeding of acetate as supplementary carbon source [48]. In the recent study of [36], it was found that 100 mg L^{-1} DCF could be degraded in 10 days by using forest soil microbial consortia. A recent report of Stylianou et al. [49] mentioned the degradation of DCF (70 mg L^{-1}) in 72 h by *Klebsiella sp. KSC*, isolated from livestock soil.

Ivshina et al. [5], explored the ability of *Rhodococcus ruber* IEGM 346, to degrade 50 mg L^{-1} DCF in the presence of supplementary source with 0.5% glucose. The strain was able to break open the central ring structure of DCF and generated linear aliphatic intermediate compounds which were less hazardous than parent compound.

A. Metabolites Produced on Microbial Degradation

DCF is reported as a recalcitrant against aerobic and anaerobic biotransformation, which suggests that all microbes are not capable of mineralizing this compound [50]. In spite of its higher resistance to degradation, a few potential microorganisms have been reported to biotransform DCF into intermediates like bacteria *Actinoplanes sp.* has been reported to degrade DCF (50 µM) into its metabolites, 4′-hydroxyDCF, 5-hydroxyDCF, and 4′,5-dihydroxyDCF, with 100% turnover in less than 5 h [51]. Another study reported rapid DCF removal in concentration (3-35 µM) with the formation of p-benzoquinone imine, derivate of 5-dihydroxyDCF [52]. DCF transformation was also investigated by using bacteria (*Geobacter metallireducens*) in a vertical flow-constructed wetland in which 5-hydroxyDCF, DCF-2,5-iminoquinone, and 1,3-dichlorobenzene were three identified intermediates [53]. In the mammalian system, DCF is broken down into carboxylation intermediate producing 1-O- acyl glucuronide [36]. Two mono-hydroxylated and one di-hydroxylated metabolites have been reported to be formed as degradation products with fungal culture treatment of *Trametes versicolor* and *Phanerochaete chrysosporium* due to presence of cytochrome P450 enzymes [54], [55]. Palyzová et al. [47] discussed the role of 24 enzymes, including catechol 1,2-dioxygenase, protocatechuate 3,4-dioxygenase, and quercetin 2,3-dioxygenase, in producing hydroxylated, methylhydroxylated, hydroxylated and oxidized, and decarboxylated DCF metabolites associated with ketoadipate, benzoate and catechol pathway during DCF degradation by *Raoultella sp.* Some intermediates formed from microbial degradation of DCF are listed in Table I. Collectively, this study summarized that microorganisms produced DCF degradation metabolites by forming hydroxylated, quinone, and dichlorobenzene compounds. Hence, bioremediation is an effective technique designed for the efficient removal of DCF and its degradation products from the aqueous environment without generating any secondary contaminants.

Table I: Common intermediates observed on microbial degradation of DCF.

DCF intermediate	Chemical Structure	Microbial Source	References
4′-HydroxyDCF		Bacteria (*Actinoplanes sp.*,and *Raoultella sp.*), fungi (*T. versicolor*, *E. nigrum)*, and yeast (*S. cerevisiae)*	[47], [51], [56], [57], [58]
5-HydroxyDCF		Bacteria (*Actinoplanes sp., G. metallireduce ns, Raoultella sp.)*, fungus (*T. versicolor)*, and yeast *(S. cerevisiae)*	[47], [51], [57], [58]
2-[1-(5-oxocyclo hexa-1,3-dienyl-2 -(3′,4′-dihydroxy-2′,6′-dichlorophen yl)imino]acetic acid.		Bacteria *Rhodococcus ruber IEGM 346, Labrys portucalensis*	[5], [15]
4′,5-DihydroxyD CF		Bacterium (*Actinoplanes sp.)*	[51]
DCF-2,5-iminoqu inone		Bacterium (*G. metallireduce ns)*	[53]
1,3-Dichlorobenz ene		Bacterium (*G. metallireduce ns)*	[53]
DCF 1- acyl Glucuronide		Bacteria *Staphylococc us sp.* *Alcaligens sp.*	[44]
2,6-dichloroanilin e		Microbial consortium	[36]

B. Analysis of Metabolites

In microbial degradation of DCF, special attention needs to be given to the quantification and detection of these metabolites formed by novel enzyme actions produced by resistant microbial community. Advanced analytical methods, such as high-performance liquid chromatography (HPLC), Ultra performance liquid chromatography (UPLC), liquid chromatography with tandem mass spectrometry (LC-MS), are of utmost importance to detect formed metabolites even at low concentrations [15].

Though different intermediates of DCF biodegradation have been reported but researches on simple structures of detoxification products are yet to be explored. However, the metabolites mentioned in numerous biodegradation reports retained the core structure without cleavage of the rigid poly aromatic ring. Therefore, more research is required in this field to find naturally occurring or engineered microorganisms that effectively break open the robust structure of DCF to generate smaller end products that are not harmful to the environment.

VII. CONCLUSION AND FUTURE PROSPECTS

Environmental monitoring studies around the world have concluded that DCF and its metabolites are ubiquitously found in surface waters, ground waters, soil, food webs, etc. The improper disposal and chronic exposure to DCF even at minimal levels lead to deleterious effects on aquatic and terrestrial domains, thereby affecting the well-being of human life and other living creatures. To combat this problem, bioremediation has emerged as an eco-friendly, safe and cost-effective alternative approach that generates innocuous intermediates on DCF degradation. Current review summarized the potential of microbial communities for the removal of toxic aromatic pollutants from the environment. It is clear that under stress conditions in presence of DCF, microbes alter their mechanisms by generating ARGs which results in outgrowth of resistant bacterial community. These microorganisms adapt and possibly transform the toxic compound that could lead to restoration of original microbial community which was disturbed by DCF exposure.

Strategies incorporated for future framework should focus on identification and extraction of novel enzymes responsible for DCF degradation. Further, studying the fate of ARGs in the environment will not only help in assessing the diversity of microbial population but also in discovering new ARGs and novel enzymes formed by adapting new resistant mechanisms and altered functional metagenomics for DCF degradation. Future studies should be based on developing approaches that help in the management of harvested crops in DCF treated soil to avoid its occurrence in food chain. The ultimate goal of this work is to completely metabolize DCF into end products either as gases (especially CO_2) or liquids (water) which can be recycled producing zero-level of pollution emissions. Therefore, this review suggests that environment friendly approaches be employed and explored further at advanced level to reduce the toxicity of DCF, producing less hazardous compounds that do not harm other parts of the environment. Hence, keeping the surroundings clean and rejuvenating healthy planet for a better future.

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