

## Review

# Bioremediation of Recalcitrant Xenobiotic Compounds: Advancements and Challenges

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### Abstract

Widespread recalcitrant xenobiotic compounds have posed serious environmental threats. These compounds are known to contaminate groundwater, crops, water bodies, and lands, affecting the lives of people living nearby. Sites polluted with organic contaminants like polycyclic aromatic hydrocarbons (PAHs), chlorinated hydrocarbons (CHC), phenols, and aromatic hydrocarbons (benzene-toluene-ethylbenzene-xylenes, BTEX) are located worldwide, in both developed and developing countries. Many pollutants have been classified as persistent organic pollutants (POPs) whose production and usage have been either limited or banned by the Stockholm Convention. In May 2001, the Stockholm Convention gave a list of 12 POPs and called them “dirty dozens,” which has now increased to 36 POPs. Much advancement has been made in the last decade; however, on-field bioremediation techniques are still not well developed. Detection of ongoing remediation in trials by using metagenomics, metatranscriptomics, metabolomics, and metaproteomics produces huge data sets. These in-silico analyses required efficient algorithms and skilled professionals to contemplate data analyses. Carbon-stable isotope analysis, microarrays, and high-throughput cultivation techniques are also promising methods that have helped researchers track bioremediation. However, challenges faced by the scientific community are numerous, which has affected the pace of advancements in achieving a defined strategy for bioremediation of xenobiotic compounds. This review summarises the need for remediation of recalcitrant xenobiotic compounds that pose a threat to the environment and human health. Also, advancements made in the field of bioremediation and the problems posed in the implementation of designed strategies have been summed up.

### Keywords

Bioremediation, Xenobiotic Compounds, PAHs, POPs

### Introduction

The problem of recalcitrant xenobiotic compound pollution has been widely compromising the human health and the environment (Kirkok *et al.*, 2020). In the course of development and industrialization, soil and groundwater contamination has affected the livelihood of the people living in the vicinity (Li *et al.*, 2021).

Inappropriate treatment of municipal and industrial waste, improper storage of chemicals, leaking of contaminants from waste pits, mine tailing, landfills and buried containers have created polluted sites worldwide (EEA, European Environment Agency, 2007). Organic contaminants mainly include polycyclic aromatic hydrocarbons (PAHs), chlorinated hydrocarbons (CHC), phenols, aromatic hydrocarbons and benzene-toluene-ethylbenzene-xylenes, (BTEX) (Vogt and Richnow, 2013). Many of these are environmentally lethal, highly persistent and carcinogenic in nature (Jayaraj *et al.*, 2017).

PAHs are a group of compounds formed due to the incomplete combustion of organic compounds, like tar, coal, oil and other fossil fuels, tobacco, smoked food, automobile exhaust, and other anthropogenic activities including geothermal reactions for extraction of fossil fuels and minerals (Ghosal *et al.*, 2016). Naturally, they are being formed due to forest fires and volcanic eruptions. Thus, their composition mainly depends on starting material and temperature of combustion. Phenolic compounds are found in water bodies due to wastewater discharge from agriculture, industrial and domestic activities (Said *et al.*, 2021). Naturally, they are accumulated due to decomposition of organic matter (Anku *et al.*, 2017). These toxic compounds pose a lethal and long lasting effect on humans and animals where they can cause damage to red blood cells and the liver. Interestingly, some microorganisms may metabolize these phenols into more toxic compounds than the parent compound (Anku *et al.*, 2017). The BTEX compounds are volatile organic compounds which are determined as industrial pollutants. They are mainly used in industries like printing, leather, gasoline and rubber (Su *et al.*, 2010). Leaching of BTEX as a part of industrial effluents is the common mode of its spread in water bodies which lead to adverse impact on water quality (Su *et al.*, 2010).

Chlorinated hydrocarbons (CHCs) are among the most recalcitrant and persistent organic compounds due to high chemical stability of C-Cl bond (Copley *et al.*, 1997). Their occurrence in consumer and industrial products such as household cleaners, dry cleaning products, glues, metal degreasers has been profoundly known. When entered in groundwater or spilled on soil, they can remain there persistently and after vaporization

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can enter into humans and livestock through air (EPA, Environment Protection Agency, 2021). Hexachlorocyclohexane (HCH) is one of the chlorinated hydrocarbons, manufactured to be used as pesticide whose only one isomer,  $\gamma$ -HCH, has pesticidal properties which accounts of 10-12% of total HCH produced and rest of the isomers are collectively called as muck and being discarded in open sinks (Lal et al., 2010). Impacts of CHCs include damage of CNS, kidneys, liver, immune system, endocrine system and reproductive system and cancer.

For effective clearance of these organic pollutants, different physical and chemical methods such as incineration, UV oxidation, solvent extraction, base-catalyzed dechlorination and fixation have been used extensively; however, they are incorporated to change the compounds from one form to another instead of solving the problem (Ghosal et al., 2016). Efficient, eco-friendly way to clean up these contaminants is bioremediation, which involves the use of microbes can transform them to less or non-toxic compounds. Microorganisms are continuously evolving in nature, thus, they are capable of transforming almost every naturally occurring compound (Gupta et al., 2016). But, synthetically made compounds or compounds released due to anthropogenic activities are not common for microbial communities; still they transform most of them due to broad substrate specificity of the enzymes which are involved in the degradation pathways of natural compounds. These degradation pathways are encoded by genes which may or may not be present in an operon. Generally, microbes work in a community in which they together can take part in transformation of a recalcitrant compound by catalyzing different steps of degradation. Knowledge of these microbes and their

ability to transform can help in building better and effective bioremediation strategies. Such strategies may include consortia of many microbes which can synergistically act upon the xenobiotic compounds. This review summarizes such bioremediation strategies which have been employed in the last few decades and their impact. It also includes the recent advancements made in the field of bioremediation and challenges faced by researchers which are required to overcome in order to develop proficient and systematic bioremediation technology.

### Worldwide scenario of xenobiotic contamination

Developed countries are better at managing these sites compared to developing countries due to the sustainable remediation approaches (Braun et al., 2020). Many of the pollutants have been classified as persistent organic pollutants (POPs), which are a group of toxic, long-lasting compounds that can persist in the environment for extended periods. That is why their production and usage has been limited or banned by the Stockholm Convention. The Stockholm Convention is an environmental treaty made between 152 states and effective since May, 2001 (Stockholm convention, 2013). Initially, 12 highly persistent and toxic compounds were identified and listed in Stockholm convention, called the “Dirty dozen” (EPA) (Table 1). POPs listed by the Stockholm Convention are divided into three categories. Category A includes chemicals whose production and use should be completely avoided, category B include chemicals whose production and use should be restricted and category C include chemicals whose unintentional release should be reduced (Table 1). Since then, PAHs and brominated carbons have been added to the list. Currently, 24 new POPs have been added into the list.

**Table 1:** Details of POPs listed as per Stockholm Convention, June 2023.

S. No.	Name of the compound	Usage	Intitial POP/New POP	Banned Since	Degrading Bacteria
<b>Category A</b>					
1.	Aldrin	Pesticide	Intitial POP	1987	<i>Cupriavidus</i> sp. Med-5 <i>Burkholderia</i> sp. Med-7 Matsumoto et al., 2008
2.	Chlordane	Pesticide	Intitial POP	1988	
3.	Chlordecone	Pesticide	New POP	2009	<i>Citrobacter_86-1</i> & <i>Citrobacter_92-1</i> Chaussonnerie et al 2016
4.	Decabromodiphenyl ether (commercial mixture, c-decaBDE)	Industrial Chemical	New POP	2007	<i>Brevibacillus</i> sp. (M1) & <i>Achromobacter</i> sp. (M2) Hu et al 2022
5.	Dicofol	Pesticide	New POP	2023	<i>Microbacterium</i> sp. D-2. Lu et al 2019
6.	Dieldrin	Pesticide	Intitial POP	1974	<i>Burkholderia</i> sp. strain MED-7 & <i>Cupriavidus</i> sp. strain MED-5.

S. No.	Name of the compound	Usage	Intitial POP/New POP	Banned Since	Degrading Bacteria
					Matsumoto et al 2008
7.	Endrin	Pesticide	Intitial POP	1984	<i>Burkholderia</i> sp. strain MED-7 & <i>Cupriavidus</i> sp. strain MED-5. Matsumoto et al 2008
8.	Heptachlor	Pesticide	Intitial POP	1974	Strain H. Qiu et al 2018
9.	Hexabromobiphenyl	Industrial Chemical	New POP	1976	<i>Arthrobacter</i> Subdivision. Kuppusamy, S. 2016
10.	Hexabromocyclododecane (HBCDD)	Industrial Chemical	New POP	August 2015	<i>Bacillus cereus</i> and <i>B. subtilis</i> . Chout et al 2021
11.	Hexabromodiphenyl ether and heptabromodiphenyl ether	Industrial Chemical	New POP		
12.	Hexachlorobenzene (HCB)	Pesticide, Industrial Chemical	Intitial POP	1966	<i>Nocardioides</i> sp. strain PD653. Takagi et al 2009
13.	Hexachlorobutadiene	Industrial Chemical	New POP	2020	<i>Serratia marcescens</i> HL1. Li et al 2008
14.	Alpha hexachlorocyclohexane	Pesticide	New POP	1970	<i>Sphingobium baderi</i> LL03 <sup>T</sup> Kaur et al., 2013
15.	Beta hexachlorocyclohexane	Pesticide	New POP	1970	<i>Sphingobium lucknowense</i> Strain F2 <sup>T</sup> Negi et al., 2014
16.	Lindane	Pesticide	New POP	1970	<i>Sphingobium lucknowense</i> Strain F2 <sup>T</sup> Negi et al., 2014
17.	Mirex	Pesticide	Intitial POP	1977	<i>Bacillus sphaericus</i> , <i>Streptomyces albus</i> . Atlanta (GA). 2020
18.	Pentachlorobenzene	Pesticide, Industrial Chemical	New POP	2010	NA
19.	Pentachlorophenol and its salts and esters	Pesticide	New POP	2018	<i>Janibacter</i> sp. Khessairi et al., 2014
20.	Polychlorinated biphenyls (PCB)	Industrial Chemical	Intitial POP	1979	<i>Cyanobacterium anabaena</i> PD-1. Zhang et al., 2015
21.	Polychlorinated naphthalenes	Industrial Chemical	New POP		No specific information
22.	Perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds	Industrial Chemical	New POP		
23.	Perfluorohexane sulfonic acid (PFHxS), its salts and PFHxS-related compounds	Industrial Chemical	New POP		
24.	Short-chain chlorinated paraffins (SCCPs)	Industrial Chemical	New POP		
25.	Technical endosulfan and its	Pesticide	New POP	2011	<i>Pseudomonas mendocina</i> ZAM1,

S. No.	Name of the compound	Usage	Initial POP/New POP	Banned Since	Degrading Bacteria
	related isomers				<i>Pseudomonas fluorescens</i> Mir et al., 2017.
26.	Tetrabromodiphenyl ether and pentabromodiphenyl ether	Industrial Chemical	New POP		
27.	Toxaphene	Pesticide	Initial POP		
<b>Category B</b>					
28.	DDT	Pesticide	New POP	2011	<i>Stenotrophomonas</i> sp. DDT-1. Pan et al., 2016.
29.	Perfluorooctane sulfonic acid (PFOS), its salts, perfluorooctane sulfonyl fluoride (PFOS-F)	Pesticide, Industrial Chemical	New POP		
<b>Category C</b>					
30	Hexachlorobenzene (HCB)	Unintentional Production	New POP	2008	<i>Nocardioides</i> sp. strain PD653. Ito et al. 2017.
31.	Hexachlorobutadiene (HCBd)	Unintentional Production	New POP	No specific information available	
32.	Pentachlorobenzene (PeCB)	Unintentional Production	New POP	No specific information available	
33.	Polychlorinated biphenyls (PCB)	Unintentional Production	New POP	1989	<i>Achromobacter</i> sp. <i>Pseudomonas aeruginosa</i> <i>Alcaligenes eutrophus</i> H850 <i>Pseudomonas putida</i> LB400 Erickson, B. D., & Mondello, F. J. (1992).
34.	Polychlorinated dibenzo-p-dioxins (PCDD)	Unintentional Production	Initial POP	No specific information available	<i>Pseudomonas</i> sp. strain CA10 Habe et al. 2001b
35.	Polychlorinated dibenzofurans (PCDF)	Unintentional Production	Initial POP	No specific information available	<i>Terrabacter</i> sp. strain DBF63 Habe et al. 2001a
36.	Polychlorinated naphthalenes (PCNs)	Unintentional Production	New POP	2025	<i>Pseudomonas</i> sp. HY. Yu et al., 2015.

Sources of such POPs include unwanted by-products of industrial processes, high temperatures and undesired/desired combustion and improper use of agro- and industrial chemicals and/or their improper disposals. These POPs are distributed in long range and tend to bio accumulate in the animal and human tissues due to their high solubility in lipids (Gautam et al., 2020). POPs can circulate over large distances and even

globally due to the cycle of evaporation and deposition, thus, the source remains untraced (Vogt et al., 2013). Evidence of the wide range transport of these particulate substances containing POPs can be accounted from the satellite image. As these compounds can exist in different phases like gas or airborne particles or a part of rain, they can be exchanged in environment media. Their transfer has also been

reported across borders through migratory species (Vogt et al., 2013).

According to the United Nations Environment Programme (UNEP) 2003, many persistent pesticides have declined from the environment since the 1990s especially, DDT, heptachlor and chlordane. However, absence in their alternative led the developing countries to continue their use, even at a lesser level. Areas where agricultural practices are done predominately like Central and South American region, East Asian region, pesticide persistence poses serious problems. dioxins and furans levels, the non-pesticide POPs are observed higher in urban and industrialized areas of Europe, East Asia, North America and Mediterranean region which may be due to the poor waste management and incomplete burning (Fact Sheet, UNEP). Mismanagement of POPs, overuse of pesticides for agricultural expansion, release of unmonitored effluents in the water bodies suggests that in the name of progress and industrial and agriculture expansion we have moved higher towards environmental degradation which is becoming uncontrollable even for the developed nations.

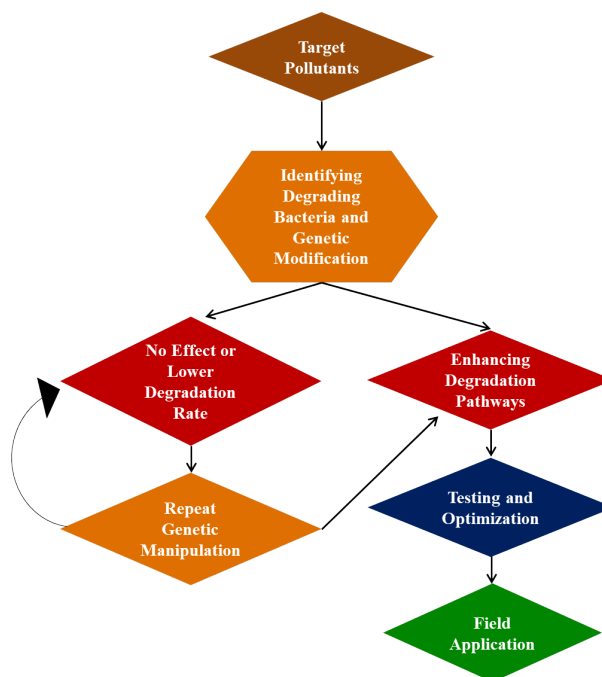
**Advancement made in development of bioremediation methods in last decade**

In the last decade advancements have been made in many ways to achieve the goal of degradation of xenobiotic compounds. First is the genetically modified microbe, often referred to as genetically engineered microorganisms (GEMs), have shown great promise in the field of bioremediation for the degradation of POPs. Genetically modifying microorganisms which are natural degrader can enhance their ability to degrade specific pollutants, including POPs, making them more effective in remediation efforts. Here's how genetically modified microbes are used for the degradation of POPs. (Figure 1):

- a) Identifying target pollutants: Researchers first identify the specific POPs present in the contaminated site. Different POPs require different enzymes and metabolic pathways for degradation. Once the target pollutants are identified, scientists can select or engineer microorganisms with the appropriate genetic traits to tackle these pollutants.
- b) Genetic modification: Scientists use various genetic engineering techniques to modify the microorganisms. This involves introducing new genes or modifying existing ones to produce enzymes that can break down the target pollutants. These enzymes may include cytochrome P450 (Singh et al., 2016) (Stein et al., 2018), dehalogenases, or other specialized enzymes capable of degrading POPs
- c) Enhancing degradation pathways: The genetic modifications aim to enhance the natural

degradation pathways of the microorganisms or introduce entirely new pathways to break down the pollutants into less harmful substances (Jaiswal, S., & Shukla, P. 2020)

- d) Testing and optimization: After genetic modification, the engineered microorganisms undergo laboratory testing to evaluate their effectiveness in degrading the target POPs. This testing helps optimize the conditions for their growth and degradation activity (Rafeeq et al., 2023).
- e) Field application: Once the genetically modified microbes are proven effective and safe in controlled laboratory settings, they may be applied to the contaminated site. This process involves carefully introducing the GEMs into the environment to degrade the POPs and clean up the pollution (Apollon et al., 2022)..



**Figure 1:** Flow chart on how genetically modified microbes are used for the degradation of POPs.

Benefits of using genetically modified microbes for POPs degradation are many and put GEM as prominent way to treat such contaminants (Nora et a., 2016). One of such benefit is specific target degradation as GEMs are tailored to target specific pollutants, increasing the efficiency of the bioremediation process. GEMs can also accelerate the degradation of POPs compared to naturally occurring microbes, which can be slower or less effective in breaking down these persistent compounds. Also, modifying organisms to thrive in the specific environmental conditions of the contaminated site can maximize their effectiveness. Genetically modified microbes can be designed to degrade pollutants into non-toxic or less toxic compounds,

reducing the overall environmental impact of the cleanup process. However, it's essential to approach the use of genetically modified microbes with caution. Potential risks and unintended consequences must be thoroughly evaluated before field application (Ang et al., 2005). Therefore, regulatory bodies often require extensive testing to assess the safety and effectiveness of GEMs before they are used in large-scale bioremediation projects.

Second advancement is Microbial fuel cells (MFCs) which involves bio-electrochemical systems that utilize microorganisms for conversion of organic matter into electrical energy. They have gained attention for their potential to treat wastewater while simultaneously generating electricity. The application of MFCs in treating persistent organic pollutants typically involves coupling the microbial degradation of these pollutants with electricity generation (Abbas et al., 2019). Process of microbial fuel cells involves, pollutant degradation in which the anode chamber of the MFC, microorganisms (such as bacteria or fungi) are grown on an electrode and fed with the persistent organic pollutants. These microorganisms are capable of breaking down some of these pollutants through metabolic processes (Thung et al., 2015). During the microbial degradation process, electrons are released as byproducts. The microorganisms transfer these electrons to the anode electrode. The flow of electrons from the microorganisms to the anode creates an electric current, which can be harvested as electricity. As the pollutants are degraded, the effluent in the anode chamber becomes cleaner, reducing the concentration of harmful organic pollutants in the wastewater.

Though the concept of using MFCs for POPs remediation is promising but, there are some challenges and limitations which should be considered (Fang and Achal, 2019). The degradation of certain persistent organic pollutants may be slow, limiting the efficiency of the process. MFCs may not be equally effective for all types of persistent organic pollutants, as different microorganisms have varying capabilities to degrade specific compounds. Designing an efficient MFC system for POPs remediation requires careful consideration of factors such as electrode materials, reactor configuration, and operating conditions. Also, while MFCs have shown promise at lab-scale, scaling up the technology for real-world applications and industrial use is still a challenge. Research in this field is ongoing, and the effectiveness of microbial fuel cells for persistent organic pollutant removal may improve with further advancements in microbial engineering, reactor design, and understanding of microbial communities' interactions with these pollutants. Nonetheless, MFCs represent an innovative approach that combines pollution control with renewable energy generation.

Third is Nano-materials which have shown promising potential for the degradation of persistent organic pollutants (POPs) due to their unique properties, high surface area, and reactivity. Nano-TiO<sub>2</sub> (Titanium

Dioxide) nanoparticles are photocatalysts that can be activated by UV light (Mandeep & Shukla 2020). When exposed to light, they generate reactive oxygen species (ROS) that can break down POPs into less harmful compounds through oxidation processes. Like TiO<sub>2</sub>, Nano-ZnO (Zinc Oxide) nanoparticles can act as photocatalysts under UV light, producing ROS to degrade POPs through similar oxidation reactions. Another such nanoparticle, Nano-Fe<sub>0</sub>, has a high surface area and can act as a reducing agent to transform POPs into less toxic compounds by reductive reactions. Some researchers have developed nano-composites, which combine different nano-materials to enhance the degradation capabilities (Liosis et al 2021). For example, a combination of TiO<sub>2</sub> and graphene can improve the photocatalytic activity and stability. Nano-Metal Organic Frameworks (MOFs) are highly porous materials with metal ions coordinated by organic ligands. They have tunable structures and can act as adsorbents to remove POPs from the environment. In some cases, they can also catalyze the degradation of POPs. Carbon nanotubes (CNTs) and graphene oxide (GO) are examples of carbon-based nano-materials that have shown potential for POP degradation (Deng et al., 2019) through adsorption and catalytic reactions.

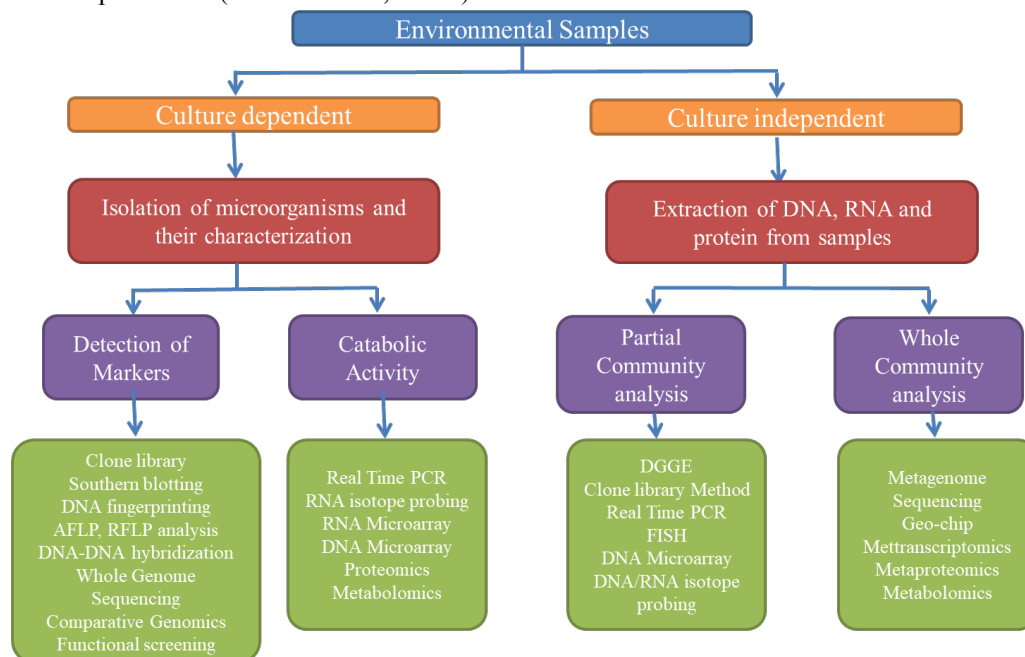
Fourth is the biofilms which offer a promising approach for the degradation of persistent organic pollutants (POPs) in the environment. A biofilm is a structured community of microorganisms (such as bacteria, fungi, and algae) which adhere to the surfaces and are embedded in a self-produced matrix of extracellular polymeric substances (EPS). Biofilms consist of diverse microbial communities that can work together in a synergistic manner (Edwards and Kjellerup, 2013). Different microbial species may possess complementary enzymatic capabilities, allowing them to collaborate in breaking down complex organic pollutants that individual microorganisms may struggle to degrade on their own. Also, within the biofilm, microorganisms create localized microenvironments with different oxygen and nutrient concentrations. This enables a wide range of microbial metabolic processes to occur simultaneously, accommodating different pollutant degradation pathways (Sonawane et al., 2022). The EPS matrix of biofilms can act as an adsorbent, trapping POPs from the surrounding environment. This sorption process concentrates the pollutants and facilitates their uptake and degradation by the microorganisms. Microorganisms within the biofilm are better protected from environmental stressors like fluctuating temperatures, toxic compounds, and predators. This protection allows them to persist and maintain their degradation activities over extended periods. Biofilm-associated microorganisms often produce specific enzymes that are highly efficient in breaking down organic pollutants (Bhatt et al 2021). Once established, biofilms can be self-sustaining, continuously removing and degrading POPs from the environment as long as favorable conditions persist.

The use of biofilms for POP degradation is an active area of research and holds significant promise for environmental remediation. However, there are challenges to consider, such as optimizing the composition of the biofilm to efficiently degrade specific pollutants and ensuring the biofilm's stability and functionality under various environmental conditions. Furthermore, biofilm-based remediation approaches need to be carefully designed and monitored to prevent potential unintended consequences, such as the release of transformed or intermediate toxic compounds during the degradation process. Proper risk assessments and management strategies are essential to ensure the safe and effective application of biofilms for POP degradation.

Fifth such advancement is constructed wetlands which are other innovative approach for the remediation of persistent organic pollutants (POPs) in the environment. They are engineered systems designed to mimic natural wetlands but are specifically constructed to treat various types of wastewaters, including those contaminated with POPs. These wetlands can effectively remove and degrade POPs through a combination of physical, chemical, and biological processes (Wang et al., 2022). As contaminated water flows through the wetland, POPs can be adsorbed or sorbed onto the surface of wetland vegetation, roots, and the substrate. The porous nature of the wetland soil and the presence of organic matter can enhance the sorption of POPs from the water. Constructed wetlands host diverse microbial communities, including bacteria and fungi, which possess the ability to biodegrade various organic pollutants, including POPs. These microorganisms break down the pollutants into less harmful substances through metabolic processes (Kumar et al., 2022).

Wetland vegetation, such as reeds, cattails, and other aquatic plants, can take up and accumulate certain POPs in their tissues. This process, known as phytoremediation, helps to remove the pollutants from the water, effectively reducing their concentration. The combination of various biological and chemical processes in the wetland environment can lead to the transformation and detoxification of POPs into less toxic forms. Constructed wetlands often have varying oxygen levels in different zones, creating redox (reduction-oxidation) conditions. These conditions can facilitate the transformation of POPs through reduction or oxidation processes, depending on the specific pollutant and its chemical properties. The slow flow of water through the wetland provides sufficient retention time, allowing the various physical and biological processes to take place and contribute to POP removal.

It's important to note that the effectiveness of constructed wetlands for POP remediation depends on factors such as the design and configuration of the wetland, the specific types and concentrations of POPs present, and the prevailing environmental conditions. Additionally, monitoring and ongoing management are essential to ensure that the wetland system remains efficient and sustainable in the long term. Constructed wetlands offer a cost-effective and environmentally friendly approach for treating POP-contaminated waters and represent an important component of strategies for managing these persistent pollutants in the environment. However, as with any remediation technology, it's crucial to consider site-specific factors and conduct comprehensive assessments before implementing constructed wetlands for POP treatment.



**Figure 2:** Schematic diagram showing the different molecular techniques for degradation studies. Source: Ghosal et al., 2016



## Role of In-silico approach in assessing bioremediation at the site

There are several high-throughput approaches to analyze bioremediation of sites which are contaminated with hazardous and/or recalcitrant wastes (Figure 2). Advancements may have occurred, but here are some of the promising techniques at the current time. High-throughput sequencing technologies enable the different meta- studies of entire microbial communities present in the contaminated soil or water, providing insights into the functional potential of these communities and the genes involved in biodegradation processes. Metagenomics provides valuable insight into the diversity, abundance, and functional potential of microbial populations involved in pollutant degradation (Achudhan *et al.*, 2023, Jadeja *et al.*, 2023). Researchers can discover novel genes and pathways involved in bioremediation processes, potentially leading to the development of more efficient bioremediation strategies. This information helps assess the suitability of a site for bioremediation and predict the efficiency of the biodegradation process. Tracking changes in the microbial community over time during bioremediation provides valuable information about the success and progress of the remediation process. Monitoring shifts in microbial populations can also help identify potential challenges and improve bioremediation strategies is another application of metagenomics.

Many studies have shown the importance of metagenomic sequencing in various ways. Amplicon sequencing [Allen *et al.*, 2007, Zhang *et al.*, 2012, Silva *et al.*, 2013], denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (t-RFLP) and has been reported to assess degrading communities of contaminated sites [Xingbiao *et al.*, 2015]. Community profiling of aromatic and oil-degrading microbes with stable isotope probing (SIP)(M.G. Dumont *et al.*, 2005, Bell *et al.*, 2011), QIIME 2 for phylogenetic inferences (Bolyen *et al.*, 2019) of reads from different platforms such as Ion Torrent sequencing platforms especially at petroleum muck harboring high concentration of aromatics and diesel contaminated Arctic waters have been reported (Bell *et al.*, 2013, Joshi *et al.*, 2014). The fourth generation Nanopore NGS platforms with advantages like low-cost genotyping, label-free, high throughput, ultra-long reads (Guerra *et al.*, 2018, Ludden *et al.*, 2017, Xia *et al.*, 2017), low material requirement and mobility of testing have been used in metagenomic analyses of polluted sites. The sites are like municipal sewage pollutants (Xia *et al.*, 2017). Further bacterial disintegration and plastic-degradation studies by DNA metabarcoding with Nanopore MinION is also reported for the Mediterranean sea (Davidov *et al.*, 2020). There are many databases, such as RDP II, Greengenes and SILVA-ngs, which are used for taxonomic and metabolic profiling. Potential microbial degraders could be identified by subjected to metagenomic analyses in

which specific pollutants and their catabolic gene pool are known (Bouhajja *et al.*, 2016, Ufarte *et al.*, 2015, Bharagava *et al.*, 2019, González-Toril *et al.*, 2023). Metatranscriptomics can get useful insights into the complete set of RNA molecules being expressed in regulatory as well as functional cellular metabolism in response to environmental stimuli (Güell *et al.*, 2011). Metabolic degradation of organic contaminants by *Micrococcineae* and *Corynebacterineae* in a complex microbial community of soil accompanied by up-regulation of oxidative stress response, DNA, O<sub>2</sub> uptake, phosphorus metabolism and down-regulation of carbohydrate (Menezes *et al.*, 2012). DNA microarrays with transcriptomics of pure cultures of *Escherichia coli* and other bacteria also provide functional information about the diversity (Güell *et al.*, 2011). RW1 perceived Dibenzofuran as a stressor and thereby involved in stress response and starvation during cell division, TCA cycle down-regulation. Gene knockout, targeted gene expression studies, Global transcriptomics by DNA-microarray has been instrumental to understanding toluene plus glucose metabolism by *P. putida* KT2440 (Castillo *et al.*, 2007). During aromatic metabolism by ZJWU it was confirmed that the principal cellular processes involved oxidative stress response enzymes, transporters, cell wall synthesis and chaperones (Yoneda *et al.*, 2016, Sha'arani *et al.*, 2019, Zampolli *et al.*, 2020). RNA-Seq authenticated the previous observations by showing up-regulation of methanogenesis, NO<sup>-3</sup> reduction, polyester synthesis and fatty acids  $\beta$ -oxidation.

Metaproteomics involves the identification and quantification of proteins expressed by microbial communities in contaminated sites. By studying the proteome, researchers can gain insights into the actual proteins involved in biodegradation and metabolic activities, complementing the information obtained through metagenomics and metatranscriptomics.

Other than Meta-, many techniques are useful in detection of ongoing rates of bioremediation. Stable Isotope Probing (SIP) is a technique used to identify the active microbial populations involved in the biodegradation of specific contaminants. It involves the use of isotopically labeled substrates (e.g., carbon-13) that are metabolized by specific microbial groups. By tracking the labeled carbon on the microbial biomass, researchers can determine which microorganisms are actively degrading the hazardous or recalcitrant wastes. Microarrays allow researchers to study the expression of thousands of genes simultaneously. Custom microarrays can be designed to target genes involved in biodegradation processes, enabling the monitoring of specific pathways during bioremediation. High-Throughput Cultivation Techniques such as microfluidics and automated plating systems, help increase the efficiency of microbial isolation and cultivation, allowing researchers to explore the biodegradation potential of a broader range of



microorganisms. In addition to these, the use of bioinformatics and machine learning for analyzing the vast amount of data generated by high-throughput approaches. These tools can help identify key microbial players, predict biodegradation pathways, and optimize bioremediation strategies.

### Challenges in developing technologies

Developing bioremediation technologies is a complex and challenging task, as it involves addressing various technical, ecological, regulatory, and ethical considerations. Certain factors should be taken care of while developing bioremediation technologies. First is specificity and efficiency, which requires ensuring that the engineered organisms or bioremediation agents target only the intended pollutants and efficiently degrade or remove them without affecting non-target organisms or causing unintended consequences. Second is the environmental complexity which attributes, complex a mix of various contaminants which may interact with each other or hinder bioremediation processes. Developing bioremediation strategies that work effectively in such complex environments is a challenge. Third is stability and persistence, which involves engineered bacteria or organisms needing to be stable and capable of persisting in the target environment long enough to remediate the pollutants effectively. Fourth is biocontainment by preventing genetically modified organisms from spreading to unintended areas and ensuring they do not become invasive or cause ecological disruptions is a significant challenge in bioremediation. Fifth is regulatory approval for development and deployment of bioremediation technologies through regulatory scrutiny to assess their safety and potential environmental impacts. Obtaining regulatory approvals for using genetically modified organisms can be time-consuming and resource-intensive. Sixth is the scale-up and deployment, which involves moving bioremediation technologies from the laboratory to real-world applications on a large scale. Factors like cost-effectiveness, logistics, and the ability to adapt to different polluted environments need to be considered. Seventh is long-term monitoring and liability to ensure that the treated areas remain free of pollutants and do not pose new risks. Determining liability for any potential adverse effects is also a complex issue. Eighth is public perception and acceptance involves addressing public concerns, building trust, and promoting understanding of technology's benefits and risks are vital for its acceptance and successful implementation.

There are possibilities to encounter problems during the development of the above strategy. This may include the evolution of pollutants and the development of resistance to bioremediation strategies over time. This necessitates continuous research and adaptation of bioremediation techniques to remain effective. Also, implementing bioremediation technologies in areas with existing infrastructure and human activities may present challenges.

Ensuring compatibility and minimal disruption to human activities is crucial. Finally, developing bioremediation technologies that are cost-effective and financially viable compared to traditional remediation methods is essential for their widespread adoption, especially in developing countries. Addressing these challenges requires a multidisciplinary approach, collaboration between researchers, regulators, and stakeholders, and ongoing research and innovation. Bioremediation has great potential as an eco-friendly solution to environmental pollution, but careful and responsible development is necessary to overcome the associated challenges and realize its full benefits.

### Conclusion

In this review, we summarized the current scenario of xenobiotic degradation along with the recent advancements in the field which seem to improve at a higher level in the past years. The understanding of degradation of various xenobiotic compounds has been enhanced in the last few decades due to the identification and characterization of new degrading organisms, development of new technologies, better understanding of the environmental pollutants, genetically modified organisms and immobilized catabolic enzymes. The role of microbes in a community can be greatly determined with the help of Omics techniques, but the problem lies in analyzing huge data which results from such studies. The successful application requires the control of various factors which affect the rate of bioremediation. This includes pH, temperature, oxygen concentration, nutrient availability, proper humidity etc. Also, it is important to recognize the microbial processes taking place at the site, the complexity of organic pollutants and long term deployment of a method and its maintenance. Bioavailability of an organic pollutant is also crucial for its biodegradation, which can be enhanced using the surfactants molecules. Some bacteria are known to produce surfactant like salicylic acid which may enhance the biosolubility of a pollutant, and in turn can enhance its rate of degradation. Apart from this, the changes in pollutants over time and the arrival of new pollutants require continuous research and improvisation in strategies. Thus, developing an efficient bioremediation strategy requires studying in depth all the possible parameters which are controlling the community profile and metabolic functioning of the habitat microbial population.

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