Microbe mediated remediation of dyes, explosive waste and polyaromatic hydrocarbons, pesticides and pharmaceuticals

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ABSTRACT

Industrialization and human activities have led to serious effects on the environment. With the progress taking place in the biodegradation field, it is important to summarize the latest advancement. In this review, we intend to provide insights on the recent progress on the biodegradation of environmental contaminants such as dyes, pesticides, pharmaceuticals, explosive waste and polyaromatic hydrocarbons by microorganisms. Along with the biodegradation of environmental contaminants, toxicity effects have also been discussed.

1. Introduction

One of the most problematic aspects of continuous anthropogenic activities and industrialization is the release of toxic waste into the environment. The release of such contaminants leads to disturbance in the nature which ultimately reflects in ecological processes. Direct effects of such contaminants may also lead to toxicity in various organisms including humans. The various kinds of environmental contaminants discussed in this review are dyes, pesticides, pharmaceuticals, explosive waste and persistent organic pollutants (POP). Dyes have been found to have carcinogenicity and allergic effects (Chung, 2016). Immuno-toxic effects of pesticides can also be found in literature (Corsini et al., 2013). The increased understanding of the dangerous effects of environmental pollutants has directed to a striking increase in research on various strategies that may be applied to clean up the environment. The physical and chemical treatment technologies presently used for the remediation of pollutants are expensive and cannot sufficiently mitigate or remediate these contaminants. Biodegradation is the natural process where substances are degraded biologically. Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds (Andeer et al., 2012). Whereas bioremediation is an engineered process of employing microorganisms to clean the environmental contaminants. It is the utilization of the biodegradation ability of microorganisms in such a manner that increases the speed of the process and provides a practical aspect of the property to clean the environment. In this review, the potential of microorganisms mediated remediation is looked upon. Various taxonomic groups such as fungi, archaeabacteria and eubacteria are abundant in members which can perform biodegradation of environmental contaminants. For performing the process of biodegradation, the microorganism first must be able to survive in the presence of particular contaminant. This review covers the research carried out in the field of bioremediation of dyes, PAHs, pharmaceuticals, explosive waste, and pesticides in the last decade and the toxicity effects of the respective pollutants in the last couple of decades.

2. Dyes

Dyes are soluble chemicals which provide color to the materials. They diffuse through the materials. Dyes have presence of at least one chromophore group in them. Dyes can be natural or synthetic. Dyes are widely used in food, pharmaceutical, leather, cosmetic and textile industries. These dyes when discharged into water bodies without any check or regulation pose a great threat to the aquatic life and consequently to the environment (B. Singh and Singh, 2016a). Among the different kinds of dyes used, the most common are azo, anthraquinone and deoxidizing dyes. An example of toxic effects of dyes is of Malachite Green (MG) a member of cationic triphenylmethane dye which has its use mostly as a fungicide or as a disinfectant agent. It is toxic to the mammalian cells in concentration as low as 0.1 mg/ml (Cleinemensen

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Table 1.
Microorganisms capable of biodegrading dyes.

| Microorganism/Co-Culture/Consortium | Dyes | Isolation from or Source | Degradation Pathway/Enzymes Involved | Degradation Product (Metabolite) | Percentage Transformation | Techniques Used | Reference |
|------------------------------------|------|--------------------------|-------------------------------------|----------------------------------|--------------------------|----------------|-----------|
| *Shewanella putrefaciencs*CN32, Bacillus circulansBW1L0161 | Sudan I | – | Synergistic effect of Azoreductase enzyme and Non-specific reductive decolorization activities of oxido-reductive enzymes lignin peroxidase, laccase and azoreductase were induced | – | 90.23% in 108 h | Drop plate method | (Liu et al., 2018) |
| *Bacillus pseudomycoides*MH229766 | Acid Black 24 (AB24) | Wastewater treatment plant in Noida, India | oxido-reductive enzymes like laccase, LiP and DCIP reductase | benzoic acid, 2(–1-oxopropyl) | 97.9% within 48 h | HPLC, FTIR, UV Spectroscopy | (Kumar et al., 2019) |
| *Fungal Strain* VITAIF-1 | Reactive Green Dye (RGD) | dye contaminated sites of Tirupur district, T.N, India | secretion of the extracellular enzymes lignin peroxidase, laccase, and azoreductase were induced | – | 90.3% in 72 h for initial dye concentration 100 mg/L | TLC, UV-vis spectroscopy, FTIR | (Sinha et al., 2016) |
| *Phanerochaete chrysosporium* | Reactive Black 5 (RB5) | – | asymmetrical cleavage of azo linkage | malachite green carbinol, (dimethyl amino phenyl)phenyl-methanone, N,N-dimethylaniline, (methyl amino)phenyl-phenyl-methanone, (amino phenyl)phenyl methanone and di benzyl methane | 90.3-97.2% at concentrations of MG 100-1000 mg/L | UV-vis GC-MS, LC-MS | (Du et al., 2011) |
| *Pseudomonas* sp. strain DY1 | Malachite green (MG) | – | Mn-peroxidase, NADH–DCIP and MG reductase were involved | 1-diazo-2-naphthol, 4-hydroxybenzenesulfonic acid, 2-naphthol and benzenesulfonic acid | 90% of decolorization of 10 mg/L of MV within 24 h | FTIR, NMR GC-MS | (Jain et al., 2012) |
| *Bacillus* sp. V1DMK, *Bacillus* sp. V3DMK, *Bacillus* sp. V7DMK, *Ochrobacterium* sp. V10DMK, *Bacillus* sp. V12DMK | Reactive Violet 5R (RV5) | Soil samples collected from Khaircutcanal, Gujarat, India | asymmetrical cleavage of azo linkage | malachite green carbinol, (dimethyl amino-phenyl)-phenyl-methanone, N,N-dimethylaniline, (methyl amino)-phenyl-phenyl-methanone, (amino phenyl)-phenyl methanone and di benzyl methane | 95% efficiency at 3% NaCl in | UV-vis TLC FTIR HPLC GC-MS and HPTLC | (R.L. Singh et al., 2015) |
| *Micrococcus luteus* strain SSN2 | Direct Orange 16 (DO-16) | textile industry near Ranipet, Tamil Nadu, India | reduction of the azo bond | 4(5-hydroxy-4-aminocyclopentane) sulfonylbenzene and 4(5-hydroxy-cyclopentane) sulfonylbenzene | 86% decolorization of azo dyes | HPLC, FTIR GC-MS and HPTLC | (Govindwar et al., 2014) |
| *Galactomyces geotrichum*MTCC 1360 | Reactive Yellow-84A | Microbial Type Culture Collection, Chandigarh, India | azoreductase, laccase and tyrosinase enzyme activities | 4(5-hydroxy-4-amino cyclopentane) sulfonylbenzene and 4(5-hydroxy-cyclopentane) sulfonylbenzene | 93.56% decolorization of 10 mg/L RV within 72 h of incubation in dark condition with agitation | UV-vis spectroscopy | (Hadhavara et al., 2013) |
| *Planerus eryngii* F032 | Reactive Black 5 (RB5) | recreational forest, UniversitiTeknologi Malaysia (UTM) | – | – | 98% dye removal observed at 0.1-0.3 g/L of dye | UV-visible spectral analysis, HPLC, and FTIR | (Prasad and Aikat, 2014) |
| *Enterobacter asburiae* strain XJUXH-4TM | Malachite green (MG) | dye-contaminated wastewater of a smallscale dyeing industry situated at Habra, West Bengal, India | significant increase in the activities of enzymes laccase, dichlorophenolindophenol reductase and malachite green reductase were observed | leucomalachite green, desmethylleucomalachite green, didemethylleucomalachite green, (dimethyl amino phenyl)-phenyl methanone, (methyl amino)phenyl-phenyl methanone, (amino phenyl)-phenyl methanone and aniline | >95% decolorization | UV-vis spectroscopy, TLC, GC-MS | (Mukherjee and Dan, 2014) |
| *Enterobacter* sp. SXCR | Congo red | from petroleum contaminated soil from Ranchi, Jharkhand, India | cleavage of azo bonds by azoreductase | – | 98% dye removal observed at 0.1-0.3 g/L of dye | UV-visible spectral analysis, HPLC, and FTIR | (Prasad and Aikat, 2014) |

(continued on next page)
| Microorganism/Co-Culture/Consortium | Dyes | Isolation from or Source | Degradation Pathway/Enzymes Involved | Degradation Product (Metabolite) Percentage Transformation | Techniques Used | Reference |
|-----------------------------------|------|--------------------------|--------------------------------------|--------------------------------------------------------|----------------|-----------|
| Acinetobacter baumannii           | Reactive red 198 | Kovalam sea shore in Tamil Nadu, India | biotransformation by various oxidative and reductive enzymes | – | 96.20% decolorization was observed in 500 mg/L of reactive red 198 after 72 h. | UV-visible spectroscopy and Fourier-transform infrared (FTIR) | (Unnikrishnan et al., 2018) |
| Arthrobacter soli BSS5            | Reactive black 5 (RB5) | effluent from textile industries located at Industrial area, Panki site 5, Kanpur, India | – | – | 98% after 120 h of incubation | Atomic absorption spectrophotometer and GC-MS | (Khan et al., 2018) |
| Staphylococcus sp. K2204          | Remazol Brilliant Blue R (RBBR) | textile wastewater | enzymes like laccase, manganese and lignin peroxidase facilitated the catalysis of the asymmetric cleavage reductive cleavage of azo groups | 4-amino-naphthalene-1-sulfonic acid; 3-amino-4-hydroxy-naphthalene-1-sulfonic acid; 3,4-dihydroxy-naphthalene-1-sulfonic acid; naphthalene-1,2,3,4-tetraol; catechol; 3,7-dihydroxy-octahydro-naphthalene-2,6-dione | complete decolorization of RBBR within 12 h. | FTIR, HPLC | (Velayutham et al., 2018) |
| Pichia sp. Strain TCL             | Acid Red B | sea mud collected in Heishijiao Beach Park (Dalian, China) | – | – | 90% of dye (100 mg/L) decolorized within 10 h | UV-vis, HPLC analysis | (Qu et al., 2012) |
| Providencia sp. SRS82             | Acid Black 210 (AB210) | Soil and wastewater samples from the vicinity of textile dyeing industries located in Indore, India | Induction of intracellular and extracellular lignin peroxidase, intracellular laccase and tyrosinase, azoreductase, and DCIP reductase | degrade 100 mg/L of dye within 90 min under optimum conditions | FTIR, HPTLC, HPLC, GC/MS and LCMS | (Agrawal et al., 2014) |
| Brevibacillus laterosporus and Galactomyces geotrichum | Reactive Red 198 (RR 198) | Microbial Type Culture Collection, Chandigarh, India | veratryl alcohol oxidase, laccase, NADH-DCIP reductase and azoreductase (Biomineralization) activities of enzymes such as tyrosinase, laccase, and manganese peroxidase were observed | (ethylsulfonyl)benzene and 1,3,5-triazine | 92% | FTIR, HPTLC, GC-MS | (Kurade et al., 2015) |
| Bacillus vietnensis sp. MSB17     | Malachite Green (MG) | continental slope of the eastern Arabian Sea | methanone, [4-(dimethylamino)phenyl] phenyl - and 2, 6-bis (1, 1-dimethylethyl) phenol | complete decolorization of dye (50 mg/L) was attained within 4 h of incubation | UV-VIS, FT-IR, and GC-MS analysis | (Kabeer et al., 2019) |
Fig. 1. Microbial degradation pathway of Malachite green. (Mukherjee and Das, 2014; J. a. Wang et al., 2012). LMG - leucomalachite green.
| Microorganism/Consortium | Pesticide | Isolated from/ Source | Degradation Pathway | Degradation Intermediates (Metabolites) | Efficiency | Technique used | Reference |
|-------------------------|-----------|------------------------|---------------------|----------------------------------------|------------|----------------|-----------|
| **Fusarium solani**     | Lindane   | Soil samples from the premises of IndiaPesticide Limited, Uttar Pradesh, India | release of chloridewhen Lindane was used as sole carbon source | – | 59.4% | Gas Chromatography | (Sagar et al., 2011) |
| **Streptomyces sp. Strain A5 and Streptomyces sp. Strain A7** | Chlorpyrifos (CP) | Soil samples were collected from a blueberry field that was located in the city of Gorbea in southern Chile | Phosphomonoesterase act in hydrolyzing O-P bonds leaving phosphorus available for uptake as a source of phosphorus and to release ethanol as a carbon source | 3,5,6-trichloro-2-pyridinol (TCP) | 90% degradation after 24 h of incubation | HPLC, Gas Chromatography | (Briceño et al., 2012) |
| **Fusarium verticilloides** | Lindane | from Agave tequilana leaves by enrichment techniques | aerobic carboxylation is suggested | gamma-pentachlorocyclohexene and benzoic acid derivatives | 86% complete removal or Chlorpyrifos in 24 h | 
| **Aspergillus terreus Strain JAS1** | Chlorpyrifos | Paddy field soil sample was collected from the top layer 0–20 cm in Vellore district, Tamil Nadu, India | chlorpyrifos employed as a sole carbon and energy source | 3,5,6-trichloro-2-pyridinol | 90% degradation after 24 h of incubation | HPLC, FTIR | (Silambareasan et al., 2013) |
| **Aliciigenes faecalis Strain JBW4** | endosulfan | activated sludge samples were collected from an endosulfan company | non-oxidative pathway | Endosulfan diol and endosulfan lactone | 87.5% of alpha endosulfan and 83.9% of beta endosulfan degraded within 5 days | GC-MS | (Kong et al., 2013) |
| **Pseudomonas aeruginosa Strain Is 6** | acephate | Composite surface soil samples were collected from agricultural sites of Tanjore, Tamil Nadu, India | The oxidative degradation of acephate was found to be due to hydrolysis of carboxyl group by carboxylesterase enzyme and releasing acetic acid residue | No accumulative products were detected; the metabolites might have formed and been immediately degraded | the strain Is-6 showed 92% degradation of acephate (1000 mg/L) within 7 days of incubation | HPLC, ESI-MS | (Ramu et al., 2014) |
| **Streptomyces sp. Strain A14** | Methoxychlor (MTX) | surface soil samples were taken from an experimental site northwest of San Miguel de Tucuman, Argentina | dominantly degraded by dechlorination, dehydrogenation and CN-replacement | 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethene, 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethene, 1-chloro-2,2-bis(4-methoxyphenyl)ethene, and 2,2-bis(4-hydroxyphenyl)acetonitrile | For conc of pesticide 8.33 and 16.60 mg/kg, bacteria reached its maximum removal percentages (40% and 76%) after 28 days of incubation | GC-MS | (Cai et al., 2014) |
| **Ochrobacterium sp. strain HZM** | quinalphos (QP) | pesticide-contaminated soil samples | hydrolysis of organophosphate compounds | 2-Hydroxyquinoline and diethyl phosphate | 84.61% | HPLC, GC-MS | (Talwar et al., 2014) |
| **Pseudomonas aeruginosa** | chlorpyrifos (CP) | bacteria were isolated from chlorpyrifos (CP) treated rice plants | enzyme catalysis | – | five isolates degraded more than 90% of CP in 24 h when the initial concentration was lower than 5 mg/L. | CLSM, GC-ECD | (Feng et al., 2017) |
| **Strain RRA, Bacillus megaterium Strain RRB, Sphingobacterium yunnanensis Strain RSA, Stenotrophomonas masepavani Strain RSB and Curtobacterium plantarum Strain RSC** | chlorpyrifos | samples were collected from isolate utilized the flubendiamide as a – | 89.06% initial pesticide was removed | | | (Jadhav and David, 2016) |
| **Chryseobacterium indologenes Strain SSJ1** | flubendiamide | – | 89.06% initial pesticide was removed | | | (continues on next page) |
| Microorganism/Consortium | Pesticide | Isolated from/Source | Degradation Pathway | Degradation Intermediates (Metabolites) | Efficiency | Technique used | Reference |
|--------------------------|-----------|----------------------|---------------------|----------------------------------------|------------|----------------|-----------|
| Bacillus sp. Strain SG2  | cypermethrin | Pesticide-contaminated soils were collected from groundnut cultivating soil of Dharwad district, Karnataka, India, sole carbon and nitrogen source | ester hydrolysis of pyrethroid takes place by the isolate with 5 days incubation period | 4-propylbenzoate, 4-propylbenzaldehyde, phenol M-tertbutyl and 1-dodecanol | bacteria degraded the compound up to 81.6% within 15 days | UV-vis Spectroscopy, HPLC | (Sharma et al., 2016) |
| Bacillus tequilensis      | trichlorfon (TGF) | Soil samples were collected from the surface layer of a pesticide-polluted field in Hubei Province, China | deoxidation and dehydration (including the cleavage of the P–C phosphonate bond and the C–O bond) | DDCV, dimethyl phosphate, Trichloroethanal, dimethyl hydrogen phosphate and chloral hydrate | degradation of 71.1% at an initial TCF concentration of 200 mg/L within 5 days | HPLC GC–MS FTIR | (Tian et al., 2016) |
| Bacillus subtilis and Fomitopsis pinicola | DDT | Culture collection | (1) dechlorination to DDD, (2) dehydrochlorination to DDE, and (3) formation of DDMU | DDD (1,1-dichloro-2,2-bis(4-chlorophenyl) ethane), DDE (1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene), and DDMU (1-chloro-2,2-bis(4-chlorophenyl) ethylene) | addition of 10 mL of B. subtilis into F. pinicola culture showed the highest DDT degradation of 86% during the 7 days incubation period | HPLC GC–MS | (Sariwati et al., 2017) |
| Citricoccus sp. TT3       | Atrazine | Soil samples were collected from the wastewater outfall of the Tianjin Huayu Pesticide Factory in China | proposed: atrazine-hydroxyatrazine-N-isopropylammonium-cyanuric acid. These steps are catalyzed by the enzymes encoded by trzN, atzB, and atzC, respectively | – | the strain removed 50 mg/L atrazine in 66 h with 1% inoculum | PCR, SEM and Agarose gel electrophoresis | (Yang et al., 2018) |
| Pseudbacilluspolymyxa    | fluazinam, BHC, PCNB, chlorpyrifos and DDT | General Microbiology Center of the China Microbial Culture Collection Management Committee | – | the main degradation products were alkanes, which are nontoxic | the degradation rates of fluazinam, BHC, PCNB, chlorpyrifos, and DDT in the medium were 94.77%, 70.34%, 77.92%, 78.30%, 66.70%, | GC–MS HPLC | (Zhang et al., 2019) |
| Rhodococcus rhodochrous sp. AQ1, Bacillus tequilensis sp. AQ2, Bacillus aryabhattai sp. AQ3 and Bacillus safensis sp. AQ4 | Metribuzin (MB) | soil samples were collected from potato vegetated field at Arifwala, Pakistan | Complete biomineralization into water and carbon di-oxide | desamino-metribuzin (DA), diketo-metribuzin (DK) and desamino-diketometribuzin (DADK) | 98.63% MB degradation was observed | GC–MS HPLC | (Wahla et al., 2019) |
| Cupriavidus sp. ISTL7     | Carbofuran | waste sampling performed at Ghazipur landfill Delhi, India | hydrolysis pathway starting from carbofuran toddegrade and form carbofuran-7-phenol and methylamine | carbofuran-7-phenol, methylamine, 2-hydroxy-3-(3-methylpropan-2-ol) benzene-N-methyl carbamate etc. | strain ISTL7 efficiently degraded approximately 98% of carbofuran (400 ppm) within 96 h | FTIR GC–MS | (Gupta et al., 2019) |
Fig. 2. Example of biodegradation pathway - Atrazine (with genes and enzymes) (De Souza et al., 1998; Mandelbaum et al., 1993; Martinez et al., 2001). AM, atrazine Monooxygenase; AC, atrazine chlorohydrolase; AH, allophanate hydrolase; BH, biuret hydrolase; CAH, cyanuric acid hydrolase; DIHA, deisopropyldihydroxyatrazine amidohydrolase; DEAM, deethylatrazine monooxygenase; EAA, N-ethylammelide amidohydrolase; HAEA, hidroxyatrazine ethylaminohydrolase; IAIA, N-isopropylammelide isopropylamidohydrolase; TC, s-triazine chlorohydrolase; TH, s-triazine hydrolase.
et al., 1984). MG has been banned in many countries but due to its low cost and efficacy it is still used in some countries. Azo dyes account for the majority of the synthetic dyes used in commercial applications. Azo dyes are aromatic compounds with one or more \(-N-N\) groups. Therefore, proper degradation or removal of these dyes from the environment is of high priority.

### 2.1. Toxicity effects of dyes

The textile effluent and their products have been found to be of toxic nature in the environment. When the textile effluent containing azo dyes is released into the environment it leads to many problems because of their teratogenic, mutagenic and carcinogenic effects (Tan et al., 2016). Various reports have observed and described the ecotoxicological effects of dyes on aquatic life (Bae et al., 2006; Merić et al., 2005). Daphnia and Danio are the most common organisms on which the evaluation of both acute and chronic toxicity is routinely done (Y. Verma, 2006). However, the results obtained from the toxicity studies on single organism cannot be extrapolated to other taxa levels because of different responses of each organism to the contaminant. The link between carcinogenic and mutagenic effects of some azo dyes has been well established as of now (Chequer et al., 2011). An indirect link was established between hypoactivity of zebrafish larvae and energy consumed. It was found that zebrafish larvae exposed with dye Basic Red 51 were less active (Abe et al., 2018). Moreover, certain metabolites produced by breakdown of dyes were found to be even more toxic than parent compounds. For example, the toxicity of Acid Violet 7 increases after biodegradation by Pseudomonas putida due to the formation of metabolites 4'-amino-acetanilide and 5-acetamido-2-amino-1-hydroxy-3,6-naphthalene disulfonic acid (Mansour et al., 2010). Natural dyes have been implemented in various uses such as cosmetics, drugs, textiles, these are more biodegradable than synthetic dyes and have less harmful effects at same concentrations as that of synthetic dyes. However, more research needs to be done to evaluate toxicity effects of natural dyes as there is less evidence available in literature on natural dyes compared to synthetic dyes (Abe et al., 2018).

### 2.2. Microbe mediated remediation of dyes

Many physical (adsorption, coagulation, flocculation, membrane filtration etc.) and chemical methods (oxidation process, Fenton’s reagent, ozonation etc.) are available for the removal of dyes. But these methods have their own disadvantages which are: low efficiency, selective over types of dyes, sludge production, generation of toxic by-products, and sometime high cost. Biodegradation using microorganisms provides a cost effective, feasible and environmental-friendly alternative to the physiochemical methods. Generally, microorganisms are isolated and characterized from anthropogenic polluted environment because they have adapted and are competent to remediate such sites (Asad et al., 2007). Examples of the microorganisms which have been isolated from such sites and were able to grow on certain optimal conditions are mentioned in the Table 1: (based on literature).

As it is evident by now, Malachite green has multiple toxicity effects on body and is a recalcitrant dye. A species of Enterobacter genus was found to degrade malachite green with more than 98% efficiency when it was provided with sucrose and beef extract (as carbon and nitrogen sources respectively) in a ratio of 5:1. Biodegradation pathway of Malachite green has been well studied, it involves enzymes such as laccase, reductase and cytochrome P450 enzymes and involves production of metabolites such as leucomalachite green, desmethyl leucomalachite green, didemethyl leucomalachite green and aniline (Ref. to Fig. 1) (Mukherjee and Das, 2014; J. a. Wang et al., 2012).

Optimization of parameters such as pH, temperature, salt concentration is done using various statistical approaches such as response surface methodology (RSM) to improve the biodegradation efficiency (Kumar et al., 2019). The genes from these tolerant strains can be characterized and can be utilized by genetic engineering to form new recombinant microorganisms which will exhibit the biodegradation capability of the donor gene microorganism. Various microorganisms such as bacteria, fungi and yeast etc. are responsible for biodegradation of dyes. However bacteria are preferred over other microorganisms due to their fast replication, easy manipulation and ability to tolerate harsh conditions (Rathour et al., 2018). Microorganisms have been utilized in various forms - pure, mixed, living and dead for biodegradation. Isolated bacteria are more often immobilized (on alginate beads) for biodegradation as it provides certain advantages such as higher degradation efficiency, capability of reuse and higher biomass loading. A great deal of advancement has been done in the development of bioreactors. Various bioreactors such as stirred tank bioreactors, airlift bioreactors, fluidized bed bioreactors, wave bioreactors, combined or sequential bioreactors have been employed (Vikrant et al., 2018). Various enzymes have been reported in literature which are linked with the process of biodegradation of dyes such as - azoreductases, laccases, tyrosinases, lignin peroxidases, Mn peroxidases and DCIP-NADH (R. L. Singh et al., 2015). The concept of simultaneous degradation of dye and production bioelectricity has been utilized in the form of Microbial Fuel Cell (MFC) (Fernando et al., 2014; Ilamathi and Jayapriya, 2018). Multiple dyes like Reactive Black 5, Reactive Orange 16, Disperse Red 78 and Direct Red 81 were degraded by a single consortium consisting of bacteria Providencia rettgeri strain HSL1 and Pseudomonas sp. SUK1 (Lade et al., 2015).

### 3. Pesticides

The growing population demands its need of food to be met unanimously. Population growth kinetics suggests that the overall population of humans by 2050 is going cross the nine billion mark. To provide the need of food to live to such an enormous number of individuals would require increase in production of food by 70 percent (According to Food and Agriculture Organization; FAO report Rome 12–13 October,2009). The first and most important thing in achieving this feat would be to minimize the losses to crops by pests. Pesticides are chemicals that are used to control the population of pest to the level at which they would cause minimalistic harm to the crops. Pesticides cover a wide range of target pests including mites, snails, insects, rodents, fungi, birds and even viruses (Velázquez-Fernández et al., 2012). They can be classified on the basis of their persistence in the environment. They can be classified as non-persistent (readily degradable) or persistent pesticides. Non-persistent pesticides include – methoxychlor, malathion, paraquat etc. Whereas persistent pesticides include – DDT, aldrin, tordon, turbacil, etc. (J. P. Verma et al., 2014).

#### 3.1. Toxicity effects of pesticides

Pesticide residues pose a great threat to the soil quality and health of living organisms. There are various effects of pesticides on aquatic life such as delayed metamorphosis, disruption of steroid metabolism, low rate of opercular movement, erratic swimming etc. (Sidhu et al., 2019). There is enough evidence of toxic effects of pesticides on both aquatic as well as terrestrial life on both plants as well as animals. Atrazine is one of the pesticides that has toxic effects on wide range of organisms including humans. It can affect central nervous system, reproductive system, cardiovascular system and immune system. There has been tremendous development in the field of statistics and artificial intelligence. To reduce the time and effort, assessment of toxicity of pesticides in rats has been done by one such example of artificial intelligence i.e. QSAR model (Quantitative Structure–Activity Relationship) (Hamadahe et al., 2016). Triazinedepon and its metabolite triadimenol affected endocrine machinery of Xenopus laevis (African frog). Triadimefon was found to be causing more toxic effects than triamidenol. Moreover, the frogs exhibited sex-linked differences liver histology, antioxidant enzyme activities and thyroid hormone levels (W. Zhang et al., 2020). At sub-lethal concentrations, flumethrin has been found to have high acute
| Microorganism/Consortium | Pharmaceutical Isolated from/ Source | Degradation Pathway | Degradation Intermediates (Metabolites) | Efficiency | Technique used | Reference |
|--------------------------|-------------------------------------|---------------------|------------------------------------------|------------|----------------|-----------|
| Trametes versicolor      | clofibric acid (CLOFI, lipid regulator) and carbamazepine (CARBA, antiepileptic/ analgetic) | American Type Culture Collection. | cytochrome P450 system may be involved in the first step of CLOFI and CARBA oxidation by T. versicolor meta-cleavage pathway. | – | CLOFI (91%) and CARBA (58%) | GC-CIRMS (M-Maro-Urirea et al., 2009) |
| Pseudomonas sp. SA01     | Phenol samples were taken from pharmaceutical plant wastewater effluent located west of Tehran | biocatalysis and biodegradation database (BBD) software developed by the University of Minnesota (UM) was used to simulate and predict the biodegradation pathway of CLF | 2-hydroxy-3-phenyl pyrazine | – | isolated strain started to degrade 0.7 g/L of phenol after an initial very short lag phase, and phenol decomposition was then rapidly completed within 30 h | UV-vis SEM (Shourian et al., 2009) |
| Mixed culture of Heterotrophic Bacteria | clofibric acid mixture of soil contaminated with several herbicides, including propanil, and soil from organic rice agriculture supplemented with (NH4)2SO4 and propanil | biocatalysis and biodegradation | 3-amino-5-methylisoxazole | 51% biodegradation (initial CLF concentration – 2 mg/L) | HPLC-DAD GC–MS (Salgado et al., 2012) |
| Pseudomonas sp. Strain CE21 and Strain CE 22 | Cefalexin Wastewater samples containing activated sludge were collected from sewage treatment plant in Hong Kong | – | Strain CE22 was able to degrade over 90% of cefalexin, while CE21 was able to remove 46.7% of cefalexin after incubation for 24 h | HPLC MS–MS (Lin et al., 2015) |
| Achromobacterdenitrificans PR1 | Sul-famethoxazole (SMX) Samples of activated sludge and treated domestic wastewater collected from a wastewater treatment plant in the North of Portugal | sulfonamide was used as sole source of carbon, nitrogen and energy | Strain PR1 was able to remove SMX at a rate of 73.6 μmolSMX/g cell dry weight | DGGE HPLC–UV–vis (Reis et al., 2014) |
| Labrys portucalensis strain F11 | Fluoxetine (FLX) Sediment sample collected from an industrially contaminated site in Northern Portugal | fluorobenzene (FB) was used as sole carbon and energy source | stoichiometric liberation of fluoride | 2 μM of racemic FLX was completely removed of both enantiomers in 30 d | HPLC analysis (Moreira et al., 2014) |
| Streptomyces MIUG 4.89 | Carbamazepine Microbial Cultures Collection of the Bioidment Research Center, ’Dunarea de Jos’ University of Galati, Romania. | extracellular laccase production thought to play role in degradation | – | 35% degradation at an initial concentration of 0.2 mg/L of carbamazepine | HPLC (Popa et al., 2014) |
| Unilago sp. SMN03 | Cefdinir Wastewater was collected from a pharmaceutical industry located in Ranipet, Vellore Dist., India | Cefdinir was utilized as a sole carbon source | six novel intermediates formed isolate was found to degrade 81% of cefdinir within 6 days and an initial cefdinir concentration of 200 mg/L when the initial Erythromycin A concentration was 200 mg/L | UV–vis LC–MS FTIR (Selvi et al., 2014) |
| Octobactremsp. Strain WX-J1 | Erythromycin A (EA) Soil contaminated by EA was collected | Strain WX-J1 can be utilized | 3-depyranoslyoxy erythromycin A, 7,12- | HPLC–UV–MS (C. Zhang et al., 2017) |

(continued on next page)
toxicity in honey bees. The toxic effects were found to be a result of increased oxidative stress and damage to the midgut by apoptosis (Qi et al., 2020). Acetamiprid is a member of neonicotinoids, it was thought to be safe in prospective of mammals but recent reports suggest that it also exhibits toxic effects towards mammals. It works by binding to nicotinic acetylcholine receptors in insects. Exposure to acetamiprid was found to be associated with decreased neurogenesis in mice and abnormal neuronal distribution in newborn mice (Kagawa and Nagao, 2018). Fenvalerate is a widely used pesticide and is known to cause impairment of male reproductive system but the mechanism is not clear. Recently, it has come to light that fenvalerate may impair male reproductive system through changes in circadian rhythm gene levels. It was found that fenvalerate inhibited testosterone synthesis, altered the expression of circadian rhythm mRNAs and increased intracellular calcium ion levels in mouse Leydig cells (Guo et al., 2017). Organophosphate pesticides have been shown to hinder various metabolic processes in plants such as photosynthesis, carbon metabolism, chlorophyll biosynthesis and nitrogen metabolism (Sidhu et al., 2019).

3.2. Microbe mediated remediation of pesticides

Earlier studies of microbial remediation of pesticide residues can be traced back to 1940. In case of DDT it was observed that if co-substrate starch (slow releasing carbon source) was added for co-metabolism, it was able to degrade over 98% of 10 ppm lindane within 10 days (Chaurasia et al., 2013). Microbial fuel cells (MFCs) are also being constructed to degrade organic waste and to simultaneously generate electricity. An experiment was conducted in which soil MFC could remove 71.15% of the hexachlorobenzene provided (Cao et al., 2015). Biofilms are already known to be important biogeochemical cycling and removal of pollutants from the ecosystem. The ability of natural river biofilm to degrade carbofuran and carbaryl was studied and effect of different seasons on the biodegradation efficiency by biofilms was also checked. It was observed that the ability of river biofilms to degrade carbofuran in four different seasons were similar (54.1–59.5%) but the biofilms showed low efficiency in degrading carbaryl (0–27.5%) (Tien et al., 2013). Genetic engineering has enormous potential in improving the process of biodegradation by making recombinant strains. Lindane is a highly persistent toxic pesticide which impairs photosynthesis, respiration and nitrogen-fixation in Anabaena. To solve this problem linA2 gene encoding dehydrochlorinase (obtained from Sphingomonas paucimobilis B90) was knocked-in and overexpressed in Anabaena genome. The resulting recombinant Anabaena was able to degrade over 98% of 10 ppm lindane within 10 days (Chaurasia et al., 2013). Microbial fuel cells (MFCs) are also being constructed to degrade organic waste and to simultaneously generate electricity. An experiment was conducted in which soil MFC could remove 71.15% of the hexachlorobenzene provided (Cao et al., 2015). Apart from soil and wastewater sources from environmentally contaminated area, the biodegrading microbes can also be isolated from higher living organisms present there. Five different bacterial strains capable of degrading endosulfan were isolated from microflora of Blatta orientalis (cockroach). The isolated bacteria were identified as Pseudomonas aeruginosa G1, Stenotrophomonas maltophilia G2, Bacillus atrophaeus G3, Citrobacter amalonaticus G4 and Acinetobacter baumannii G5 based on morphological, biochemical and fatty acid profile analysis (FAME). They were capable of degrading endosulfan and had efficiency range between 56 and 89%. Similarly five different strains of endophytic bacteria were isolated from rice plants and were found to be capable of degrading chlorpyrifos both invivo as well as invitro (Feng et al., 2017).

4. Pharmaceuticals

Pharmaceuticals comprise a class of relatively emerging contamnats compared to others. Pharmaceuticals are utilized all over the world to treat diseases or to maintain the health humans or animals. Various kinds of pharmaceuticals contaminating the environment include antibiotics, analgesics, antacids, tranquilizers, stimulants, 

| Microorganism/Consortium | Pharmaceutical | Isolated from/Source | Degradation Pathway | Degradation Intermediates (Metabolites) | Efficiency | Technique | Reference |
|---------------------------|----------------|----------------------|---------------------|--------------------------------------|------------|-----------|-----------|
| Citrobacter amalonaticus | Paclitaxel     | at a site near a pharmaceutical factory, Henan, China samples were collected from wastewater chamber of the Sobhan oncology pharmaceutical company | The isolate utilized Paclitaxel as the sole carbon source. Aerobic degradation pathway is suggested by authors | Erythromycin A concentration was 100 mg/L, 97% of Erythromycin A was degraded 87–93% efficacy under aerobic condition | HPLC | (Zamani et al., 2016) |

Table 3. (continued)
Table 4.
Biodegradation of explosive waste by microbes.

| Microorganism                          | Explosive waste | Isolated From                  | Degradation pathway                                                                 | Degradation product                                                                 | Efficiency/ Specific degradation rate | Technique used          | Ref.                  |
|----------------------------------------|-----------------|--------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------|-------------------------|------------------------|
| Phanerochaetechrysosporium             | TNT             | Forest Products Laboratory     | Degradation occurs by reduction of nitro groups                                       | 2amDNT 4amDNT                                                                       | The initial concentration of TNT was 30 mg/L. Thin concentration of TNT was reduced to less than 60 μg/L at the end of the 96 h incubation specific degradation rate was a value of 0.22 mmol of N per s/kg of protein | HPLC NMR GC-MS            | (Bumpus and Tatarko, 1994) |
| Stenotrophomonas maltophiliaPB1        | RDX             | Soil and water samples         | isolate from the culture used RDX as a sole source of nitrogen for growth                | methylene-N-(hydroxymethyl)-hydroxylamine-N′-(hydroxymethyl) nitrosoimine            | specific degradation rate gave a value of 1.03 mmol of PETN/g of protein per hour | HPLC NMR Mass Spectrometry | (Binks et al., 1995) |
| Enterobacter cloacaePB2                | PETN            | Soil and water samples         | Isolate was found to use PETN as a sole source of nitrogen for growth                    | pentaerythritol dinitrate, 3-hydroxy-2,2-bis[[nitrooxy] methyl]propanal, and 2,2-bis[[nitrooxy] methyl] propanal | specific degradation rate gave a value of 1.03 mmol of PETN/g of protein per hour | Mass Spectrometry NMR HPLC | (Binks et al., 1996) |
| Pseudomonas putida strain TP1 and Pseudomonas aeruginosa strain TP6 | TNT             | Soil samples collected from a TNT-contaminated site located in southern Taiwan | Both strains demonstrated the ability to grow on the medium containing TNT as a carbon, energy, and nitrogen source | –                                                                                   | More than 90% of the TNT in the growth medium was degraded by both strains after 22 days incubation | HPLC                  | (Chien et al., 2014) |
| Mixed culture                         | NTO             | Soil Samples                   | degradation occurred via reduction of nitro-groups                                        | 3-amino-1,2,4-triazol-5-one (ATO) and 3-hydroxymino-1,2,4-triazol-5-one (HTO)        | –                                                                                   | HPLC-DAD QToF-MS         | (Krzmarzick et al., 2015) |
| Mixed Culture                         | TET and PETN    | textile wastewater treatment plant activated sludge | PETN degradation in the aerobic condition follows a successive reductive degradation pathway with the release of NO2 in each denitrification step. TNT biodegradation involved reduction of one nitro group to form a hydroxylamino group and subsequent reduction of the other nitro group to an amino group | Addition of rhamnolipid surfactant (50 mg/L) increased the removal efficiencies of TNT and PETN from 53% and 57% to 98% and 91%, respectively | –                                                                                   | HPLC LC-MS              | (Karami et al., 2017) |

antipyrctic, lipid regulators, anti-depressants, and other various prescription and non-prescription drugs (Rana et al., 2017). Although pharmaceuticals have been present in water bodies for decades the paradigm of considering them environment contaminants has shifted towards the start of 21st century. Pharmaceuticals are excreted out of the human system after being transformed into a metabolite or without transformation. The excreta from humans as sewage carries these pharmaceuticals towards wastewater treatment plants (WWTP). If the wastewater is not treated properly, the effluent from WWTP becomes a cause of concern for the aquatic ecosystem after being release into the water bodies (Rivera-Utrilla et al., 2013). Aquaculture, hospital wastewater and illegal drug disposal can be the other sources of contamination (Caracciolo et al., 2015). Pharmaceuticals as emerging contaminants are unique in the sense that they are designed to be active even at low concentrations. Moreover, their target includes enzymes or receptors which can be conserved among evolutionary distant organisms. Impact of these contaminants in the range of ng/L to μg/L have shown to cause sub-lethal effects in non-target organisms in literature (Hampel et al., 2010; Mimeault et al., 2005).
4.1. Toxicity effects of pharmaceuticals

It is quite obvious how pharmaceuticals play a major role in increasing the life span of humans by decreasing the potential risk of diseases and ultimately treating them. The possible effects on other organisms and environment are becoming clearer with the help of research focusing on ecotoxicology effects of pharmaceuticals. NSAID (non-steroidal anti-inflammatory drugs) is the most important class of drugs – Ibuprofen, ketoprofen and aspirin are its most important members. These drugs are incompletely degraded and their discharge into sewage or release into surface water ultimately leads to their accumulation which poses a threat to aquatic life (Gomez-Olivan et al., 2014). Ibuprofen is linked with nephrotoxic effects in Rhamdia quelen (Mathias et al., 2018). NSAIDs are also linked with decreased photosynthetic and respiratory rates in green algae Scenedesmus obliquus (H. Wang et al., 2020). Salicylic acid, primary metabolite of acetylsalicylic acid (aspirin), was found to be responsible for oxidative stress and neurotoxicity in Mytilus galloprovincialis (mussel). It was observed that the acetylcholinesterase activity was decreased when treated with ketoconazole and erythromycin both singly as well as in combination, providing indication of potential neurotoxicity to the animal (Liu et al., 2017). When wistar rats were treated with pharmaceutical wastewater, necrosis of renal epithelial cells in kidney, inflammation in endocardium and cellular swelling in liver were observed (Sharif et al., 2016). Pharmaceutical wastewater constitutes mixture of pharmaceuticals in low concentrations rather than isolated drugs. Therefore, studies focusing on treatment with mixture of pharmaceuticals might provide a more suitable approach to find potential toxicity in organisms present in environment. In environment, drugs may interact with each other and interfere with the mode of action or work independently. This may lead to increased or decreased effect on the non-target organisms (Geiger et al., 2016). For example, the toxic effect on Lissodelphis peronii in the form of loss of tactile response was relatively higher on exposure to mixture of Naproxen, Carbamazepine and Sulfamethoxazole as compared to when individual compounds were tested, however the concentrations of drugs used were much higher than those found in the environment (Melvin et al., 2014). To predict potential targets of drugs on evolutionary close or distant species, databases such as ECOdrug (www.ecodrug.org) can also be utilized.

4.2. Microbe mediated remediation of pharmaceuticals

As it is evident by now that microbes play a major role in biodegradation of xenobiotics, pharmaceuticals have also been found to be degraded by the microbes. In fact, some microbes utilize these contaminants as source of their energy by complete mineralization. Biodegradation provides a feasible method of removing contaminants because the physical methods, advanced oxidation process, activated carbon are limited by high energy requirement and production of toxic by-products (Homem and Santos, 2011; Schwarzenbach et al., 2006).
Table 5. Biodegradation of different PAHs by various microbes.

| Microorganism/Co-Culture/Consortium | PAHs                  | Isolation from or Source                  | Degradation Pathway/Enzymes Involved                                                                 | Degradation Product (Metabolite)                          | Percentage Transformation | Techniques Used | Reference                      |
|-------------------------------------|-----------------------|-------------------------------------------|-----------------------------------------------------------------------------------------------------|----------------------------------------------------------|--------------------------|------------------|---------------------------------|
| Halomonas sp.                       | Phenanthrene (Phe), pyrene (Pyr), naphthalene (NaP), and benz[a] pyrene (BaP) | Brackish water sample from Pichavaram mangrove, Tamil Nadu, India, [1, 2, 3, 4, 5, 6] | Extracellular ligninolytic enzymes (laccase and non-specific peroxidases) | variable                                   | naphthalene 34–73%, phenanthrene 9–67%, fluorene 11–64% | GC-MS            | Torres-Farradé et al., 2019     |
| Ganoderma sp.                       | Naphthalene, phenanthrene and fluorene | –                                          | Naphthalene dioxygenase and ligninolytic enzymes                                                   | u, β-naphthol, salicylic and benzoic acid                | 86.47%                                  | HPLC and Thin layer chromatography (TLC) | Elhusseiny et al., 2019 |
| Pleurotus ostreatus                | naphthalene            | Pharmaceutical Microbiology Laboratory (NCRRT-Egypt) | variable                                                                                           | 94.80, 90.16, and 93.80%, respectively, after 10 days   | GC-MS                          | (Santupalli et al., 2019) |
| Aspergillus terricola var americanus | Benz (a) Anthracene, Dibenzo (a, h) Anthracene and Indeno [1, 2, 3-cd] Pyrene | crude oil was collected from Dayag Oilfield, Tianjin Province, Northern China | Aeroxygenases through dioxygenase enzyme system pathways                                       | 4,5-dihydroxy-4,5-dihydropyrene, 4-phenanthrol, 1-hydroxy-2-naphtholic acid and phthalate | 82.88% after 25 d | GC-MS                          | Jin et al., 2016 |
| Pseudomonas sp. JPN2               | pyrene                | cultural collection from Dayag Oilfield, Tianjin Province, Northern China | Degradation of pyrene through aeroxygenase enzyme system                                           | variable                                                 | 30, 47, and 5%, respectively | GC/MS                  | Liang et al., 2016            |
| Pseudomonas sp. JPN1               | benzo[a]pyrene (BaP), fluorenone, and phenanthrene | –                                          | Anaerobic biodegradation with nitrate as the electron acceptor                                     | variable                                                 | 30, 47, and 5%, respectively | GC/MS                  | Liang et al., 2016            |
| Ulva prolifera                      | Phenanthrene          | coastal water (Rushan City, China)         | –                                                                                                   | 91.3%                                                   | –                         | GC/MS                  | C. Zhang et al., 2017         |
| Chlorocella vulgaris                | fluorenone            | Collection of Algae of Bushehr Shrimp Research Institute, Iran center for conservation and utilization of blue green algae, iari, new delhi, india | dioxygenase enzyme system based degradation                                                        | N-Hydroxymethylcarbazol, Dibutyl phthalate, Hexadecanoic acid, ethyl ester, 1,2-Benzenedicarboxylic acid, diocetyl ester | –                         | GC-MS                  | Asghari et al., 2019          |
| Anaabaena fertilissima             | anthracene (ant) and pyrene (pyr) | –                                          | –                                                                                                   | degraded product for ANT was 2, 4-Dimethyl-1-heptene and for PYR it was 2, 3, 4-Trimethylhexane | degradation of ANT by 46% and PYR by 33%, at 5.0 mg/L and 3.0 mg/L, 78.8% was observed in 13 days | GC/MS                  | Patel et al., 2016             |
| Cellulosimicrobium cellulans CWS2  | benzo[a]pyrene        | PAH contaminated soil                      | Anaerobic degradation under nitrate-reducing conditions                                             | pyrene, 1-amino-pyrene, phenanthrene, 1-methylphenanthrene, 1,7-dimethylphenanthrene, 1-(2-hydroxypropyl) naphthalene, 1-methyl-naphthalene, 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione, diethyl phthalate, and 2-acetyl-3-methoxybenzoic acid | 92.8% after 14 days | GC/MS                  | Qin et al., 2018              |
| Achromobacter xylosidovorans Strain DN002 | Fluoranthene          | petroleum-contaminated soil               | Aerobic degradation through dioxygenases (catechol 1,2 dioxygenase and catechol 2,3 dioxygenase) | –                                                      | 92.8% after 14 days | GC/MS                  | Ma et al., 2015               |
| Hydrogenaphaga sp. PYR1            | pyrene and benz[a]pyrene | river sediments in the east area of Taihu Lake (a large shallow lake in China) | Anaerobic degradation under ferric iron reduction conditions                                        | benzoic acid, 2-hydroxy-phenyl ester and naphthalene, 1,2,3-trimethyl-4-propenyl | 94% pyrene within 15 d | GC-MS                  | Yan et al., 2017               |

(continued on next page)
Some of the examples of biodegradation by microbes are shown in Table below:

An important pharmaceutical contaminant is Paracetamol (PAM). It is a common over-the-counter drug used most commonly as antipyretic. Microbial fuel cell was coupled with Fenton oxidation process to provide a method by which PAM could be degraded without external power supply (L. Zhang et al., 2015). MFC generally consists of anode and cathode. Microorganisms are grown on anode and are known as electrogenic. They promote the electron transfer to cathode and the oxidized pollutants on cathode are reduced (Logan, 2009).

Nootropic drugs as environmental contaminants and their ecotoxicological effects have sparked little interest. These drugs are not readily metabolized in the system and as much as 90% of the administered drug is reported to be excreted out through urine (Mache et al., 2012). Piracetam (2-oxo-1-pyrroolidine acetamide) is an example of nootropic drugs which was found to be completely mineralized by two species of Ochrobactrum. Its biodegradation occurs through cleavage of the heterocyclic ring at the C–N bond (Woźniak-Karczewska et al., 2018). It was the first report on complete biodegradation of piracetam. However, more insight is needed to elucidate its complete biodegradation pathway. A strain of thermophilic microorganism, Thermus thermophilus C419 was found to be capable of biodegrading members of fluoroquinolones such as ciprofloxacin, ofloxacin, norfloxacin, enrofloxacin. This suggests that microbes can be also utilized to treat harsh environments which are also contaminated (Pan et al., 2018).

Acrylonitrile degrading Corynebacterum sp. D5 utilized nitrile hydratase and amidase in a two-step reaction to generate acrylamide and acrylic acid, although it couldn’t completely mineralize it (Sunarko and Sulistinah, 2019). Over 90% of Iopromide and 70% of Carbamazepine was found to be degraded by fungi Gymnopus luteofolius and Stropharia rugosoannulata respectively when

Table 5. (continued)  

| Microorganism/ Co-Culture/ Consortium | PAHs | Isolation from or Source | Degradation Pathway/Enzymes Involved | Degradation Product (Metabolite) | Percentage Transformation | Techniques Used | Reference |
|--------------------------------------|------|-------------------------|--------------------------------------|-------------------------------|--------------------------|----------------|----------|
| Mycobacterium gilvum                 |      | activated sludge from a coking wastewater treatment plant of SGS Songshan Co., Ltd., China | Aerobic degradation through dioxygenases | Phthalic acid, 1-Naphthol, 4-Phenanthrenol, 4-Phenanthrene carboxylic acid | 95% of pyrene (50 mg L−1) in 7 days | (Wu et al., 2019) |

Fig. 4. Different pathways for biodegradation of PAHs by microbes (Bogan et al., 1996; Cerniglia, 1992; Eaton and Chapman, 1992; Gibson and Parales, 2000; Mueller et al., 1995).
It wouldn’t be surprising for anyone to consider the residues from explosives as environmental contaminants. Explosives are used worldwide most commonly for the use as military ammunition. Explosives are also used in underground mining, demolition work and in construction industry for new roads. Explosives have high content of nitrogen and oxygen which on explosion leave toxic waste in the environment.

Various classes of explosives include nitrate esters, nitroaromatics, and (B. Singh et al., 2012). Conventional methods of treating explosive contaminated site (incineration and composting) suffer limitations such as high expenditure of energy and cost as well as exposure of workers to toxins (Esteve-Núñez et al., 2001).

5.1. Toxicity effects of explosive waste

Most of the data concerning toxic effects of explosive waste comes from TNT and RDX. Dissolved oxygen (DO), chemical oxygen demand (COD), total dissolved solids (TDS) and conductivity are some of the most important parameters that are studied in context to understand and evaluate the toxicity due to the effluents from production of explosives.

TNT wastewater has been shown to have poor biodegradability because of high COD (Ye et al., 2011). Effect of TNT (provided in oral form) was studied in wild cotton rats (Sigmodon hispidus), histopathological studies revealed that TNT caused splenic congestion, lymphoid hyperplasia and increase in liver weight in both males and females. The number of erythrocytes and level of hemoglobin were decreased in both sexes, whereas increase in level of methemoglobin and enhanced activity of glutathione S-transferases (GST) were observed in males (Reddy et al., 2000). Recent advancement in technology provided direct manner for studying toxicity effects of environmental contaminants. Single Plane Illumination Microscopy (SPIM) provided a 3D approach to visualize toxicity effects in zebrafish in developmental stages due to exposure to TNT. The resultant toxic effects observed were – high level of apoptosis in actively developing tissues, cardiac looping defects and hypoplastic heart chamber formation (Eum et al., 2016).

5.2. Microbe mediated remediation of explosive waste

Microbes have been found to successfully metabolize the residual from explosive waste as the source of their growth. Biodegradation of nitrate esters occurs through denitration reaction (Christodoulatos et al., 1997). PETN reductase enzyme was isolated from Enterobacter species (Ref. to Table 4) which was able to transform PETN (pentaerythritol tetranitrate) into nitrites and nitrates (Binks et al., 1996). TNT (2,4,6-trinitrotoluene) is member of the class nitroaromatic compounds. The main pathway by which TNT is biodegraded starts with initial hydrogenation reaction to yield hydride-Meisenheimer complex of TNT (H-TNT) (Vorbeck et al., 1998). Environmental factors also contribute to the degree of biodegradation. For example, it was found that using initial neutral or slightly acidic medium (pH 6) favored the biodegradation of TNT by Yarrowia lipolytica AN-L15 which grew and added to the acidity of the medium by release of organic acids (Ziganshin et al., 2010). It was observed that if co-substrate starch (slow releasing carbon source) was added for co-metabolism by a developed microbial consortium led to complete mineralization and detoxification of DDT under...
degraded via sequential reduction of the N-nitramines class of explosives has been widely used in military munitions. This method is exposed to PAHs affects human fertility and has been associated with the formation of reactive metabolites such as diol-epoxides, radical cations, which lead to DNA mutations, ultimately resulting in teratogenicity. Toxicity arises due to metabolism of PAHs leading to formation of mono-, di- and tri-nitroso derivatives (Ref. to Fig. 3). Alternatively, RDX can also proceed to degradation via direct ring cleavage pathway (Halasz et al., 2002). Another study pointed towards the role of electron transport machinery in degradation of RDX. cymA gene was disrupted by transposon sequence in RDX-defective strain of Shewanella oneidensis. This isolated defective strain degraded RDX at a minimal rate (10% of the wild type) compared to the wild strain providing evidence that cymA (c-type cytochrome) has a major role to play in anaerobic reduction of RDX (Perreault et al., 2012). Several species capable of biodegradation have been isolated, enriched and identified from contaminated area as shown in Table 4. Whereas some species have also been identified by the use of stable isotope probing which is a culture independent method that targets only active organisms. The method involves uptake of labelled substrate and subsequent incorporation of labelled atoms into nucleic acids (Andeer et al., 2012). Members of the classes Spirochaetes, Bacteroidia and α-Proteobacteria which were not previously observed were found to be capable of RDX degradation using this method.

6.1. Toxicity effects of PAHs

Various PAH pollutants pose severe threat to the environment and human health because of their potential toxicity. Most important sources of exposure to PAHs include food containing PAHs, smoke from open fireplaces, and cigarettes (Abed-Shafy and Mansour, 2016). Some PAHs are well known to have properties of carcinogenicity, mutagenicity, and teratogenicity. Toxicity arises due to metabolism of PAHs leading to formation of reactive metabolites such as diol-epoxides, radical cations and o-quinones (active carcinogens). These reactive metabolites result in DNA adducts, which lead to DNA mutations, ultimately resulting in alteration of gene expression profiles (Moorthy et al., 2015). Increased exposure to PAHs affects human fertility and has been associated with incidences of male sterility, loss of ovarian functions (Bidgoli et al., 2011), and early onset of natural menopause (Yun Y. Huang et al., 2018). Epidemiological studies indicate that the ability of PAHs to cross the placental barrier can lead to developmental toxicity. Prenatal or early postnatal exposure to PAHs can lead to various complications like intrauterine growth retardation, low IQ, problems with behavior, allergies or asthma (Drwal et al., 2019). Exposure to PAHs is also linked with diabetes (Yang et al., 2017), oxidative stress (Wang et al., 2015), hepatotoxicity (F. Li et al., 2020b), cutaneous toxicity (Prieux et al., 2019) and many short-term health effects including nausea, vomiting, and inflammation (Gao et al., 2018).

6.2. Microbe mediated remediation of PAHs

A large variety of bacteria, fungi and algae have been isolated that are capable of degrading PAHs using varying metabolic pathways (Ref to Table 5). Aerobic bacteria as well as algae use mono and di- oxygenases for the activation and cleavage of benzene rings, whereas anaerobic bacteria follow PAH catabolism by entirely different pathways and enzymes with metal- and/or flavin-containing cofactors. Various lignolytic and non-lignolytic fungi are able to oxidize PAH and the enzymes involved in the initial attack are mainly Cytochrome P-450 mono oxygenase and lignin degrading enzymes such as manganese peroxidase, lignin peroxidase, phenoloxidases (laccases, tyrosinases), and H2O2-producing enzymes (Kadri et al., 2019) (Ref to Fig. 4).

Most hydrocarbon-contaminated sites such as soil or groundwater system are anoxic, but anaerobic hydrocarbon biodegradation rates are extremely low. Activated carbon (AC) has been shown to act as conductive material facilitating direct interspecies electron transfer (DIET) between bacteria attached to the AC particles (Lovley, 2017). Under anaerobic conditions the biodegradation potential of naphthalene was strongly stimulated (96%) by the AC addition and the diversity of microbial communities increased and was structurally changed with increase in abundance of Geobacter, Thiothrix, Sulfitocurvum, and methanogenic archaea (Bonaglia et al., 2020). Microbial co-metabolism has also been found to increase degradation rate of PAHs. Microbacterium sp. strain mediated degradation of BaP (benzo[α]pyrene) increased notably with the addition of PHE (phenanthrene), and was not accelerated by PYR (pyrene) under denitrifying conditions (Qin et al., 2017).

Low molecular weight (LMW) PAHs are more water-soluble and therefore more readily biodegradable than high molecular weight (HMW) ones. Some bacterial species have been reported to produce emulsifier substances called biosurfactants which can adeptly increase the solubility of these contaminants resulting in increased bioavailability and biodegradation of slightly soluble PAHs. Thermophilic strain Aeribacillus pallidus SL-1 evaluated for the biodegradation of crude oil and PAHs at 60 °C was found to produce SL-bioemulsifier which improved the solubility of PAHs (Tao et al., 2020). Recently, electro-bioremediation has emerged as a promising method for in situ remediation with ability to enhance the removal efficiency. Application of electric potential gradient among electrodes located in a contaminated site forms the basis of this method (Refer to Fig. 5). Electro-bioremediation used for field-scale remediation of a coking plant site resulted in 29.3% and 44.4% increase in degradation of the total and 4–6–ring PAHs, compared to bioremediation alone. Also, the total toxicity equivalent concentrations of total PAHs and 4-, 5- and 6-ring PAHs decreased 49.0%, 63.7%, 48.2% and 30.1%, respectively (F. Li et al., 2020).

7. Conclusions and future perspectives

There has been great progress in the field of bioremediation, although the complete utilization of microbes for bioremediation of sites looks like a possibility of near to midterm future. Despite the high energy and expenditure required, conventional methods of treatment of
environmental contaminants are mostly still in use. There is obvious need for biomediaion to take leaps in success to make it more suitable options compared to conventional methods. Some species have been found to be performing biotransformation rather than complete mineralization of toxic wastes which ultimately leads to production of metabolites of low, equal and sometimes even of higher toxicity than the initial toxic compound. Recent studies have made it clear that mixed consortia utilization can be more advantageous compared to pure cultures for complete mineralization. Co-metabolism using co-substrate can be a good method to improve the efficiency of biodegradation. The recent rise in the field of bioinformatics and the use of statistics can pave a way for easier optimization of culture conditions of microorganisms achieving optimum efficiency of biodegradation. There is still a lot of scope in this field to be covered, for example there are still many species for which biodegradation pathways are yet to be elucidated. Elucidation of these pathways can provide genetic engineering to improve the potential of biodegradation using microorganisms drastically.

Credit author statement

Paramdeep Kaur and Deepshusha Monga: Software, Data curation, Writing- Original draft preparation, Visualization, Investigation. Baljinder Singh: Conceptualization, Methodology, Supervision, Editing, Validation

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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