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Approaches in Bioremediation of Dioxins and Dioxin-Like Compounds – A Review on Current and Future Prospects

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ABSTRACT

Waste generation is becoming increasingly prominent in the environmental arena due to the increase in population and living standards of life. Dioxin and Dioxin-related compounds are a set of hazardous chemicals that are ubiquitously distributed. Polychlorinated dioxins are introduced into our surroundings by both spontaneous and induced activities like combustion, incineration of waste, recycling of e-waste, and paper and pesticide manufacturing. They are chloroaromatic compounds that are found to be lethal and possess carcinogenic properties and are one of the primary examples of persistent environmental pollutants (POP). Removal of these compounds from the environment is very challenging due to their recalcitrant nature. An alternative technique is the use of microbial technology which includes the use of bacteria and fungi to detoxify the dioxins that are considered to be a more effective, economical, and environmentally sustainable alternative. Different microbial interactions were studied for their degradation potential. Polychlorinated dibenzo-p-dioxin and furans (PCDD/F) are found to be degraded by bacteria by adopting either aerobic or anaerobic pathways and the details regarding the diversity, distribution, bioremediation potential, metabolic pathway have been analyzed. This review provides an overview of the source of contamination, its potential toxicity assessment, and various bioremediation techniques that are employed are discussed in detail. It also highlights the nanoremediation technique - a promising tool in which nanoparticles are used in the treatment of toxic organic pollutants.

INTRODUCTION

The environment is highly contaminated by various toxic chemicals due to several anthropogenic and industrial activities that often release organic and inorganic pollutants into the surrounding environment that accumulate as highly toxic pollutants. Any substance uncommonly present in the environment in a higher concentration than the acceptable level that is hardly subjected to biodegradation is termed xenobiotics (Mathew et al. 2017). In the present world scenario, the term dioxin is gaining much importance due to its persistent and toxic characteristics. Dioxins and Dioxin-like compounds (DLC) are chemical compounds that include PCDD/F depicted by the chlorine atom arrangement (Kulkarni et al. 2008) and polychlorinated biphenyls (PCB) that are considered to be persistent organic pollutants. These compounds are found to be highly toxic and possess carcinogenic properties. Once these toxic compounds have been generated, they can pertain to the soil, sediment, and dump yards of E-waste for the distant future. Dioxins possess the potential to sustain in the environment for a longer duration when absorbed onto clay and the organic content of the soil matrix due to its hydrophobic nature (Thi et al. 2019, Srogi 2008).

Dioxin structure consists of two benzene rings linked by an oxygen atom. For PCDD, the aromatic ring is linked by two oxygen atoms and for PCDF, the ring is connected by a carbon bond and oxygen ring. Dioxins have a high melting point and low vapor pressure (Srogi 2008a). Among various categories of dioxin congeners, only 7 out of 75 PCDD congeners, and 10 out of 135 PCDF congeners have dioxin-like toxicity. Their level of toxicity is calculated by the positioning of chlorine atoms (Kulkarni et al. 2008b). Among the dioxin derivatives, the highly toxic compound known is 2,3,7,8- tetrachlorinated dibenzo-p-dioxin (Nguyen et al. 2021) and is labeled as a human carcinogen by the International Agency for Research on Cancer (ICAR) (Srogi 2008b). These chemicals are top-ranked as one of the persistent organic pollutants (POP) often specified as the "Dirty dozen" (Weber et al. 2008). Dioxins are found to possess toxic, carcinogenic, and mutagenic properties (Saibu et al. 2020a). Polychlorinated dibenzo-furan isomers have also been reported in various media like soil, and sediment in many industrialized areas. Fig. 1 depicts the structure of PCDD and PCDF with their possible number of congeners.

SOURCE, ORIGIN AND TOXICITY OF DIOXINS

PCDD/F are formed through various natural and human activities. Various sources of dioxin contamination are summarized in Fig. 2. Accidental events such as volcanic eruptions, forest fires, and house fires result in dioxin emissions to a limited extent (Kulkarni et al. 2008, Ruokojärvi et al. 2000). Dioxins are unintentionally released as a by-product during various anthropogenic activities which include combustion of chlorinated compounds, incineration of residues from household and production sectors (Mukherjee et al. 2016, Tuppurainen et al. 1998), bleaching of pulp and paper mill (Rosenberg et al. 1994, Thacker et al. 2007) metallurgical process (Buekens et al. 2001, Łechtańska et al. 2017), dumping of e-waste (Dai et al. 2020). Burning of PCDD and PCDF are also produced as a micropollutant during the chlorination of Pentachlorophenol (PCP) which was initially used as a wood preservative. Agent Orange – a known herbicide containing dioxin profusely used during the Vietnam war in the1960s remains a significant environmental threat and greatly affects human beings (Thi et al. 2019). In the Luoi Valley of Vietnam, soil, animal tissue, and human blood were found to contain increased levels of the most toxic dioxin molecule, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Dwernychuk et al. 2002, Thi et al. 2019). Toxic equivalence quantities (TEQs) are used to express the toxicity of dioxin where TCDD is rated as 1 and the fraction of this for other less toxic congeners (Kulkarni et al. 2008d). The concentration of Dioxin-like compounds (DLC) in the environment is determined by the Dioxin toxicity equivalence.

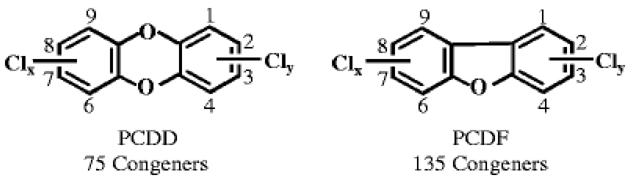

Fig.1: Chemical structure of PCDD and PCDF with their possible congeners (Bunge & Lechner 2009a).

HEALTH HAZARDS DUE TO DIOXIN-POLLUTED ENVIRONMENT

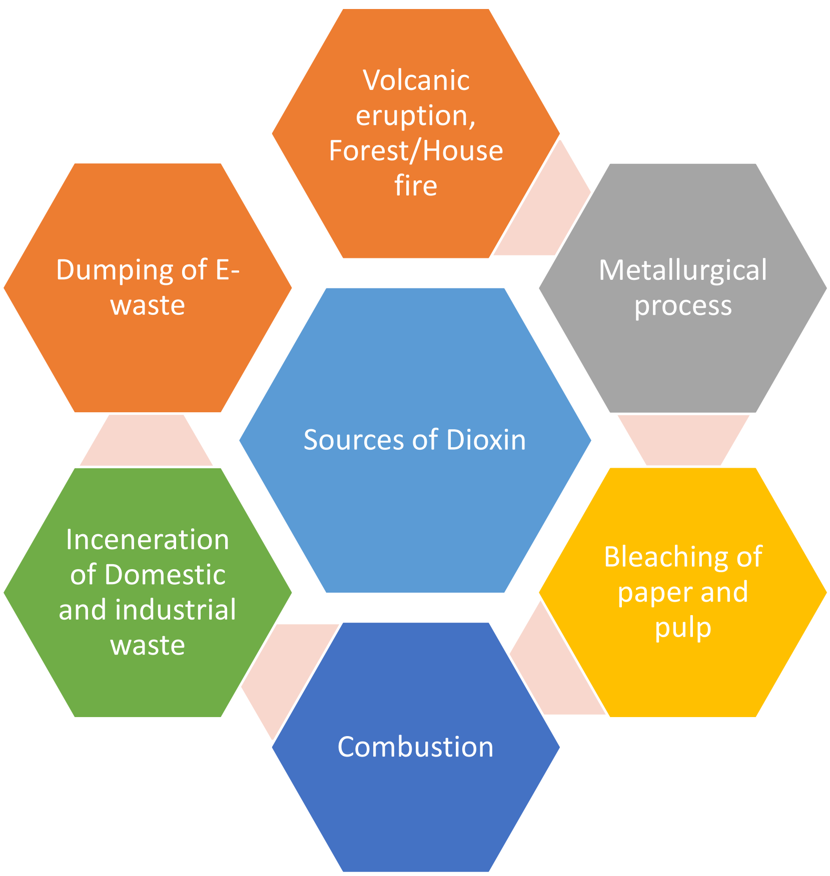

Fig. 2: Major sources of dioxins in the environment.

Dioxin being inadequately incinerated is released into the soil and aquatic sediment bioaccumulates in the fatty tissues of animals. Humans are exposed to dioxin mainly through food, accounting for more than 90% of dioxin exposure. The lipophilic nature of dioxin is high in the fat of animals and fishes and that results in the presence of dioxin in food and dairy products (Rathoure 2018). Exposure to TCDD is reported to cause epigenetic modification and relative toxicity. Similarly, the experimental data conducted in cell line and animal studies has resulted in a decreased male/female ratio in offspring (Viluksela & Pohjanvirta 2019). The et al. (2020), studied and proved that the perinatal exposure to dioxin subsequently affected the learning ability of children particularly boys. It has also been reported that exposure to dioxin has a detrimental effect on human health which includes simple skin lesions to altered hepatic function, as well as the immune, nervous, and endocrine systems functioning adversely. Dioxin can percolate the blood-brain barrier and attack the nerve cells directly exerting a toxic effect on the central nervous system (Miyazaki et al. 2016).

IMPORTANCE OF BIOREMEDIATION FOR DIOXIN-CONTAMINATED SOIL

Removal of highly recalcitrant dioxin and dioxin-like compounds from the environment is much demanding. Their persistent nature has become a major global concern. Therefore, remediation of such highly contaminated areas is required. Diverse methods and technologies have been devised and adapted which can be categorized into three broad groups: Physical, chemical, and biological treatments (Binh et al. 2016). Physical and chemical treatments include incineration and thermal desorption method, photolysis, radiolytic treatment using gamma rays, alkaline dehydrochlorination, Fenton treatment, and usage of different oxidants and reductants (Haglund 2007, Hrabák et al. 2016). Although incineration has been an inexpensive technique for maximum destruction of dioxin when compared to other physico-chemical techniques, treating a massive amount of contaminated soils and sediment has its own disadvantages. This paved a way for a biological alternative (Wittich 1998). Biological treatment which includes biodegradation of dioxin-contaminated soil using microbial diversity which is capable of degrading dioxin is given more importance, especially from the ecological and economical point of view(Binh et al. 2016). It is one of the promising alternatives to clean up dioxin-contaminated soil due to its low-cost performance when compared to others. The microbiological approach involves a sustainable and cost-effective treatment for the PCDD/F contaminated soil (Chen et al. 2013). Nam et al. (2008) evaluated the removal of PCDD/F from the fly ash, a major source of dioxin contamination using a biocatalyst that contains bacterial and fungal consortia capable of degrading dioxins. The above treatment resulted in 68.7% removal of PCDD/F in 21 days.

MECHANISM AND APPROACHES OF MICROBIAL REMEDIATION OF DIOXINS

Biodegradation utilizes microorganisms to break down the potentially hazardous chemical substances into less/ non-toxic smaller compounds using its metabolic or enzymatic mechanism. The extent of biodegradability greatly relies on the toxicity, the concentration of the contaminants, the type of microorganism involved, and the properties of the contaminated soil. The concentration of the contaminants can be reduced by both aerobic and anaerobic mechanisms. (Kao et al. 2004). The key difference between them is that the aerobic degradation takes place in lower chlorinated congeners while the anaerobic pathway takes place in highly chlorinated congeners(Kulkarni et al. 2008e). The microbial consortium and fungal remediation are also found to be useful in degrading chlorinated DD/F(Chen et al. 2013, Dao et al. 2021). The products of the catabolic reaction should be further mineralized because incomplete degradation of such compounds can result in more toxic metabolites than the primary substrate (Kao et al. 2004).

Aerobic Biodegradation of Dioxins

In aerobic conditions, the microorganism involved in degrading the dioxins undergoes an oxidative degradation pathway. The two main pathways in oxidative degradation are lateral and angular dioxygenation. The initial is the addition of two hydroxyl groups into the ring structure using the enzyme dioxygenase; the resultant is transformed into cis-hydrodiols. Both angular and lateral deoxygenation systems are found in species of different phylogenetic groups (Nojiri & Omori 2002, Saibu et al. 2020b)

Bacterial strains possessing the ability to degrade DD and DF were isolated and characterized (Habe et al. 2001). Sphingomonas wittichii strain RW1 was the extensively researched organism that can degrade dioxin based on metabolic processes. The organism isolated from the enriched culture was inoculated with the water sample obtained from the Elbe river. The organism underwent angular dehydroxylation and the catechol thus obtained was found to be cleaved by *Sphingomonas wittichii* strain RW1(Wittich et al. 1992). Saibu et al. (2020a) isolated dioxin degrading bacterial strains from polluted soil. Among 17 strains isolated, two strains displaying better metabolic competencies were selected and the organisms were identified as *Bacillus* sp. SS2 and *Serratia* sp. SSA 1 and the percentage of removal of dibenzofuran were found to be 93.87% for SS2 and 2,8 – dichlorodibenzofurans and 2,7 – dichlorodibenzo-p-dioxin it was 86.22% and 82.30% respectively. Other bacterial strains that are found to be capable of DD/DF biodegradation and their congeners belong to *Rhodococcus* and *Terrabacter* (Iida et al. 2002), *Geobacillus* sp. UZO 3 (Suzuki et al. 2016), *Burkholderia cenocepacia* strain 869T2 (Nguyen et al. 2021), and *Pseudomonas mendocina* NSYSU (Lin et al. 2017). Quensen & Matsumura (1983) studied the oxidative degradation of 2,3,7,8 TCDD by *B. megaterium* and *Norcardiopsis sp.* They concluded that the solvent used greatly influences the degree of metabolism. *Janibacter terrae* strain XJ-1 isolated from sediment degraded dibenzofuran aerobically by using them as a sole source of carbon and energy and produced gentisic acid as final products with several metabolites as intermediates (Jin et al. 2006).

Anaerobic Biodegradation of Dioxins

One of the most favorable techniques for detoxification of highly chlorinated dioxin is reductive dechlorination. Highly chlorinated congeners are difficult to be degraded by aerobic pathways. Such compounds can be successfully degraded by an anaerobic mechanism. The reductive dechlorination process removes the chlorine from the aromatic ring anaerobically. Microorganism uses different molecular strategies to cleave the carbon-halogen bond. In the absence of oxygen, the microbial communities metabolize the organic compound using alternate electron acceptors (dehalorespiration). The absence of the electron acceptor has a direct impact on the degradation of the organic compounds (Bossert et al. 2005). Microbial dehalogenation of the various organic compounds has been observed in organisms from anoxic estuarine and marine sources (Rodenburg et al. 2017, Vargas et al. 2001). The corresponding fully dechlorinated or partially dechlorinated product is more susceptible to further degradation by both aerobic and anaerobic techniques. Bacteria related to the genus *Dehalococcoides* were found to undergo reductive dechlorination of highly chlorinated dioxins to a much lower chlorinated compound (Bunge & Lechner 2009b). In another study, a microcosm was built to analyze the dechlorination mechanism of sediments contaminated with PCDD/F taken from the historically contaminated site of the Kymijoki River. It was spiked with 1,2,3,4-Tetrachloro dibenzofuran and the result showed that the sediment contained indigenous microorganisms capable of dechlorinating the dioxin (Kuokka et al. 2014).

Mycoremediation of Dioxins

Degradation of dioxin is found to be carried out by various ligninolytic fungi isolated from soil (Dao et al., 2019). The organisms that are capable of degrading the most complex polymer, lignin, using an extracellular enzyme which includes lignin peroxidase and manganese peroxidase, have the potential to degrade most recalcitrant compounds including polychlorinated dioxins (Field et al. 1993). Furthermore, the fungus has the ability to absorb and biotransform hazardous contaminants. Biodegradation of dioxin by microorganisms was first reported by white-rot fungi *Phanerochaete sortida* YK-624 (Kanan & Samara 2018). Bumpus et al. (1985) evaluated the biodegradation potential of *P. chrysosporium* in degrading the 2,3,7,8-TCDD. Takada et al. (1996) reported that *P. sortida* could degrade highly chlorinated dioxins and furans. Table 1. gives the details of various fungi and their biodegradation ability of various dioxin congeners.

Table 1: Dioxin degrading fungi with their degradation potential of various congeners.

Organism	Compound degraded	Duration	Percentage of degradtion	Reference
Rigidoporus sp. FMD21	2,3,7,8- Tetrachlorodibenzo-p-dioxin	50 days	79.9%	(Dao et al. 2021)
Phanerochaete chrysosporium OGC101	2,7- Dichlorodibenzo-p-dioxin	27 days	50 %	(Valli et al. 1992)
P. velutina, *Stropharia rugosoannulata,* *Gymnopilus luteofolius*	Polychlorinated dibenzo-p-dioxin/Furan	77 days 70 days	67% 61% 69%	(Anasonye et al. 2014)
Phlebia brevispora	1,3,6,8- Tetrachlorodibenzo-p-dioxin	90 days	50%	(Kamei et al. 2009)
P. sordida YK-624	Polychlorinated dibenzo-p-dioxin/Furan	14 days	76% 70%	(Takada et al. 1996b)
Pleurotus pulmonarius	Polychlorinated dibenzo-p-dioxin/Furan	72 days	96%	(Kaewlaoyoong et al. 2020)
Panellus styticus	2,7- Dichlorodibenzo-p-dioxin	40 days	100 %	(Sato et al. 2002)

Dioxin Removal by Composting Microorganisms

Compost-mediated bioremediation is a biological process based on combining the feedstock with contaminated soil, where the compost matures, which ultimately results in the degradation of the pollutant (Semple et al. 2001). Co-composting is regarded as one of the efficient methods for the degradation of the recalcitrant compounds into less toxic compounds with the aid of microorganisms (Covino et al. 2016, Mattei et al. 2016). The potential composting relies on various factors like microbial metabolism, moisture content, temperature, pH, nutrients, and the density of bulking agent (Semple et al. 2001). Lin et al. (2018) studied the degradation of dioxin-contaminated soil by a co-composting technique using food waste. The results showed that 70% of PCDD/F was successfully degraded in 49 days with initial TEQ 6048 ng-TEQ/kg was reduced to 1604 ng-TEQ/kg. The author concluded that thermophilic degradation can be used to degrade dioxin. Huang et al. (2019) examined the organisms involved in co-composting and found that after 42 days of composting the degradation was around 75% and the toxic equivalent quantity (TEQ) was reduced from 16.004 ng-TEQ kg^{-1} to 1916 ng-TEQ kg^{-1} day^{-1}. Beesley et al., (2010) state that the use of compost enhances the efficiency of degrading the PAH-contaminated soil by 25%, and their phytotoxicity test ensures that the amendment has increased the shoot length. Narihiro et al. (2010) used a developed fermentor which yielded a 15 – 16% reduction during remediation. Molecular studies revealed three major phylogenetic groups including *Acidaminobacter, Dehalococcoides,* and *Rhizobium* were present. Tran et al. (2020) suggested that the biodegradation of dioxin-contaminated sites can be carried out using bacteria obtained from aerobic composting with the removal efficiency of 81% in 35 days. The 16s RNA sequencing indicated that the organism belongs to *Bacilli, Actinobacteria, Clostridia, Gammaproteobacteria,* and *Alphaproteobacteria*. It provides an easy and safe method for remediation of dioxin-contaminated soil.

DEGRADATION OF DIOXINS IN MICROCOSMS

A microcosm is a miniaturized ecosystem model representing a portion of the natural environment under controlled laboratory conditions, therefore, representing microbial diversity (Cravo-Laureau & Duran 2014, Grenni et al. 2012). Microcosm experiment provides some advantages namely i) simple, cost-effective ii) maintaining replicates easily and manipulating the growth condition for maximum degradation results iii) identifying microbial community present in the natural environment inheriting the ability to degrade the pollutant (Salam et al. 2015). Chen et al. (2013) conducted an experiment in the PCDD/F contaminated soil to detect the bioremediation potential of various microorganisms by constructing a microcosm. Microbial activity was stimulated in a closed microcosm by adding oxygen and additional nutrients. Nearly 100% and 95-98% reduction was observed in Octachlorodibenzofurans (OCDF) and Heptachlorodibenzofurans (H_PCDF) respectively after six weeks and no significant reduction in Octachlorodibenzo-p-dioxin (OCDD) within 12 weeks. In the second batch microcosm, the bacterial consortium from the first batch was added to the fresh medium with 50 mg OCDF powder and the degradation efficiency for OCDF was 82.1% and 87.9% for OCDD demonstrating the microbial community present in the microcosm enhanced the degradation of highly chlorinated PCDD/F.

In an experiment conducted by Lin et al. (2017) the biodegradation potential of a novel strain, *Pseudomonas mendocina* NSYSU in OCDF polluted soil was studied through a microcosm under anaerobic conditions. A batch of seven different microcosms was set up. Upon incubation of 64 days, it was observed that 59% of OCDF was degraded in a batch containing the *P. mendocina* NSYSU and sterile soil, a 23% reduction was observed in the batch containing *P. mendocina* NSYSU and unsterile soil, and no reduction was observed in control batch.

NANOREMEDIATION OF DIOXINS

The concept of accelerating the removal of hazardous environmental contaminants (organic or inorganic pollutants) using a cost-effective treatment process led to the development of nanobioremediation (Cecchin et al. 2017). Nanobioremediation uses a combined effort of nanoremediation with microorganism-mediated biological treatment to clean up the recalcitrant environmental pollutants. In recent times, the use of nanoparticles is gaining greater importance in different fields of research due to their unique physicochemical properties which include a large surface area, the surface modification which ultimately results in increased reactivity, and better remediation performance (Chauhan et al., 2020, Guerra et al. 2018). Zero-valent(ZVI) iron is a widely used nanomaterial to treat sites contaminated with hazardous compounds (Galdames et al. 2020). Kim et al. (2008) evaluated the ZVI potential of nanoparticles in PCDD degradation. The results obtained show that the palladized ZVI shows three times increased dechlorination potential when compared to unpalladized ZVI. Binh et al. (2016) studied the 2,3,7,8- TCDD biodegradation using sequentially enriched microorganisms obtained from dioxin-contaminated soil with Carboxymethylcellulose (CMC) coated NZVI in a sequential anaerobic degradation followed by aerobic treatment. The results showed 58% degradation of the toxic compound.

CONCLUSION AND FUTURE PROSPECTIVES

The past century has witnessed an uncontrolled growth of industries and rapid urbanization that resulted in the massive contamination of the environment and one among them are dioxins. Their persistence is a global threat and immediate measures are to be taken to reduce their level in the environment. Their structural complexity makes them resistant to degradation. The environment is a niche containing a richness of species which is a reservoir of thousands of diverse microorganisms having various potentials including degradation of hazardous environmental pollutants. The current status of the bioremediation of dioxin shows promising results for biodegradation and detoxification of highly chlorinated dioxins. This review provides an opportunity to study microorganism and their mechanism involved in degrading the pollutant. Different techniques in bioremediation are also discussed in detail. The uncovered part of the study is the isolation of microorganisms from extremes of the environment and conduct of transcriptome, metagenomics, and proteomics studies, and the use of engineered organisms for carrying dioxin metabolizing enzymes for better efficiency of remediation. Further research has to be extended by involving molecular techniques to find a wide range of microorganism that possesses the potential to reduce high levels of dioxin in the soil. The Nanobioremediation technique is an emerging versatile technique that possesses the potential in treating the most toxic environmental pollutant. The use of nanomaterials along with the microorganism has proven to show high efficiency in degrading hazardous pollutants. Therefore, the advancement in the use of nanobioremediation would also promising technique for remediation of environmental challenges.

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